CHARACTERIZATION OF HYDROPHOBIC SEAWEED FILM REINFORCED WITH KENAF BAST CELLULOSE NANOFIBERS

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by

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LIST OF SYMBOLS

±	Plus-minus
<	Less than
>	Greater than
°C	Degree Celsius
°	Degree
%	Percentage
mm^2	Millimetre square
Pa	Pascal
g	Gram
s	Second
m^2	Meter square
m	Meter
α	Alfa
T_m	Melting temperature
H_m	Enthalpy of melting
T_{onset}	Initial degradation temperature
T_{max}	Maximum degradation temperature
M_o	Initial weight
M_f	Final weight
lo	Original length
e	Extension
θ	Tangent

LIST OF ABBREVIATIONS

CA	Contact angle
CF	Cellulose fibres
CNC	Cellulose nanocrystals
CNF	Cellulose nanofibres
CNW	Cellulose nanowhiskers
EAB	Elongation at break
FT-IR	Fourier Transforms Infrared Spectroscopy
GPa	Gigapascal
HCI	Hydrochloric acid
KCI	Potassium chloride
KBr	Potassium bromide
MAC	Moisture absorption capacity
MCC	Microcrystalline cellulose
MPa	Megapascal
NCC	Nanocrystalline cellulose
RC	Refined carrageenan
RH	Relative humidity
Rpm	Revolutions per minute
SEM	Scanning electron microscopy
SF	Seaweed film
TGA	Thermogravimetric analysis
TS	Tensile strength
WS	Water solubility
WVP	Water vapour permeability
WVTR	Water vapour transmission rate
XRD	X-ray diffraction
YM	Young modulus

PENCIRIAN FILEN RUMPAI LAUT HIDROFOBIK DIPERKUAT DENGAN SELULOSA GENTIAN NANO DARI KULIT KENAF

ABSTRAK

Plastik amat kukuh dan tahan kepada proses biodegradasi dalam persekitaran semula jadi. Oleh itu pelbagai kajian telah dijalankan pada masa lalu untuk membangunkan filem dari sumber semula jadi. Dalam hal ini, filem berasaskan rumpai laut menunjukkan ciri-ciri fungsian yang sangat baik, jadi terdapat keperluan untuk menyiasat prestasi dan kesannya terhadap persekitaran untuk menentukan jangka hayat serta kesan pada persekitaran. Penggunaan nanoselulosa sebagai tetulang pengisi amat terkenal untuk meningkatkan sifat mekanikal filem. Sifat hidrofilik rumpai laut boleh diatasi dengan rawatan silane menggunakan triethoxymethyl silane untuk memberikan sifat hidrofilik kepada filem. Filem rumpai laut berasaskan nanoselulosa telah disediakan menggunakan proses tuangan pelarut. Dalam projek penyelidikan ini, siasatan telah dibuat untuk mengkaji sifat fiziko-kimia filem terbiodegradasi dan prestasinya. Kajian ini merangkumi pemahaman tentang sifat mekanikal, fizikal, kimia, haba, morfologi, biodegradasi dan hidrofilik filem. Kajian terperinci telah dijalankan untuk mengenal pasti ukuran optimum hidrofilik filem untuk memeriksa tahap rintangan pada lembapan. Jumlah nanoselulosa yang berbeza yang digabungkan dalam filem rumpai laut juga dikaji dan dibandingkan antara satu sama lain. Kadar interaksi air akan diukur dengan mengira pertambahan berat film yang selari dengan masa sewaktu kajian. Selain itu, maklumat tentang perubahan fiziko-kimia yang berlaku dalam sampel filem akibat rawatan silane di bawah keadaan terkawal telah diperoleh oleh analisis spektroskopi inframerah transformasi. Mengimbas analisis imej mikroskop elektron memberikan bukti yang sangat baik pada tahap mikroskopik yang menjelaskan sifat morfologi filem. Juga dilaporkan bahawa kehabluran sangat mempengaruhi prestasi mekanikal oleh itu analisis pembelauan sinar-X bagi semua sampel telah disiasat. Projek ini membantu memahami dengan teliti terhadap kesan penggabungan nanoselulosa dan kesan rawatan silane ke atas prestasi filem rumpai laut. Filem rumpai laut berasaskan nanoselulosa yang diubah suai menggunakan silane telah berjaya direka dengan sifat hidrofobisiti yang baik tanpa mengurangkan sifat mekanikal dan fizikal filem tersebut.

CHARACTERIZATION OF HYDROPHOBIC SEAWEED FILM REINFORCED WITH KENAF BAST CELLULOSE NANOFIBERS

ABSTRACT

Plastics are durable and resistant to biodegradation processes in the natural environment. Intensive researches have been conducted in past to develop the film from the natural source. The seaweed-based film showed excellent functional properties, so there is a need to investigate its performance and impact on surroundings to determine its longevity and harmfulness. Using kenaf nanocellulose as filler reinforcement is well known to increase the mechanical properties of films. The hydrophilicity nature of the seaweed can be countered by silane treatment using triethoxymethyl silane to give hydrophobicity properties to the film. The nanocellulose based seaweed film were prepared using solvent casting process. In this research project, study on physicochemical properties of the biodegradable film and its performance has been completed. This study include the understanding of the mechanical, physical, chemical, thermal, morphology, biodegradability, and hydrophilicity properties of the film. Detail study has been carried out to identify the optimum measure of silane surface treatment on seaweed films hydrophobicity. Different amounts of nanocellulose incorporated within the seaweed film also has been studied and compared to one another. The rate of water interaction with the seaweed films were measure by calculate the weight increase with respect to time. Information about physicochemical changes that occurred in film samples due to silane treatment has also been determined by Fourier transform infrared spectroscopy analysis. Scanning electron microscopy image analysis provide very good evidence at the microscopic level in favor of film morphological properties. It was also reported that crystallinity strongly affects mechanical performance based on X-ray diffraction analysis on films sample. This project contribute to understanding the effect of incorporating nanocellulose and the effect of silane treatment on seaweed films performance. The silane-modified nanocellulose based seaweed film has successfully fabricated to have good hydrophobicity properties without reducing the mechanical and physical properties of the film.

CHAPTER 1

INTRODUCTION

1.1 Overview

The development of a chemical procedure for creating synthetic polymers (plastics) from crude oil was a breakthrough in both chemistry and material sciences, and it led the way for the manufacture of one of the most versatile groups of materials ever created in the history of humanity. These novel materials combined characteristics such as strength, flexibility, lightweight, ease of manufacture, and cheap cost of manufacture (Sivan, 2011). However, it was discovered that these materials were exceedingly durable and were believed to be among the most non-biodegradable synthetic materials available. These characteristics made it possible to use plastics in practically every industrial, agricultural, or residential sector.

The handling of synthetic plastics is also one of the most serious problems facing the world today. It causes serious environmental problems through contaminated natural resources, such as water quality and soil fertility (Bhatnagar & Kumari, 2013). To date, more than 267 species in the marine environment have been affected by the entanglement or ingestion of synthetic plastic fragments (Derraik, 2002). Pollutants leached from synthetic plastics tend to accumulate in marine organisms that absorb or ingest them. This synthetic plastic pollutant poses a threat to the foundation of every food chain, and has a profound impact on non-marine species such as polar bears, seabirds, and humans who consumed the marine-grown resource (Hammer et al., 2012).

Data show that, due to population growth and huge demand, approximately 140 million tons of synthetic plastics are delivered globally every year (Rao et al., 2014). Approximately 50% of synthetic plastics are used in packaging applications, and 90% of them are used as municipal waste (Sangawar & Gadre, 2013). Compared with other plastics such as polyvinyl chloride, polypropylene, and polystyrene, polyethylene such as low-density polyethylene (LDPE) and high-density polyethylene (HDPE) is one of the most commonly used synthetic plastics. It is estimated that the global production of polyethylene, polypropylene, polyvinyl chloride and polystyrene in 2012 will be 30%, 19%, 11% and 7% respectively (Pathak, 2017). The production of synthetic plastics is increasing, and its consumption is increasing at a rate of 12% per year (Koutny et al., 2006; Sivan, 2011). Plastic like polyethylene is the most widely used synthetic polymer, having a worldwide manufacturing capacity of around 140 million tonnes per year at the time of writing. Plastic trash accumulates in the environment due to a lack of effective techniques for properly disposing of it, providing an ever-growing ecological hazard to both land and aquatic wildlife (Shimao, 2001).

Naturally occurring biopolymers include starch, cellulose, gum, alginate, carrageenan, chitosan, and pectin. Among these, starch and cellulose are two of the most well-known polysaccharide-based biopolymers due to their widespread availability, abundance, and low cost (Hills, 2012). Seaweed is a versatile organism that generates a variety of polysaccharides including agar, carrageenan, and alginate. These polysaccharides are widely used in the development of biopolymers. Biopolymers generated from seaweed polysaccharides have several advantages, including their renewable nature, biodegradability, biocompatibility, and environmentally friendly (Jumaidin et al., 2018). Seaweeds (or marine macro-algae) are plant-like creatures that often reside in coastal locations adhering to rock or other hard substrates. Due to its high carbohydrate content, seaweed species have been increasingly used industrially as a source of hydrocolloids (seaweed derivatives) in industries such as food technology, biotechnology, microbiology, and medicine, as well as the plastics industry (El-Said & El-Sikaily, 2013; Gade et al., 2013). Carrageenan is a naturally occurring polymer that is derived from edible red seaweeds of the *Rhodophyceae* family. It has been utilized as a thickener or gelling agent in a variety of sectors, including food, pharmaceuticals, and other industries (Zia et al., 2017).

There are a few disadvantages in using natural polymers such as carrageenan and cellulose. Because of their hydrophilic nature, these natural materials are only suitable for a limited range of applications (Thakur et al., 2013). Biopolymers must possess physico-chemical characteristics that are equivalent to those of synthetic polymers in order to be considered viable prospective substitutes for synthetic polymers in the marketplace (Thakur et al., 2014). The current study has thus been carried out by the use of chemical modification such as silane surface treatment in order to improve the films performance, especially in term of their hydrophobicity. Their mechanical performance, however, is often poor in terms of high-end applications, which might restrict their potential for usage. Plasticizers can be added to biopolymers to give them the workability they need in order to overcome the problem (Vieira et al., 2011). With further enhancement of the biopolymer, the performance of the resulted materials is comparable with the conventional synthetic polymer.

Nanocellulose as a reinforcing material in nanocomposites has been a significant area of study. Apart from its numerous benefits, such as affordable, low density, renewability, high specific properties, biodegradability, and relatively good surface reactivity, nanocellulose exhibits superior properties as a reinforcing phase in nanocomposites when compared to micro- or macro-cellulose composites. The tailorability, design flexibility, and processability of nanocellulose–polymer composites enable their widespread use in a variety of sectors, including automotive, packaging, electronics, and biotechnology (Kargarzadeh et al., 2017). The term "nanocellulose" refers to nanocellulose-derived nanomaterials. Nanocellulose is classified into three basic types: nanocrystalline cellulose (NCC), nanofibrillated cellulose/cellulose nanofiber (CNF), and bacterial nanocellulose (BNC). Although these nanocelluloses are chemically identical, their shape, particle size, and crystallinity may vary owing to their varying sources and extraction techniques (Lavoine et al., 2012; Phanthong et al., 2018). CNF which is made by breaking cellulose fibres, is an example of a biodegradable, renewable nanofiller (biopolymer) with a minimal carbon footprint. Each year, plants produce around 75 billion tonnes of cellulose, making it a highly abundant resource (Feng, Cheng, et al., 2018).

Natural cellulosic fibres, like kenaf, have the potential to be used in polymeric composites in replacement of synthetic fibres. The increasing depletion of synthetic resources such as petroleum and increased awareness of global environmental concerns associated with synthetic materials all contribute to the acceptability of natural fibres as biocomposite components. Kenaf (*Hibiscus cannabinus L.*) is a versatile hibiscus species that is used to manufacture engineered timber, garments, packaging material, rope, and twine. Kenaf is mostly composed of cellulose (about 70%), which explains for its superior mechanical properties (Asyraf et al., 2021). Kenaf is a fast-growing plant that is very adaptable to its surroundings. It is one of the most traditional sources of natural cellulosic fibre. However, the high lignin concentration and tiny fibre size exclude its use in the manufacturing of textile fibres and bioenergy refineries (Song et al., 2018). This study focuses on examining the properties of the used materials and the modified/unmodified composite films in relation to their water barrier and mechanical performance.

Packaging materials comprised of edible components are known as edible films. Due to their environmental friendliness, safety of consumption, and convenience of use, edible films are increasingly in demand nowadays (Maftoonazad & Badii, 2009). This is due to the fact that edible films can lengthen the quality, freshness, and shelf life of meals. The edible films cover the packed food product to form a semipermeable barrier, increasing its barrier characteristics by reducing moisture, lipid, gas, and volatile exchange (Kumar et al., 2021).

1.2 Problem statement

Kappaphycus alvarezii is the world's fifth most farmed macro algae, thanks to its significant contribution in the production of carrageenan, which is extensively utilized in the food sector. However, *Kappaphycus alvarezii* is an underdeveloped biomass for green bio refinery conversion, further study is needed since it contains important proteins, lipids, and phenolic chemicals (Rudke et al., 2020). Carrageenan is a naturally occurring polymer produced from seaweed that has been extensively utilized as a food ingredient due to its appealing physicochemical features during the last decade (Liu et al., 2015). The extraction and purification of carrageenan are expensive, time intensive, and need a large amount of water and chemicals throughout the process (Hernandez-Carmona et al., 2013). Raw seaweed was utilized instead of purified carrageenan in the production of composite films due to its lower cost and ease of processing. Several prior investigations have shown the capability of fabricating composite films from raw seaweed (Khalil, Tye, Saurabh, et al., 2017).

Despite this, their low mechanical strength and poor barrier qualities have restricted their application possibilities.

Seaweed based films offer a number of benefits, including excellent transparency, good tensile strength, gelling ability, and film forming capabilities (Sánchez-García et al., 2010). Due to the rising need for raw materials throughout time, k-carrageenan is now mostly extracted from the cultivated species *Kappaphycus alvarezii* (Rhein-Knudsen et al., 2015). The use of carrageenan based film from seaweed as packaging material gave several benefit because of the suitable properties the materials had to offer especially being eco-friendly and biodegradable. However, in comparison to plastics, they have lower mechanical and water barrier properties, limiting their industrial use. As a result, research is being conducted to discover ways for optimizing the mechanical and barrier qualities of these biodegradable packaging materials (Savadekar et al., 2012). By utilizing the CNF as a filler, the functionality especially mechanical properties of the film can be enhanced. In comparison to other inorganic reinforcing fillers, CNF provide additional benefits, including a low ecological impact, low density, ease of recycling, and relative simplicity of processing owing to their nonabrasive nature (Azizi Samir et al., 2005; Podsiadlo et al., 2005). For hydro-soluble matrices, such as starch are used, the nanocomposites are usually formed by the casting process. Numerous researches have reported that solution cast nanocomposites based on starch and cellulose nanofibers exhibit excellent dispersion and overall performance (Angles & Dufresne, 2000; Chen et al., 2009; Mathew et al., 2008). Incorporating the CNF as a filler for carrageenan film also exhibit similar characteristic and properties because of their chemical compatibility shared by the carbohydrate.

The utilization of kenaf bast as the resource for the nanocellulose give several advantages. Kenaf is a fibrous plant, like wood and bamboo, is professionally grown as a fibre crop attributed to its beneficial properties (Khalil et al., 2010). Nanocellulose fibres have a high mechanical strength, a high strength-to-weight ratio, a huge surface area, and are very flexible. For these reasons, it's' become the focus of study and industrial use, as the novel advanced material promotes the development of promising biodegradable polymers for a variety of applications, including textiles, paper, engineering, medicine, and construction (Dufresne, 2013). Kenaf bast fibre is a non-woody lignocellulosic substance that is an excellent precursor material for CNF extraction (Kargarzadeh et al., 2012). Kenaf bast fibre is high in cellulose and has a low specific gravity also have superior properties as reinforcement agents (Anuar & Zuraida, 2011). The high specific strength and modulus of the fibre have attracted considerable attention because to its superior mechanical qualities, large specific surface area, and low coefficient of thermal expansion, including a high aspect ratio (Sulaiman et al., 2020).

The issue of having poor water barrier properties is a crucial task to be settle. Packaging materials are supposed to have a good barrier properties which become a challenge for bio-based material which usually very hydrophilic. Carrageenan is a water soluble polymer comprised of a linear chain of partly sulfonated galactans. These hydrocolloids are long-chain hydrophilic polymers (polysaccharides) that when dispersed in water create viscous dispersions and gels (Khalil, Tye, Saurabh, et al., 2017). Chemical modification can be made to increase the hydrophobicity of the material. Performing silane surface treatment using triethoxymethylsilane on carrageenan based film can improve the water barrier properties of the film. There are previous studies that have reported the fabrication of hydrophobic cellulose based material by silane modifications which tend to improve the performance and properties of the material (Chen et al., 2015; Jiang & Hsieh, 2014; Jiménez-Saelices et al., 2017; Zhang et al., 2014). Hence, this study is designed to explore comprehensively on the optimum measure of silane surface treatment condition and different reinforcement filler loading quantity in producing better hydrophobic macro algae film on physical, mechanical, morphology, thermal and biodegradability properties for packaging applications.

1.3 Research Objectives

The present research embarks on the following objectives:

1) To analyze the chemical properties of raw seaweed and cellulose nanofiber for biocomposite films fabrications.

2) To study the effect of silane surface treatment with different concentration and treatment period on the water barrier properties of seaweed films.

3) To investigate the effect of CNF loading on physical, mechanical, morphological, thermal and biodegradability properties of the modified and unmodified seaweed biocomposite films.

1.4 Thesis Layout

This thesis was dividing into six respective chapters, as shown below:

Chapter 1: Introduction: provide a summary of the research's background, justification, and objectives.

Chapter 2: Literature review: studied prior research and literature on a variety of topics relating to the overall area of the research.

Chapter 3: Methodology: described in detail the procedures and materials used in this study, as well as the experimental design. The production of nanocellulose, seaweed films and the silane surface treatment was discussed in detail. Techniques for characterizing raw materials and composite films were addressed in detail.

Chapter 4: Results and Discussion: provide the results of the investigation based on variety of scientific approaches and analysis for the raw materials, silane modification parameter and the modified/unmodified CNF seaweed film. Each study's results were carefully analyzed and discussed.

Chapter 5: Conclusions and Recommendations: summarized the research's overall findings and made positive future projections and suggestions for further research.

CHAPTER 2

LITERATURE REVIEW

2.1 Macro algae

Macro algae or seaweed are found in marine ecosystems ranging from the arctic to tropical latitudes and from the intertidal zone to the euphotic zone's deepest depths. These algae provide vital ecological functions, including facilitating a variety of invertebrate recruitment processes, acting as autogenic ecosystem engineers by providing three-dimensional habitat structure, and providing critical structural strength to coral reef ecosystems. Calcified macro algae play a crucial role in carbonate deposition in coastal areas (Nelson, 2009). Apart from other biomass sources, algae produce a high amount of biomass per unit of light and area, may include a large amount of starch or oil, does not need fresh water or agricultural land, and can be sustained with either wastewater or ocean. Algae produce a diverse range of chemical compounds, most notably carbohydrates and lipids (Rajkumar et al., 2014).

Macro algae grow rapidly and may reach lengths of up to 60 m (McHugh, 2003b). Macro algae grow at a pace that is far faster than terrestrial plants. For example, brown algae biomass production ranged between 3.3 and 11.3 kilograms dry weight m^{-2} yr^{-1} in non-cultured algae and up to 13.1 kg dry weight m^{2} during a seven month period in cultured algae, compared to 6.1 to 9.5 kg fresh weight m^{-2} yr^{-1} in sugar cane, the most productive land plant (Kraan, 2013). They are only obtainable seasonally in natural water basins. Macro algae cultivation at sea, which does not need arable land or fertilizer, may provide a solution to the energy issue. macro algae are primarily used for food production and hydrocolloid extraction (Goh & Lee, 2010). In marine ecosystems, macro algae are at the bottom of the food chain and contribute between 30% and 50%

of the estimated net quantity of world oxygen through photosynthesis (Conti & Iacobucci, 2008).

Macro algae is a generic term that encompasses red algae (*Rhodophyta*), green algae (*Chlorophyta*), and brown algae (*Ochrophyta*). Although these algae are evolutionarily distinct, they are linked by the endosymbiotic processes (for which numerous hypotheses exist) that resulted in the formation of plastids (Lobban et al., 1994). Multicellular algae often grow vertically, toward the source of light, in order to generate larger and larger thalli and gather inorganic nutrients from a larger volume of water. Between the top of the mediolittoral belt and the greatest depth to which sufficient light (to fuel photosynthesis and growth) may penetrate, benthic microalgae reside attached to the rocky bottom by a basal holdfast (Lobban et al., 1994). Due to the absence of roots in algae, only a few species can survive on sedimentary substrates, such as certain siphonous green algae (e.g., *Halimeda*, *Caulerpa*, and *Udotea*) that develop root-like penetrating holdfasts that are also employed for nutrient absorption (Littler & Littler, 1988).

At the present, deterioration of benthic habitats is regarded as the greatest significant danger to micro algae biodiversity. It is well acknowledged that human-induced coastal changes, sediment loading, and seawater pollution have diminished the area of critical benthic habitats such as seagrass meadows and micro algae beds, hence impairing the ecosystem's functioning (Mangialajo et al., 2008; Stefani & Vincenzi, 2005; Turley, 1999). However, considerable variation in micro algae assemblages might be attributed to the interaction of human and natural ecological factors (Rinne et al., 2011). Marine plant and algae species are also critical for global carbon and nitrogen cycling, and they offer several ecosystem services, such as support for invertebrate and fish populations that are consumed by humans (Conti & Iacobucci, 2008; Hemminga & Duarte, 2000).

Rhodophyta's red colour is due to the predominance of phycoerythrin and phycocyanin pigments, which obscure other pigments such as chlorophyll a, beta-carotene, and a number of xanthophylls. Coralline algae (orders Corallinales and Peyssonneliales) are a vast group of red algae that are capable of mineralizing the cell wall with calcium and, to a lesser degree, magnesium carbonate (Mann & Jahns, 1996). According to the six kingdoms of life (Cavalier-Smith, 2004), Rhodophyta and Chlorophyta are classified in kingdom Plantae, whilst Ochrophyta is classified in kingdom Chromista. Rhodophyta, or red algae, may be grown in temperate, subtropical, and tropical climates. *Eucheuma denticulatum, Kappaphycus alvarezii, Chondrus crispus*, and *Sarcothalia* crispate are the most significant red algae species for industrial use, since they are sources of carrageenan (Rudke et al., 2020). They represent a large array of species that are found in tropical, temperate, and cold-water coastal and continental shelf environments. Red algae are ecologically crucial because they are primary producers, offer structural habitat for other marine animals, and are critical in the formation and preservation of coral reefs (Kumar et al., 2008).

2.1.1 Kappaphycus alvarezii

Kappaphycus alvarezii, more often referred to as "cottoni," is the primary source of kappa carrageenan, whereas Eucheuma denticulatum, more commonly referred to as "spinosum," is the primary source of iota carrageenan. Both species account for roughly 88 percent of raw material processed for carrageenan production, generating around 120,000 dry tonnes per year^{-1}, with the majority of output coming from the Philippines, Indonesia, and Tanzania (McHugh, 2003b). *Kappaphycus* is a genus of red algae (Rhodophyta). There are six species of *Kappaphycus*, namely *Kappaphycus alvarezii*, *Kappaphycus cottonii*, *Kappaphycus inermis*, *Kappaphycus malesianus*, *Kappaphycus procrusteanus* and *Kappaphycus striatus* (Liao, 1996; Nguyen & Huynh, 1995; Tan et al., 2014). The global production of *Kappaphycus alvarezii* for commercial extraction of kappa carrageenan reveals that the BIMP-EAGA region produces 96.5% of the total production, of which 55% comes from the Philippines, followed by Indonesia (38%) and Malaysia (2.5%) (Hayashi et al., 2010).

Over the last five decades, much attention has been paid to the red seaweed *Kappaphycus alvarezii* (Rhodophyta, Gigartinales, Areschougiaceae), a carrageenophyte of economic significance. According to reports, seaweed cultivation began in southern Mindanao in the latter part of the 1960s in the Philippines, adopting indigenous species selected from the wild (Krauss, 1977). *Kappaphycus alvarezii* provides for around 80% of Philippine seaweed exports, which are sold fresh and dried; although dried seaweed is more in demand, fresh seaweed is highly prized in restaurants (Hurtado, 2003). The Philippines is home to Asia's biggest carrageenan refinery, which facilitates the product's entry into other Asian nations such as Indonesia, Malaysia, Japan, China, India, and Vietnam. Farming continues to expand into new areas. At the mid-1990s, an Indian *Kappaphycus alvarezii* agriculture was established in Okha on the west coast with the purpose of producing kappa carrageenan (Mairh et al., 1995).

Table 2.1 Types and varieties of *Kappaphycus alvarezii* in Sabah, Malaysia (Ling, 2014).

Types/ Varieties	Processing method
"White" *Kappaphycus alvarezii* var. giant 	Seaweed was placed in a clear plastic bag and left it under the sun until it turns yellow-white colour. Then, the seaweed was further placed under the sun until constant weight obtained. The technique was known as "sauna- dried."
"Purple" *Kappaphycus alvarezii* var. giant 	Seaweed was hanged and dried under the sun until the seaweed turned into purplish colour. Then, the seaweed was dried to constant weight obtained.
"Green" *Kappaphycus alvarezii* var. tambalang hijau 	This type of "green" seaweed was obtained by drying the seaweed directly under the sun until a constant weight achieved.
Kappaphycus alvarezii var. green flower 	This type of "green" seaweed applied the same processing method as " white seaweed," but an artificial food-grade dye was mixed with this seaweed

Locally, red seaweed *Kappaphycus alvarezii* is also referred to as "white," "purple," and "green" seaweeds, depending on their drying technique, as shown in Table 2.1 (Limantara & Brotosudarmo, 2015). *Kappaphycus alvarezii* may be classified according to their pigmentation into red, brown, and green forms. It is feasible to use the pigments to make food dyes that are safe for humans and provide considerable functional health benefits (Limantara & Brotosudarmo, 2015). Pharmaceutical substances such as fucoxanthin, carotenoid, zeaxanthin, and astaxanthin, polyphenols such as phenolic, flavonoids, and tannins are abundant in seaweed and have been shown to have significant antioxidant and anti-prostate cancer activities (Ling, 2014).

Kappaphycus alvarezii is a robust, dense, and hard marine algae/seaweed that may reach a height of two meters. Its thallus, a plant body that is not separated into root, stem, and leaf, but rather comprises of the holdfast, stipe, and blade, is coarse, with axes and branches ranging in diameter from 1-2 cm. Meanwhile, its principal axes are rather straight and devoid of secondary branches around their apices/tips. *Kappaphycus alvarezii* are one of the most abundant red algae in the tropics, growing at a fast rate that may double its biomass in 15 to 30 days (Trono Jr, 1992). These species may spread by vegetative fragmentation as well as sexual reproduction by spreading spores into the water column and allowing them to be carried by prevailing water currents (Azanza-Corrales et al., 1992). This species of alga may be reddish, yellowish, brown, or green in color, depending on the quantity of the phycoerythrin pigment. It is relatively suitable for the cultivation and grows rapidly, at a rate of around 4.5 percent daily (Gereniu et al., 2017).

This plant has been introduced internationally into a variety of maritime nations for experimental and commercial production as a viable alternative livelihood for coastal communities. Along with the introduction, there is growing concern about the impact of species on the biodiversity of endemic habitats. The introduction of non-endemic cultivars required the adoption of quarantine techniques to prevent more invasive species (Bindu & Levine, 2011). These marine red algae, specifically *Kappaphycus alvarezii*, are economically significant because they generate the hydrocolloid carrageenan, which is widely used in the food, pharmaceutical, cosmetic, baking, and daily product industries due to its unique functional properties such as gelling, thickening, stabilizing, and water-holding capabilities (Gade et al., 2013; Rhein-Knudsen et al., 2015). This polysaccharide makes up the majority to 37% of the algae on dry base (Lechat et al., 1997). Concerns about *Kappaphycus alvarezii* non-endemic nature, impact on biodiversity, and disruption of local ecological community health and balance have been raised as a consequence of its worldwide introduction into new environments. Commercial invasive cultivars are gaining attention and are being seen as a possible threat to algae culture as a source of economic growth for coastal fishing communities (Barrios, 2005; Rodgers & Cox, 1999; Woo et al., 1989).

Kappaphycus alvarezii has a high concentration of minerals, vitamins, bioactive compounds, proteins, lipids, and polyphenols that have antibacterial, antiviral, antifungal, and other medical benefits (Hayashi & Reis, 2012). Carrageenan is the most abundant chemical component in *Kappaphycus alvarezii*, accounting for between 35.3 and 61.7 % of the total (de Góes & Reis, 2012). Kappa-carrageenan is obtained mostly from *Kappaphycus alvarezii*, whereas iota- and lambda-carrageenan are isolated from Eucheuma, Gigartina, and Chondrus species (Bixler & Porse, 2011). Carrageenan was originally obtained from wild red seaweed *Chondrus crispus*, which is still used in small amounts today (Abdou & Sorour, 2014). Over time, the increased demand for the raw material (carrageenan) resulted in the cultivation of the *Kappaphycus alvarezii* and

Eucheuma denticulatum species (McHugh, 2003a). *Kappaphycus alvarezii* is mostly used for the extraction of kappa-carrageenan, while *Eucheuma denticulatum* is used primarily for the extraction of iota-carrageenan (Cunha & Grenha, 2016).

Previous (Matanjun et al., 2009) reported a studies on the nutritional content of Malaysian *Kappaphycus alvarezii*, formerly known as *Eucheuma cottonii*, where its proximate, fatty acid, and amino acid compositions. The tendency of *Kappaphycus alvarezii* to absorb and accumulate trace elements in its tissue is well documented (Kumar et al., 2007). This indicates that the mineral and trace element composition of micro propagated *Kappaphycus alvarezii* may differ from that of field-grown specimens. (Graham, 2009) discovered that the amount of compounds contained in seaweeds might vary depending on a variety of parameters such as the season, the age of the population, the species, the geographic location, and the temperature. Table 2.2 shows the differences in chemical compositions in *Kappaphycus alvarezii* based on prior investigations.

Table 2.2 Chemical compositions of *Kappaphycus alvarezii*.

Composition(wt %)	*Kappaphycus alvarezii* (%)	*Kappaphycus alvarezii* (%)	*Kappaphycus alvarezii* (%)	References
Carbohydrate	59.58	57.30	65.20	(Abd-Rahim et al., 2014) (Abirami & Kowsalya, 2011) (Abdul Khalil et al., 2018)
Protein	5.74	4.5	3.40	
Lipid	0.75	0.89	1.10	
Ash	19.70	28.9	11.57	

Generally, the red seaweeds *Kappaphycus alvarezii, Eucheuma denticulatum,* and *Betaphycus gelatinae* are the three most important red seaweeds for the manufacture of commercial carrageenan (Kılınç et al., 2013). Nonetheless, some species from the *Gigartina, Iridae, Hypnea,* and *Furcellaria genera* have also been utilised, resulting in the production of several kinds of carrageenan extracts from these plants (De Ruiter & Rudolph, 1997; McHugh, 2003a). *Chondrus crispus* is less commercially viable than *Kappaphycus alvarezii* because they tend to generate a combination of kappa and lambda carrageenan, which is difficult to extract and separate from the rest of the carrageenan mixture (Cunha & Grenha, 2016). The decline of gel strength that occurs when either kappa or iota carrageenan is mixed with lambda carrageenan occurs throughout applications, resulting in a weaker gel that is less suited for industrial applications (McHugh, 2003b).

A strong demand for carrageenan has resulted in the expansion of the cultivation of the seaweed *Kappaphycus alvarezii* in Malaysia, which has led in the cultivation of the seaweed. In response to rising demand for *Kappaphycus alvarezii,* a shortfall of seedling supply has emerged while micro propagation is one of the possible solutions (Yong et al., 2015). Since 1978, the cultivation of *Kappaphycus alvarezii* has been established in East Malaysia, specifically in Sabah (Sade et al., 2006). Since then, seaweed has emerged as one of the most commercially valuable natural resources in the country. Meanwhile, two plants for semi-refined carrageenan manufacture have been founded in Semporna and Tawau, both in the Sabah province. Also in Peninsular Malaysia, the cultivation of Kappaphycus (of the tambalang variety) has been introduced and successfully established (Phang et al., 2010).

2.2 Chemical and rheological properties of carrageenan

Carrageenan, formerly referred to as 'carrageenin,' was discovered in 1862 by British chemist Stanford, who isolated it from Irish moss (Chondrus crispus). Later, the name was altered to carrageenan to conform to the '-an' suffix required for polysaccharide names (Pereira, 2011). Carrageenan, a polysaccharide derived from marine algae, is rapidly gaining recognition as a potentially renewable biomaterial with significant promise as a replacement for current synthetic plastics. Carrageenan-derived materials have been extensively researched in recent decades for applications ranging from pharmacological and biomedical to culinary films and coatings. Carrageenans have limitations in the field of flexible films, principally due to its inherent hydrophilicity (Sedayu et al., 2019).

Carrageenan is a hydrophilic linear sulfated galactan found only in the cell walls of red sea algae (Rhodophyceae). It is derived predominantly from the genera Chondrus, Eucheuma, Gigartina, and Iridaea (Jouanneau et al., 2010). Carrageenan has a rather large molecular weight, ranging between 100 and 1000 kDa on average. Along with the ester sulphate and galactose groups that form the primary backbone of the structure, it contains other polysaccharide residues and substituents such as glucose, xylose, uronic acids, methyl ethers, and pyruvate groups (Aldalbahi, 2012). Carrageenan is a hydrocolloid mostly composed of potassium, sodium, magnesium, and calcium salts of sulfated galactose esters and copolymers of 3,6-anhydro-galactose (De Ruiter & Rudolph, 1997). Carrageenan is a sulfated polygalactan that contains between 15% and 40% ester-sulfate and an average relative molecular mass of well over 100 kDa. It is composed of alternating units of d-galactose and 3,6-anhydro-galactose (3,6-AG), which are linked by 1,3 and 1,4-glycosidic linkages.

Carrageenan is categorized into six basic forms: Iota (ι)-, Kappa (κ)-, Lambda (λ)-, Mu (μ)-, Nu (ν)- and Theta (θ)- carrageenan. This nomenclature is essential both chemically and commercially, since the various carrageenans subtypes are isolated from different weed sources (Campo et al., 2009). This categorization is based on the fact that it is soluble in potassium chloride. The key changes affecting the carrageenan type's characteristics are the quantity and location of ester sulphate groups, as well as the amount of 3.6-AG (Necas & Bartosikova, 2013). These designations do not refer to particular chemical structures but rather to generic changes in the composition and degree of sulfation at various sites within the polymer. The sulphate groups on the disaccharide repeating unit indicate the 3 major carrageenan type: lambda (λ), kappa (κ), and iota (ι) (Al-Alawi et al., 2011) as depicted in Figure 2.1 below. Increased ester sulphate content results in a lower solubility temperature and a weaker gel. Carrageenan of the kappa type contains around 25%–30% ester sulphate and approximately 28–35% 3,6-AG. Carrageenan of the iota type contains around 28 to 30% ester sulphate and approximately 25 to 30% 3,6-AG. Lambda-type carrageenan contains about 32–39% ester sulphate and no 3,6-AG (Barbeyron et al., 2000).

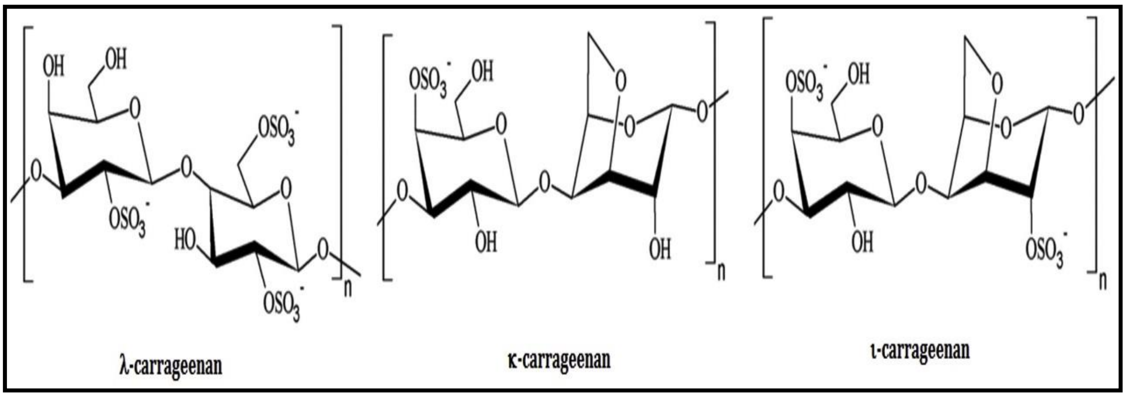

Figure 2.1 Chemical structure carrageenans (Cunha & Grenha, 2016).

Kappa (κ)-carrageenan is a polysaccharide comprised of alternating 3-linked β-d-galactose 4-sulfate and 4-linked 6-anhydro- α-galactopyranose is a disaccharide repeating unit with one negative charge (Campo et al., 2009). Each disaccharide repeating unit of Iota (ι)-carrageenan contains two sulphate groups (Pavli et al., 2011). Iota and kappa carrageenan exhibit similar properties, including the ability to undergo thermoreversible conformational rearrangement in aqueous solution at high temperatures, while combining and forming networks at lower temperatures via sulphate groups and the 3,6-anhydro-d-galactopyransyl ring (Gu et al., 2005). Additionally, ι and κ carrageenans exhibit gelling capabilities in the presence of cations, which is affected by the concentration and type of the cations (i.e. K^+ and Ca^{2+}, respectively) in the solution, as well as the concentration of the biopolymer (Uruakpa & Arntfield, 2004). Additionally, various salts have different impacts on the phase transitions and gelation of ι- and κ-carrageenan gels. It has been observed that in the presence of KCl, stronger κ-carrageenan gels were formed compared in the presence of the other salts, particularly NaCl, $MgCl_2$, LiCl, $SrCl_2$, and $CaCl_2$ (Kara et al., 2006). The gel-sol transition temperatures of κ-carrageenan were strongly related to KCl, NaCl, and $CaCl_2$ contents (Pekcan & Tari, 2008).

Lambda (λ)-carrageenan contains three sulphate groups per disaccharide unit, but unlike κ and ι-carrageenan, it lacks a 3, 6-anhydride bridge (Pavli et al., 2011). Throughout all temperatures, λ-carrageenan is incapable of forming gel and has a random coil shape (Gu et al., 2005). The addition of salts may reduce the viscosity of a solution by lessening the electrostatic repulsion between the sulphate groups, which is permitted by an increase in the concentration of macromolecules in the solution. For instance, this is what occurs for lambda fraction (Campo et al., 2009). In the presence of sulphate groups orientated towards their exterior section, the kappa- and iota-carrageenans form a network of three-dimensional double helices, which is formed by the "crosslinking" of adjacent spiral chains that include sulphate groups directed towards their external part as shown in Figure 2.2. The 2-sulfate group of λ-carrageenan is directed towards the interior half of the molecule, preventing this "crosslinking" from occurring (Morais et al., 1989).

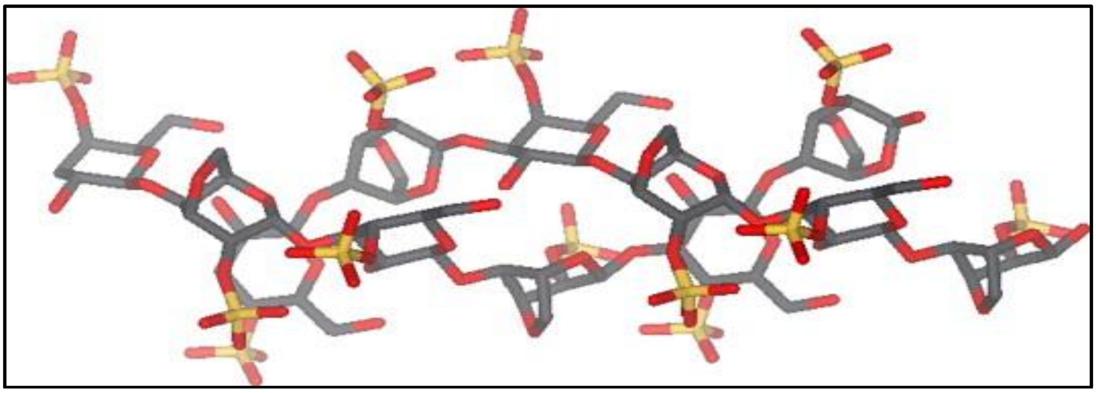

Figure 2.2 Double helix model of ι-carrageenan(Campo et al., 2009).

Along with the distinct carrageenan kinds discussed above, there are also various hybrid carrageenan types to consider. In nature, seaweeds do not often generate pure carrageenan, but rather a variety of diverse sulfated polysaccharides in their structures, including hybrid carrageenans, which are a kind of sulfated polysaccharide (van de Velde, 2008). The amount of carrageenans present in a particular seaweed is governed by a variety of variables, including the seaweed's biological stage (Gómez-Ordóñez & Rupérez, 2011), gender of the gametophyte, environmental conditions, as well as the extraction process used in the recovery of carrageenan (Hilliou, 2014; Larotonda et al., 2016; Souza et al., 2011). The vegetative phase of the Gigartinales seaweed order generates highly sulfated forms of μ-carrageenan, which are non-gelling, but the reproductive stage produces κ- and ι-carrageenans that are gelling, as well as ν- and η-carrageenans. Additionally, the immature seaweed of *Kappaphycus striatum* has less gelling carrageenan types than the matured seaweed (Hilliou, 2014).

Regarding carrageenan's rheological characteristics, all three commercially significant hydrocolloids exhibited significantly diverse features. Kappa- and iota-carrageenan shared the characteristic structure of a 4-linked galactose residue's anhydro-galactose bridge, which adopts the $^{1}C_{4}$ – chair conformation, as seen in Figure

2.3(a). This conformation is critical for the production of helical structures and gels (Campo et al., 2009). Also non-gelling carrageenans are the naturally occurring precursors (Mu- and Nu-carrageenan) of kappa- and iota-carrageenan, which include D-galactopyranosil units in the $^4C_1$ conformation as shown in Figure 2.3(b), which are non-gelling carrageenans. The occurrence of disaccharide units without the 3, 6-anhydro ring and possessing a $^4C_1$ conformation, according to (Campo et al., 2009), results in twists in the regular chains, which eventually prevents the development of helical strands. Carrageenan is prevented from gelling as a result of this effect.

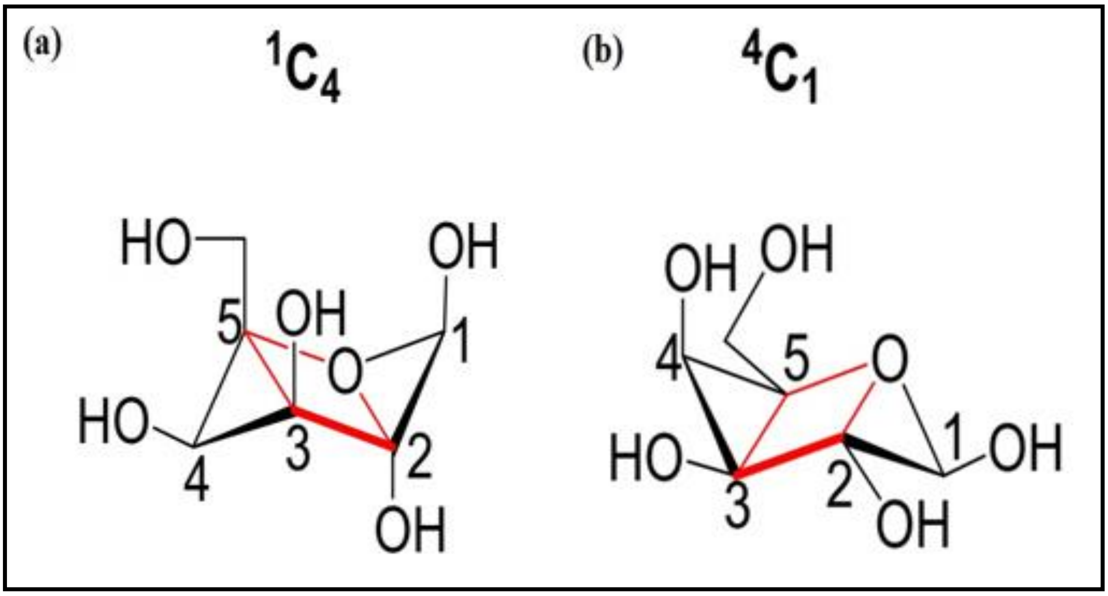

Figure 2.3 (a) 1C4 conformation of β-D-galactoyranose (b) 4C1 conformation of β-D galactoyranose (Campo et al., 2009).

The conformations ($^1C_4$ and $^4C_1$) shown in Figures 2.3(a) and 2.3(b) are defined by the use of numbers to indicate ring atoms that are either above or below a specified reference plane (red) that contains the carbon atoms C-2, C-3, C-5, and O. Figure 2.5 (a) shows that the C-1 is positioned above the reference plane and the C-4 is placed below it, resulting in the symbol $^1C_4$ being used. Meanwhile, in Figure 2.5(b), the C-4 plane is located above the reference plane and the C-1 plane is located below the plane, resulting in the sign $^4C_1$ for the C-4 plane. There is a possibility that the carrageenan's conformations contribute to the rheological characteristics of the substance in distinct ways.

2.3 Extraction of carrageenan from seaweed

The extraction of seaweed hydrocolloids is a time-consuming and complex procedure that involves many phases of processing in order to be effective. Due to the water soluble nature of ordinary seaweed hydrocolloids, hot water is often the most preferred solvent for their extraction, with the exception of alginate/alginic acid extractions, which need hot alkali as the primary solvent (Minghou, 1990). Traditionally, carrageenan powder was obtained from red seaweed by following the stages of impurity removal, hot water extraction, and drying to get a fine powder as shown in figure 2.4. The primary disadvantage of this approach is that an extraction solvent is required during the extraction process to precipitate the carrageenan, and then the precipitate is dried and crushed to get carrageenan powder. Carrageenan powder must be dissolved prior to the manufacturing of carrageenan fibres. Adding the solvent during the extraction process and dissolving the carrageenan powder during the preparation of carrageenan fibre uses a significant quantity of water, ethanol, and energy (Dong et al., 2018).