BIODEGRADABLE SEAWEED-BASED COMPOSITE FILMS INCORPORATED WITH CALCIUM CARBONATE GENERATED BY *BACILLUS SPHAERICUS*

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by

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LIST OF SYMBOLS

A	Area
Cr	Crystallinity
MPa	Mega pascal
g	Gram
θ	Theta
°	Degree
˚C	Degree Celsius
cm $^{-1}$	Reciprocal centimeter
cm/min	Centimeter per minute
%	Percent
cm	Centimeter
µm	Micrometer
mm	Millimeter
nm	Nanometer
L*	Lightness
a*	Redness
b*	Yellowness
C*	Chrome
g	Gram
g/m^2	Gram per meter square
g/cm^3	Gram per cubic centimeter
g/L	Gram per litter
µL	Micro liter
+	Addition

=	Equal
×	Multiply
<	Less than
>	More than
mg	Milligram
cm^2	Centimeter square
ml	Milliliter
min	Minute
s	Seconds
h	Hour/hours
min^{-1}	Reciprocal minute
L	Litter
rpm	Revolutions per minute
wt. %	Weight percentage

LIST OF ABBREVIATIONS

AgNPs	Silver nanoparticles
ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
C-$CaCO_3$	Commercial Calcium Carbonate
DTG	Derivatives Thermogravimetric Analysis
EDX	Energy Dispersive X-ray
FT-IR	Fourier-Tranform Infra-Red
FESEM	Field Emission Scanning Electron Microscope
HDPE	High density polyethylene
LDPE	Low density polyethylene
M-$CaCO_3$	Microbially Induced Calcium Carbonate Precipitates
MICP	Microbially Induced Calcium Carbonate Precipitation
MMT	Montmorillonite
PE	Polyethylene
PLA	Polylactic acid
PVA	Polyvinyl alcohol
SEM	Scanning Electron Microscopy
TGA	Thermogravimetric Analysis
WVP	Water vapour permeability
WVTR	Water vapour transmission rate
XRD	X-Ray Diffraction

FILEM KOMPOSIT TERBIODEGRADASI BERASASKAN RUMPAI LAUT DIISI KALSIUM KARBONAT YANG DIHASILKAN OLEH *BACILLUS SPHAERICUS*

ABSTRAK

Filem berasaskan rumpai laut telah menjadi tren sejak kebelakangan tahun ini disebabkan manfaat nutrisi, kuantiti, keserasian dan sifat kebolehdegradasi. Walau bagaimanapun, sifat hidrofilik filem rumpai laut telah mengehadkan prestasi penghalang air, mekanikal dan haba. Oleh itu, kajian ini bertujuan untuk meningkatkan prestasi filem berasaskan rumpai laut merah mentah (*Kappaphycus alvarezii*) dengan menggunakan pengisi kalsium karbonat mendakan mikroba (M-$CaCO_3$). Untuk menentukan peningkatan prestasi filem, filem komposit berasaskan rumpai laut diisi dengan pengisi M-$CaCO_3$ dengan muatan yang berbeza [0,06, 0,08, 0,10, 0,15, 0,20 dan 0,50 (wt.%)] dan diciri berdasarkan sifat fizikal, mekanikal, haba, kebolehdegradasi, morfologi dan kehabluran dengan menggunakan pelbagai teknik pencirian seperti FESEM, EDX, FT-IR XRD dan TGA. Sifat filem kemudian dibandingkan dengan filem yang diisi dengan kalsium karbonat komersial (C-$CaCO_3$). Pemuatan pengisi yang optima telah dicapai oleh 0.15 wt. % M-$CaCO_3$ dan 0.10 wt.% C-$CaCO_3$ berdasarkan keputusan sifat fizikal, mekanikal dan haba. Ini telah dibuktikan bahawa penyerapan kelembapan dan kebolehtelapan wap air dikurangkan dengan ketara ($p<0.05$) sementara sudut sentuh, kekuatan tegangan, modulus tegangan, pemanjangan pada takat putus dan kestabilan haba ditingkatkan dengan ketara ($p <0.05$) apabila pembebanan pengisi meningkat dari 0.06 wt. % hingga 0.15 wt. % M-$CaCO_3$ dan 0.10 wt.% C-$CaCO_3$, masing-masing. Hasil kajian juga menunjukkan bahawa filem yang diisi dengan pembebanan 0.15% M-$CaCO_3$ mencapai sudut sentuh tertinggi (100,94°); penyerapan kelembapan (98.69%) dan kebolehtelapan wap air terendah (2.45×10^{-10} gm / m^2. s. Pa) sementara filem yang diisi dengan pembebanan 0.10 wt.% C-$CaCO_3$ menunjukkan kekuatan tegangan (44.31 MPa), modulus tegangan (228 MPa) dan pemanjangan pada takat putus (16.82%). Secara perbandingan antara pembebanan optimum C-$CaCO_3$ (0.10 wt.%) dan pembebanan optimum M-$CaCO_3$ (0.15 wt.%), filem komposit diisi dengan pengisi 0.15 wt.% M-$CaCO_3$ menunjukkan penyerapan kelembapan sebanyak 10.27 % lebih rendah, sifat penghalang air sebanyak 31.56% lebih baik, sudut sentuh sebanyak 9.21% lebih tinggi dan sifat kebolehbiodegradasi sebanyak 0.85% lebih baik daripada filem yang diisi oleh C-$CaCO_3$ (0.10 wt.%). Selain itu, hasil kajian juga menunjukkan bahawa peratusan kebolehbiodegradasi filem komposit berasaskan rumpai laut yang diisi dengan pengisi M-$CaCO_3$ adalah 40% lebih tinggi daripada filem mulsa konvensional. Oleh itu, penemuan kajian ini menunjukkan bahawa filem diisi dengan M-$CaCO_3$ yang dihasilkan daripada kaedah yang lebih mesra alam mempunyai potensi yang menjanjikan bukan sekadar menjadi pengisi alternatif bagi $CaCO_3$ komersial tetapi juga berpotensi sebagai alternatif bagi filem mulsa berasaskan petroleum yang sedia ada di pasaran pada masa yang terdekat.

BIODEGRADABLE SEAWEED-BASED COMPOSITE FILMS INCORPORATED WITH CALCIUM CARBONATE GENERATED BY *BACILLUS SPHAERICUS*

ABSTRACT

Seaweed-based films have been trending in the recent years due to its nutritional benefits, abundance, compatibility and biodegradability. However, the hydrophilic nature of seaweed film has been limiting its water barrier, mechanical and thermal performances. Therefore, this study is purposed to develop biodegradable film using raw red seaweed (*Kappaphycus alvarezii*) as a matrix and incorporated with microbially induced calcium carbonate precipitates (M-$CaCO_3$) to further enhance the film performances. In order to determine the enhancement of film properties, seaweed-based composite films incorporated with different filler loading [0.06, 0.08, 0.10, 0.15, 0.20 and 0.50 (wt. %)] of M-$CaCO_3$ were characterized based on physical, mechanical, thermal, biodegradability, morphological and crystallinity using various characterization techniques such as FESEM, EDX, FT-IR XRD and TGA. The properties of the films were then compared with the films incorporated with the commercial calcium carbonate (C-$CaCO_3$). The optimum loading was attained by 0.15 wt. % M-$CaCO_3$ and 0.10 wt.% C-$CaCO_3$ based on the results of physical, mechanical and thermal properties. It has proven that moisture absorption and water vapour permeability was significantly ($p<0.05$) reduced while the contact angle, tensile strength, tensile modulus, elongation at break and thermal stability were significantly enhanced upon increasing filler loading from 0.06 wt. % up to 0.15 wt. % M-$CaCO_3$ and 0.10 wt.% C-$CaCO_3$ loadings, respectively. Results also showed that films incorporated with 0.15 wt.% of M-$CaCO_3$ attained the highest contact angle (100.94°);

lowest moisture absorbtion (98.69%) and water vapour permeability (2.45×10^{-10} $g.m/m^2$. s. Pa) while the films incorporated with 0.10 wt.% of C-$CaCO_3$ showed the highest tensile strength (44.31 MPa), tensile modulus (228 MPa) and elongation at break (16.82%). In comparison between the optimum loading of C-$CaCO_3$ (0.10 wt. %) and the optimum loading of M-$CaCO_3$ (0.15 wt.%), the composite films incorporated with 0.15 wt.% M-$CaCO_3$ filler promoted lower moisture absorption by 10.27%, better water barrier by 31.56%, higher contact angle by 9.21% and better biodegradability properties by 0.85%. Apart from that, the results revealed that the percentage of biodegradability of the seaweed-based composite films incorporated with M-$CaCO_3$ filler were higher than the conventional mulch film by 40%. Hence, these findings suggested that M-$CaCO_3$ produced from a more environmental friendlier method has a great potential not merely to serve as alternative filler to the commercial $CaCO_3$ but also serve as a promising alternative to the existing conventional petroleum-based mulch film in the near future.

CHAPTER 1

INTRODUCTION

1.1 General background

The global shift to the use of bio-based materials has gained considerable interest due to their spectrum of application in food packaging, plasticulture practices and biomedical sciences. Demand of polysaccharide-based composite film is expected to increase in the modern applications as the global demands of plastic films especially in the agricultural sector had increased by 69% from 4.4 million tons in 2012 to 7.4 million tons by the year of 2019 (Sintim and Flury, 2017). PE bags, mulching films and greenhouse covers can be observed in plantation areas for their significance in preventing weeds growth, managing fertilizer, controlling temperature and improving crops growth (Aquavia et al., 2021). However, due to the non-biodegradable properties of PE films, plenty of unrecyclable waste has been generated. Thus, research on using polysaccharides-based materials to form composite film has been increased in the recent years.

Composite film features the combination of two or more constituent materials to produce new composite system with enhanced properties (Wang et al., 2011). The interest of research on composite films using various types of raw materials has advanced tremendously in the recent years mainly attributed to ubiquitous applications including super capacitors, drug release, wound dressing, packaging and agricultural mulch (Wang et al., 2018; Tsai et al., 2018; Chin et al., 2018; Kakroodi et al., 2017; Zhao et al., 2017). In order to achieve an ideal composite film with excellent physical, mechanical and thermal properties for a certain application, the types of matrix and filler used are among the important factors to be taken into considerations. Filler with appropriate content is usually incorporated into a base matrix as integration of polymer matrix and filler inherently possess better properties compared to single material due to the synergic effect formed between the components (Sun et la., 2014).

Polyethylene (PE), high-density polyethylene (HDPE) and low-density polyethylene (LDPE) was once a promising base matrix used to fabricate composite film. However, research related to petroleum-based material has slowly faded because of escalating awareness of fossil fuels depletion and the issues of environmental pollution (Bilck et al., 2010). Therefore, preliminary development and characterization of biodegradable film using biopolymers such as starch, chitosan, seaweed's polysaccharides, cellulose, pectin as matrix have gained tremendous interest in the research of composite films with the hope to replace the existing synthetic plastics. This is mainly due to their renewability, availability, biodegradability, biocompatibility, low toxicity, non-antigenic and non-carcinogenic characteristics (Abdul Khalil et al., 2018b; Tye et al., 2018; Abdul Khalil et al., 2017a; Cazón et al., 2017).

Since the past decade, seaweed is considered as one of the high potential biopolymers and is on trending due to their impressive phycocolloids (ie: carrageenan, alginate and agar) with natural gel-forming properties (Das et al, 2021; Abdul Khalil et al., 2017a). They have the ability to form colloid system either in a gel form or solubilized particles even in the presence of water (Cazón et al., 2017; Abdul Khalil et al., 2016; Siah et al., 2015). Seaweed's polysaccharides have been widely applied in cosmetic, packaging, pharmaceutical, food and agricultural industries (Abdul Khalil et al., 2017b). In the recent years, the use of seaweed in agriculture field has gained wider acceptability than the use of excessive chemical fertilizer, herbicides and pesticides as seaweed itself is considered organic, biodegradable, non-toxic, and non-hazardous to organism and environment. The advantages of using seaweed extracts to improve agricultural productivity have been well-documented (Arioli et al., 2015).

Previous studies have successfully developed seaweed-based films. However, they usually exhibit poor mechanical and water barrier properties (Abdul Khalil et al., 2018b; Siah et al., 2015; Zarina and Ahmad et al., 2015). This is because seaweed is usually hydrophilic in nature and the film fabricated can be very brittle (Siah et al., 2015). Therefore, enhancement by modification through grafting/blending with other polymers or incorporating with fillers to increase their competitiveness with the commodity polymers is a necessary.

Inorganic fillers, also known as mineral fillers have attracted considerable attention. Wide spectrum of applications in pharmaceutical, food, textile and paper have been reported (Xu et al., 2016; Sun et al., 2014; Mbey et al., 2012; Alves et al., 2010). Many studies elucidated that incorporation of inorganic fillers into a polymer matrix has more than a function to reduce the cost of the polymer (Wang et al., 2011; Topalömeret et al., 2019). Inorganic fillers are commonly incorporated to enhance properties such as mechanical, thermal, water barrier, and optical although the use of fillers is relatively lower in quantity of weight compared to the polymer matrix (Wang et al., 2011).

Among the many types of filler, calcium carbonate ($CaCO_3$) is one of the oldest and prominent mineral fillers, which has been used conventionally in the paper, paint, plastic, chemical, pharmaceutical, agriculture and metallurgical industries owing to its availability, low cost, non-toxicity, non-abrasiveness, compatibility and antimicrobial properties (Ataee et al., 2011; Ramakrishna et al., 2016; Browning et al., 2021). Recent work has focused on the biological approaches more than chemical or mechanical approaches to obtain $CaCO_3$ in order to save time and their associated ease of process. This process is commonly known as microbially induced calcium carbonate precipitation (MICP). Compared to the conventional method of mining $CaCO_3$, it is said to save time and energy as it takes only about 4 to 24 hours to precipitate $CaCO_3$ without using complex machine (Ortega-Villamagua et al. 2020; Rahman et al., 2020). Besides, it is regulated by the physiological of microorganism in a controlled environment that helps to precipitate $CaCO_3$ crystals with 100% purity (Castro Alonso et al., 2019). According to Dhami et al. (2013), it is the most studied branch of biomineralisation that is applied in various fields from biotechnology to engineering. It is defined as a process of producing minerals through passive surface-mediated mineralization by organism basic metabolic activities (Dhami et al., 2013).

$CaCO_3$ as a by-product can be formed through varied mechanism such as via photosynthesis, urea hydrolysis, biofilm, anaerobic sulfide oxidation, sulfate reduction and extracellular polymeric substances. However, the most common $CaCO_3$ precipitation is by urea hydrolysis (Anbu et al., 2016; Chae et al., 2021). The advantages of using urea hydrolysis include the high chemical conversion efficiency up to 90%, straightforward process and easily control parameters compared to the other pathways (Rahman et al., 2020). This method is assisted by ureolytic bacteria such as *Bacillus sphaericus*, *Bacillus pasteurii* and *Bacillus cereus* which promote precipitation $CaCO_3$ of under high calcium environment. *Bacillus sphaericus* is amongst the most common bacteria agents used in MICP to produce calcium carbonate in calcite polymorph due to its high yield of $CaCO_3$ precipitates (Achal and Mukherjee, 2015). MICP method has been employed in diverse field of applications such as cement, plastic, rubber fluorescent particles in stationery ink and fluorescent marker (Dhami et al., 2013). However, there is a lack in research on using MICP technique in polysaccharide-based materials. Considering the demand and higher research and industrial focus on green production, it is indeed necessary to study the incorporation of fillers produced from a more environmental-friendlier method into seaweed-based films to improve film performances.

1.2 Problems statement

Seaweed extracts or phycocolloids can be extracted via alkaline or acid hydrolysis using chemical such as ethanol and sodium hydroxide (NaOH). However, the extraction of seaweed phycocolloids often requires chemical and energy consumption which is not environmental friendly and it is time and energy consuming. Chemical such as sodium hydroxide or potassium hydroxide is required to extract the phycocolloids through heating (Abdul Khalil et al., 2017c). In order to reduce such issues, Siah et al. (2015) fabricated films from raw edible red seaweed (*Kappaphycus alvarezii*) without the additional steps of extraction. Siah et al. (2015) proposed several applications including food wrap, facial mask, sachet and pouch. Although the study implied the feasibility of using raw seaweed to form film without the process of extracting phycocolloids, principal drawbacks were encountered by the raw seaweed-based film as the films turned out to be brittle, weak in mechanical, thermal and water barrier properties due to its hydrophilic nature, which limits the films' functionalities (Siah et al., 2015). Therefore, further enhancement on mechanical, water barrier and thermal properties is still a necessary to expand the application of seaweed-based material

Recently, the usage of raw red seaweed (*Kappaphycus alvarezii*) as the base matrix in film forming has been explored. Film properties including water barrier, hydrophobicity, mechanical and thermal properties were enhanced significantly after incorporating with organic fillers such as oil palm nano-fillers and microcrystalline cellulose (MCC) or/and by blending with other polymer such as starch (Kontopoulou, 2014, Abdul Khalil et al., 2016, Abdul Khalil et al., 2017a, Abdul Khalil et al., 2017b Abdul Khalil et al., 2018b).

Calcium carbonate is usually acquired from excavating carbonate-contained rocks, eggshells and marine organism, skeletons, stalagmites, stalactities (Wang et al., 2015a). However, obtaining $CaCO_3$ using the conventional method of excavating rocks can generate sound, air, water and land pollution, which is not a sustainable method for a long period. Such environmental impacts have to be overcome by implementing a greener and sustainable approach. Hence, microbially induced calcite precipitation (MICP) was employed in this study. MICP is an alternative way to reduce energy consumption and produce highly purified $CaCO_3$ in a short time (Anbu et al., 2016).

Although MICP has been regarded as an economical technique and a novel strategy to resolve continuous erosive impact on the limestone surface, there is still a deficiency of report on the characterization of microbially induced $CaCO_3$ precipitates (Wang et al., 2017). Hence, it is necessary to characterize and identify the properties of microbially induced $CaCO_3$ on the entire composite system. Direct implementation of MICP treatment on cement has shown great interest in the application of bio-concrete or bio-cementation with the function to re-mediate fractures within the structures and improve durability of bricks (Anbu et al., 2016). However, the application of $CaCO_3$ from the production of MICP remains elusive. Instead of using direct MICP treatment on the film, this study used the end product of MICP ($CaCO_3$ precipitates) as the filler to eludicate its properties when compared to the commercial $CaCO_3$.

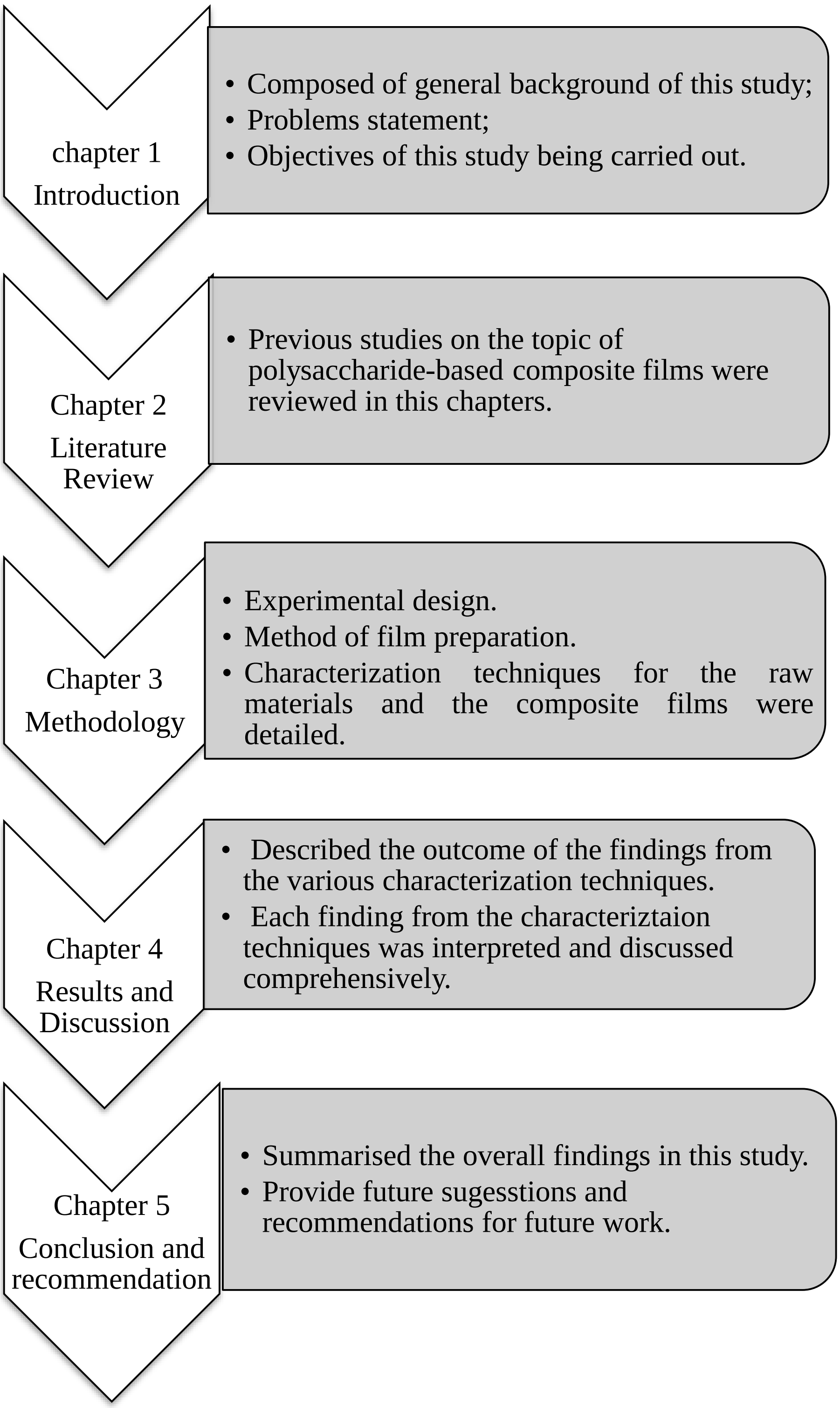
Upon thorough research and investigations, incorporating of either commercial $CaCO_3$ or microbially induced $CaCO_3$ in raw seaweed film has not been reported elsewhere. This study highlights the feasibility of using versatile seaweeds as the matrix and $CaCO_3$ as inorganic fillers to enhance its functional properties and to identify its potential application as mulch film.

1.3 Research objectives

- To characterize the physicochemical and thermal properties of microbially-induced $CaCO_3$ generated by *Bacillus sphaericus* and the commercial $CaCO_3$ precipitates.
- To determine the effect of the microbially-induced $CaCO_3$ precipitates on physical, mechanical, chemical, thermal and biodegradable properties of the seaweed-based composite films with different $CaCO_3$ loadings.
- To compare the physical and mechanical properties of fabricated seaweed-based composite films with the conventional mulch film.

1.4Thesis layout

This entire thesis comprised of five main chapters as listed in Figure 1.0.



chapter 1
Introduction

- Composed of general background of this study;
- Problems statement;
- Objectives of this study being carried out.

Chapter 2
Literature Review

- Previous studies on the topic of polysaccharide-based composite films were reviewed in this chapters.

Chapter 3
Methodology

- Experimental design.
- Method of film preparation.
- Characterization techniques for the raw materials and the composite films were detailed.

Chapter 4
Results and Discussion

- Described the outcome of the findings from the various characterization techniques.
- Each finding from the characteriztaion techniques was interpreted and discussed comprehensively.

Chapter 5
Conclusion and recommendation

- Summarised the overall findings in this study.
- Provide future sugesstions and recommendations for future work.

Figure 1.1 Thesis layout

CHAPTER 2

LITERATURE REVIEW

2.1 Constituents and processing of polysaccharide-based composite film

Matrix plays a crucial role as a continuous phase in a composite material (Wang et al., 2011). Recently, polysaccharide-based matrices are trending because it is more sustainable towards the ecosystem as compared to the petroleum-derived polymer. Table 2.1 shows the properties, characterizations and processing of polysaccharide-based composite films.

Polysaccharides are high molecular weight biological molecules of carbohydrates that composed of long polymers of monosaccharides molecules and their derivatives such as glucose, fructose, galactose and mannose, joining the multiple sugar molecules together by glycosidic bonds (-O-). They can be formed either linear or branched, composed merely one type of monosaccharide (homopolysaccharides or homoglycans) or more than one type (heteropolysaccharides or heteroglycans) as well as semi-crystalline and amorphous which are normally insoluble in water at ambient temperature. Examples of homopolysaccharides are starch and cellulose while examples of heteropolysaccharides are agar, alginate and carrageenan (Matahwa, 2008). Polysaccharide can be differed in the type of sugar, forming by joining glucose molecules together in different ways. Polysaccharides can be sub divided into anionic and cationic (Prajapati et al., 2014).

Polysaccharides exhibit remarkable properties include renewability, availability, biodegradability, inexpensive, non-antigenic, non-carcinogenic and immunogenic (Abdul Khalil et al., 2013, Abdul Khalil et al., 2017d). Besides, polysaccharides are more stable than the other biopolymers such as lipids and proteins as they are not irreversibly denatured via heating (Avella et al., 2005). Most of the polysaccharides are obtained from plants and composed of monosaccharide units bound by glycosidic bonds. Hence, they are usually not toxic and are biocompatible ascribed to their structural similarity of glycosaminoglycans (GAGs) (Cazón et al., 2017). With diverse properties of low, intermediate and high molecular weights, linear or branched structures as well as high level of chirality, it makes polysaccharide an ideal matrix for green composite/eco-friendly production (Avella et al., 2005; Cazón et al., 2017).

However, the principal drawbacks encountered by polysaccharide-based matrices are usually weak in mechanical, thermal and water vapour barrier properties compared to synthetic composites due to their hydrophilic nature which limits the functionalities and applications of the composites (Rhim et al., 2013; Othman et al., 2015). Therefore, many studies have incorporated inorganic fillers into polysaccharide-based matrices with the aim to enhance film properties. For examples, chitosan-based films were enhanced by adding fillers such as clay, Montmorillonite (MMT), AgNPs, carbon nanotubes and graphene-based materials as a reinforcing agent to stimulate chemical reactions and modify the polymer interface to improve the properties of the composites (Dong et al., 2014; Moura et al., 2016).

Table 2.1 Properties, characterizations and processing of polysaccharide-based composite films

Matrices	Fillers	Plasticisers	Techniques	Film properties and characterizations	References
Alginate	Calcium chloride ($CaCl_2$)	Glycerol, fructose, sorbitol, polyethylene glycol (PEG-8000)	Solution casting	➢ Film thickness ➢ Mechanical ➢ Water vapour permeability (WVP) ➢ Moisture sorption isotherm	Olivas et al., 2008
Chitosan	Clay	NM	Solution casting	➢ Film thickness ➢ Water solubility ➢ WVP ➢ Differential scanning calorimetry (DSC) analysis ➢ Thermogravimetric analysis (TGA) ➢ SEM	Casariego et al., 2009
Cellulose	Silver (AgNPs)	NM	Solution casting	➢ Film thickness ➢ WVP ➢ Microbiological analysis ➢ FT-IR ➢ Mechanical	de Moura et al., 2012
Starch	Clay	Glycerol	Solution casting	➢ XRD ➢ SEM ➢ FT-IR ➢ Transparency ➢ DSC ➢ Dynamic mechanical thermal analysis (DMTA) ➢ Water uptake	Mbey et al., 2012

K-carrageenan	Nanoclay/ cellulose nanocrystal	Glycerol	Solution casting	➢ Film thickness ➢ Mechanical ➢ Morphology [using scanning electron microscope (SEM)]	Zakuwan et al., 2013
K-carrageenan	Montmorillonite (MMT) /AgNPs	Glycerol	Solution casting	➢ Mechanical ➢ Contact angle ➢ WVP ➢ TGA ➢ Antibacterial activity ➢ Colour and transparency	Rhim and Wang, 2014
Starch	$CaCO_3$	Glycerol	Solution casting	➢ Film thickness ➢ Mechanical ➢ Water vapour permeability (WVP) ➢ XRD ➢ DSC ➢ Optical ➢ SEM	Sun et al., 2014
Alginate	Silicon dioxide (SiO_2)	Glycerol	*In situ* synthesis	➢ Film thickness ➢ Mechanical ➢ Water solubility and water content ➢ Swelling degree test ➢ Water solubility evaluation ➢ WVP ➢ FT-IR ➢ Light Transmission and Transparency of the Films ➢ Surface Color Measurement ➢ XRD ➢ SEM	Yang et al., 2016

Starch	Zinc Oxide (ZnONPs)	Glycerol	Solution casting	➢ Mechanical ➢ SEM ➢ TGA ➢ FT-IR	Yunus and Fauzan, 2017
Starch	$CaCO_3$	NM	Solution casting	➢ Film thickness ➢ Mechanical ➢ Oxygen permeability ➢ Biodegradablility (using soil burial test) ➢ FT-IR ➢ XRD ➢ TGA	Swain et al., 2018
Potato starch	$CaCO_3$	Glycerol	Solution casting	➢ Mechanical ➢ Water absorption capacity ➢ Coefficient of friction ➢ Solubility ➢ SEM	Dawale and Bhagat, 2018
K-carrageenan	ZnONPs	Glycerol	Solution casting	➢ Mechanical ➢ Solubility	Saputri et al., 2018
Soluble soy-bean polysaccharide	Titanium oxide (TiO_2)	Sorbitol	Solution casting	➢ Mechanical ➢ Contact angle ➢ Atomic-force microscopy (AFM) ➢ SEM ➢ Anti-bacterial activity ➢ Anti-mold activity ➢ Migration test	Salarbashi et al., 2018

(Note: NM = Not mentioned

Filler is playing an important role in enhancing or reinforcing the entire composite system albeit the small amount of fillers is used. Fillers act as a discontinuous phase in the composite, they are usually scattered and distributed within the matrix. A complex structure of interphase is created when the fillers are incorporated into the matrix phase, where the configuration and interaction between the fillers and the matrix will determine the properties of a composite. Synergic effect between filler and the matrix phases occurred as both filler and matrix are complementing one another, thus producing a composite with enhanced properties (Wang et al., 2011).

Numerous inorganic fillers have been incorporated into alginate-based films, such as magnesium aluminium silicate (MAS), calcium chloride, and clay to improve rheological and mechanical properties, retard water uptake and drug permeability of alginate gels and films (Pongjanyakul and Puttipipatkhachorn, 2007). The addition of Montmorillonite (MMT) into pectin-based matrix had enhanced the mechanical properties of the entire composite system (Chen et al., 2013). Similar properties enhancement had attained in carrageenan matrices where the clay and chitosan were incorporated (Park et al., 2001).

Aside from the matrix and the filler, the fabrication of polysaccharide-based composite film usually involves the mixture of base matrix, filler and water with or without the presence of plasticizer. The common reasons to add plasticizer are to reduce film rigidity by enhancing the mobility of the polymer chains. Thus, enhanced film with lower second order transition temperature (Tg) are usually noticed in a plasticized-film (Sanyang et al., 2015). Polyols such as glycerol, sorbitol, mannitol and sugars are among the common plasticizing agents used in hydrophilic polymer and polysaccharides films (Vieira et al., 2011; Souza et al., 2012).

Glycerol is one of the widely used bio-epoxies in polysaccharide-based composite films. It is usually viscous, odourless, and colourless with syrupy-sweet taste coupled with excellent adhesion properties. The main component of glycerol is triglycerides which can be discovered in vegetable oil, crude oil or animal fat (Thakur et al., 2014). It consists of three hydrophilic hydroxyl groups that inherently make it hygroscopic and soluble in water. Moreover, it is miscible in many substances including alcohol, phenol, ethylene glycol, propylene glycol and trimethylene glycol monomethyl ether (Quispe et al., 2013).

There are a range of processing methods to fabricate composite film including physical methods such as melt compounding and solution casting as well as chemical methods such as *In situ* polymerization and *In situ* condensation as stated in Table 2.1 previously. Nonetheless, solution casting is amongst the most extensive method used in forming composite films particularly polysaccharide composite film due to its simplicity and ease of processing (Li et al., 2010). Homogeneous composite film is formed after the solvent is evaporated by subsequent treatment in oven or coating process as shown in Figure 2.1.

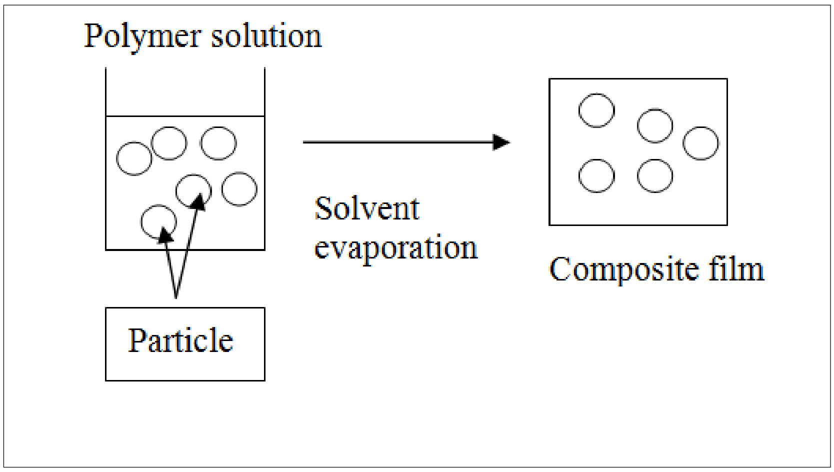


Figure 2.1Schematic drawing of solution casting/film casting method

Properties such as mechanical (Tensile, Tensile modulus, Elongation at break), thermal, water vapour permeability, biodegradability, solubility, water absorption ability and opacity are among the properties tested and determined in the study of composite films. The study of structural analysis including Fourier-transform infrared spectroscopy (FT-IR) and X-ray diffraction analysis (XRD) are also performed to determine the existence of functional group and crystalline phase respectively. Besides, some of the studies observed the morphology of the composite films using Scanning electron microscope (SEM).

2.2 Relevance of seaweeds as the composite matrix

2.2.1 Background of seaweed

In the past, seaweed had been misunderstood as mere weeds in the ocean. Recently, seaweed-based materials have emerged as an upfront research material particularly in fabricating composite films. Multifaceted usages of seaweed have recognized in food, pharmaceutical, agriculture and other end-user applications worldwide (Tiwari and Troy 2015).

Seaweed is subject to a larger group of algae that live in marine or saline water environment. It grows easily in shallow marine water, estuaries and sub tidal-region up to a depth where 0.01% photosynthetic light is available (Tiwari and Troy 2015). It does not have true real roots, stem or leaves but it consists of holdfast, stipe and blade (Figure 2.2). Holdfast functions as an anchor or attachment for the seaweed; the stipe functions as support to the blade and absorption of nutrients; and the blade is essential for photosynthesis process as well as absorbing nutrients from its surrounding (Dhargalkar and Kavlekar, 2004).

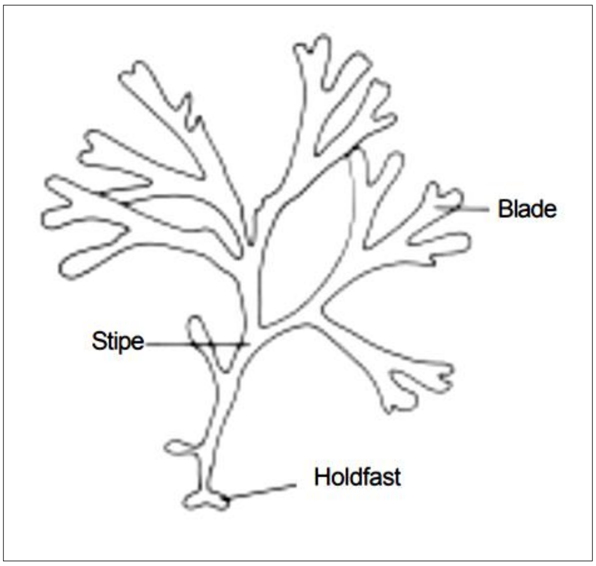


Figure 2.2Thallus of seaweed (Dhargalkar and Kavlekar, 2004).

Seaweed can be classified into red seaweed (Rhodophyceae), brown seaweed (Phaeophyceae) and green seaweed (Chlorophyceae) according to the colour of their pigments, morphology and anatomical characteristics as described generally in Table 2.2.

Based on the Table 2.2 as displayed, red seaweed is usually found in warmer waters and tropical areas, brown seaweed is usually found on rocky intertidal while green seaweed can be found in fresh water, ocean surface or marine sediments (Vera *et al.*, 2011; Meinita et al., 2012; Tiwari and Troy 2015).

Table 2.2 Classification of seaweeds and their characteristics

	Red seaweed	Brown seaweed	Green seaweed
Class	*Rhodophyceae*	Phaeophyceae	Chlorophyceae
Phycocolloids	Agar, carrageenan	Alginate, fucoidans and laminaran	Ulvans
Habitat	inhabits warmer waters and tropical seas	found on the rocky intertidal	fresh habitat, ocean surface or marine sediments
Photosynthetic pigments	chlorophyll *a* with accessory red/blue phycobilin pigments, predominantly the red-colored phycoerythrin and phycicyanin	xanthopyll pigment called fucoxanthin and β-carotenoids in addition to chlorophyll α and c.	chlorophyll α and β and contained chromatophores
Thallus	filamentous, simple or branched, free or compacted to form pseudoparenchyma with uni or multiaxial construction	Simple, freely branched filaments to highly differentiated forms.	free filaments to definetely shaped forms. Moderate to highly calcified appearing in fan shaped/ feather like or star-shaped branches
Size	Usually small, ranging from a few centimetres to approximately a meter in length.	Large and approximately 20 m long, 2-4 m long.	Usually small and size range similar to red seaweeds.
Reproduction	vegetative, asexual and sexual method	vegetative, asexual and sexual methods	vegetative, asexual and sexual method
Storage form of food	Floridean starch and floridosides sugar.	laminarin starch, manitol (alcohol) and some store iodine also.	Starch

(Phang, 2006; Vera *et al.*, 2011; Meinita et al., 2012; Tiwari and Troy 2015).

Seaweed contains carbohydrate, protein, minerals, vitamins, dietary fibre and lipids. It also contains secondary metabolites such as monoterpenes, sesquiterpenes, diterpenes meroterpenoids, phlorotannins and steroids that promote functional properties including anti-bacterial, anti-inflammatory, anti-viral, anticoagulant and anti-tumour (Tiwari and Troy 2015). Seaweed is famous with its sulfated polysaccharides, namely phycocolloids which play essential role in both the cell wall and the intercellular matrix. These biopolymers are attractive in composite films applications owing to its film-forming ability and excellent mechanical properties (Jumaidin et al., 2017). Nevertheless, the content of chemical compositions may vary with the distribution, environment of growth and types of seaweeds.

2.2.2 *Kappaphycus alvarezii*

In Malaysia, red seaweed attained the highest number of taxa with 186 taxa followed by 105 taxa from chlorophyta and 73 taxa phaeophyta. *Gracilaria and Kappaphycus* species are among the most popular seaweeds found from lower intertidal to upper sub-tidal areas in Sabah and around islands in Peninsular Malaysia (Asmida et al., 2017).

Kappaphycus alvarezii, previously known as *Eucheuma cottonii* is one of the red seaweed species (*Rhodophyceae*) which can be found and cultivated in Phillipines, Indonesia, Mexico, Brazil, Fiji, Tanzania, Kiribati, Kenya, Madagascar and in Malaysia, particularly the east coast of Malaysia, Sabah. It has been cultivated for over 40 years in the tropical regions mainly for carrageenan production (Jumaidin et al., 2018; Zhang et al., 2015).

Kappaphycus alvarezii is not merely marketed to make salad, soup and pudding but also served as a promising biomass with regards to its high growth rate which can be doubled up within 15 to 30 days, high yield per area and high efficiency in CO_2 capture (Mondal et al., 2017). For the past four decades, *Kappaphycus alvarezii* became economically and industrially important as the source of carrageenan. This is because it contains mostly kappa-carrageenan (ie: for gel formation abilities and viscosifying) and not more than 10% of iota-carrageenan (Ilias et al., 2017). Besides, it is more preferred over *Chondrus crispus* (the original source of carrageenan) due to ease in processing to obtain kappa-carrageenan (Chunha and Grenha, 2016).

2.2.2 (a) Physical Properties

Physical properties are generally measurable and observable parameters that are frequently determined before considering a new natural material as potential filler or matrix for polymer composites. This is to avoid impractical and wastage of end product produced using the raw material. For instances, high moisture content is not preferable for a composite due to weak stability in terms of dimensions, tensile strength and porosity formation (Jumaidin et al., 2017).

Physical characterization of raw *Kappaphycus alvarezii* is still in lack since most research works are emphasizing on the phycocolloid, carrageenan and characterization of the end product (ie: the composite film) instead of the raw seaweed. However, study done by Jumaidin et al. (2017) on the physical properties of raw *Kappaphycus alvarezii* stated that the moisture content of raw *Kappaphycus alvarezii* was low which was 1.13% compared with other natural fibres (Kenaf and jute), which attained around 3 to 5%. The authors explained that lower moisture content could be due to preliminary heating of seaweed prior to storage.

The colour of *Kappaphycus alvarezii* can be varied depending on the variant of the red seaweeds. Most *Kappaphycus alvarezii* in Malaysia is shiny green to yellow orange in colour as seen by naked eyes (Jumaidin et al., 2017). Morphologically, *Kappaphycus alvarezii* looks like a spiny and bushy plant with many irregular smooth surface branches. The cell wall comprised of two layers: outer cell wall which is amorphous embedding matrix with cellulose fibers and phospholipid; and inner cell wall which consists of fibrillar skeleton made up of sulphated polysaccharides. The polysaccharides can be broken down during extraction to obtain kappa-carrageenan (Dewi et al., 2015).

2.2.2 (b) Chemical compositions

In comparison to physical properties, more research works have been conducted to characterize chemical composition of *Kappaphycus alvarezii*. The widely characterize chemical compositions are carbohydrate, lipid, protein, ash, sulphated groups and minerals content (Table 2.3).

Table 2.3 Chemical composition of *Kappaphycus alvarezii*

Chemical compositions	References
Carbohydrate	
52.3%	Abirami and Kowsalya, 2011
50.1%	Kumar et al., 2015
56.1%	Ariffin et al., 2017
57%	Hong et al., 2007
65.20%	Abdul Khalil et al., 2018b
Protein	
6.2%	Xieren and Aminah, 2017
9.81%	Yong et al., 2015
2.5%	Masarin et al., 2016
12.69 to 23.61%	Kumar et al., 2015
4.5%	Abirami and Kowsalya, 2011
2.5%	Ariffin et al., 2017
3.0%	Hong et al., 2007
3.4%	Abdul Khalil et al., 2018b
Lipid	
2.06%	Yong et al., 2015
0.6%	Masarin et al., 2016
0.39 to 0.91%	Kumar et al., 2015
1%	Xieren and Aminah, 2017
0.5%	Ariffin et al., 2017
0.89%	Abirami and Kowsalya, 2011
0.7%	Hong et al., 2007
1.1%	Abdul Khalil et al., 2018b
Fibre	
5.3%	Ariffin et al., 2017
6.3%	Hong et al., 2007

Ash	
16.3%	Xieren and Aminah, 2017
16%	Masarin et al., 2016
38.86%	Jumaidin et al., 2017
33.16%	Yong et al., 2015
20.99% to 33.81%	Kumar et al., 2015
21.4%	Ariffin et al., 2017
28.9%	Abirami and Kowsalya, 2011
11.57%	Hong et al., 2007
Cellulose	Jumaidin et al., 2017
5.30%	
Hemicellulose	Jumaidin et al., 2017
0.39%	
Lignin	Jumaidin et al., 2017
6.73%	
Minerals	Yong et al., 2015; Kumar et al., 2015
Sodium (Na), Potassium (K), Calcium (Ca), Magnesium (Mg)	

The major component that can be found in Kappaphycus alvarezii is the carbohydrate content which contains about 50% to 65.20% of dry weight. The high proportion of carbohydrate in the seaweed is usually contributed by the hemicelluloses, cellulose and the long-chain sulfated polysaccharides from the group of galactons. These are the main components that made up the cell wall of seaweed (Masarin et al., 2016).

Carrageenan is an anionic sulphated linear polysaccharide formed by a straight chain backbone structure of alternating 1,3-linked β-D-galactopyranose and 1,4-linked α-D-galactopyranose units (Fig. 2.3) (Vankatesan et al., 2015). The 3-linked units occur as the 2- and 4-sulphate or the unsulphated derivative, while the 4-linked units occur as the 2-sulphate, 2,6- disulphate, the 3,6-anhydrid and the 3,6-anhydride-2-sulphate. Although there are about 15 different types of carrageenan reported, the three isomers of carrageenans being most industrially relevant are the iota (ι), kappa (κ), and lambda (λ) carrageenans. The differences among these three are the number and position of the organosulphate groups with one, two, and three repeating galactose units and disaccharide units respectively (Cunha and Crenha, 2016). Besides that, kappa-carrageenan is also commonly known by its strong and firm gelling properties, iota-carrageenan is known by its elasticity while lambda-carrageenan is a non-gelling polysaccharide due to the absence of helical structure. The characteristics of carrageenan are influenced by the sulphate ester group of 3,6-anhydro-galactose unit (Zarina and Ahmad, 2014). Nevertheless, only kappa-carrageenan and iota-carrageenan can be found in *Kappaphycus alvarezii* (Ilias et al., 2017).

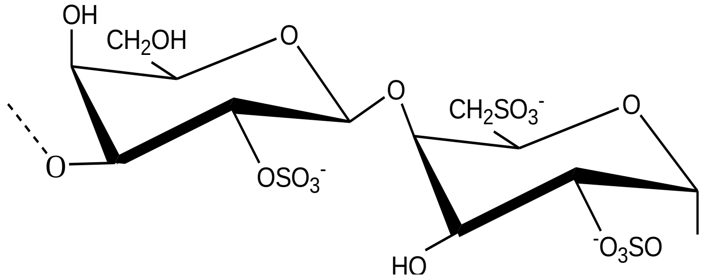


Figure 2.3 Chemical structure of carrageenans (Venkatesan et al., 2015).

Carrageenan is soluble in boiling water mainly attributed to their sulfate and hydroxyl groups (Wanyonyi et al., 2017). The solubility of carrageenan is above 80˚C (Cunha and Grenha, 2016). The gelling point and melting point of carrageenan are in the range of 30-50˚C and 50-70˚C respectively. Although carrageenan liquefies when it is heated to the melting point, it can gel again while cooling, which attributed to its thermo-reversible properties (Abdul Khalil et al., 2018b). The gel strength is the range of 100 to 350 g/cm^2 while the viscosity is approximately 30 to 300 cP. Among the three isomers, κ-carrageenan is relatively less hydrophilic and soluble compared to the other three isomers due to the presence of 3,6-anhydro-galactose unit and fewer sulfates group content, which is found mostly in the species of *Kappaphycus alvarezii*.

Carrageenan has been used broadly in many fields as a coating base to prolong the shelf life of foods, in packaging films as an alternative to the current petroleum-based packaging, in the pharmaceutical industry as thickening, gelling, stabilizing and suspending agents (Abdul Khalil et al., 2017b). Furthermore, it is recommended as the source of bioethanol production due to the high content of carbohydrate (ie: the galactose content). The good gelling properties are attributed to the negative charge on each disaccharide which makes it a prominent matrix in film forming. Moreover, the ability to form double helix network during the gelation is able to contribute to the physical and mechanical properties of the matrix. Nevertheless, in order to surmount the problem of its hydrophilic nature, researchers have incorporated different fillers such as clay, silver nanoparticles (AgNPs) and silicon dioxide (SiO_2) into carrageenan-based matrices to enhance the water barrier and mechanical properties (Park et al., 2001, Rhim and Wang, 2014; Venkatesan et al., 2017; Tabatabaei et al., 2018).

The second largest component in *Kappaphycus alvarezii* is the ash content which attained from 11.57% to 38.86% depending on its variant. The high proportion of ash content indicated that seaweed contains high amount of macro minerals such as sodium (Na), calcium (Ca), magnesium (Mg) and some trace elements such as iron (Fe), zinc (Zn), Copper (Cu) and (Mb). Previous report showed that the ash of *Kappaphycus alvarezii* exhibited high calcium (ie: 0.16%; 159.5 mg/100g), followed by iron (0.33%, 33.8 mg/100g) and zinc (0.016%; 1.58 mg/100g) (Rajasulochana et al., 2010; Hayashi and Reis, 2012). It is also reported that the amount of minerals and trace elements of the seaweed were higher than the terrestrial plants due to its metabolic system which enable it to absorb minerals and elements from the sea water (Rajasulochana et al., 2010, Masarin et al., 2016). The minerals and trace elements can be one of the major contributions of crystallinity.

Although there are some controversies on the presence of lignin and hemicelluloses content in seaweed, the existence of lignin and hemicelluloses in

Kappaphycus alvarezii have been confirmed by a number of studies including Martone et al. (2009), Wi et al. (2009), Mahdi et al. (2016) and Jumaidin et al. (2017). It was reported in detail that low lignin and hemicelluloses contents were identified in *Kappaphycus alvarezii* with only 6.73% and 0.39% respectively. The presence of lignin was further validated by the stretching of aromatic group (1520 cm^{-1}) while the presence of hemicelluloses was confirmed by the presence of hydroxyl (3443 cm^{-1}) and carbonyl group (1648 cm^{-1}) via Fourier-transform infrared spectroscopy (FT-IR) analysis (Jumaidin et al., 2017). Low content of lignin and hemicellulose is preferable in the bioethanol industries as it eases the process of pre-treatments and hydrolysis.

Protein is the third highest content in *Kappaphycus alvarezii* obtained from 2.5% to 23.61% mainly enriched with about 18 amino acids including alanine, arginine, asparagine, aspartic acid, glutamic acid, cystine, glycine, histideine, isoleucine, lysine, leucine, methonine, praline, phenylalanine, serine, theronine, tyrosine, tryptophan and valine. The physico-chemical properties of protein including solubility and gelation are influential towards the functional properties of gel-forming and film forming abilities (Kumar et al., 2014; Abdul Khalil et al., 2018b). In fact, protein is more hydrophobic compared to polysaccharides (eg: carrgeenan, cellulose) albeit its poor water resistance. It has contributed to mechanical stability in film forming (Mellinas et al., 2016).

The lowest content of chemical composition in *Kappaphycus alvarezii* is the lipid which ranged from 0.39% to 2.06%. Lipid is also more hydrophobic than polysaccharides. Usually, lipid-based films are able to reduce water vapour permeability (Mellinas et al., 2016). Hence, the low constituents of protein and lipid in *Kappaphycus alvarezii* could be one of the reasons that caused weak water barrier properties in the films produced by Siah et al. (2015) and Abdul Khalil et al. (2018b).