PROPERTIES AND CHARACTERIZATION OF LIGNIN NANOPARTICLES REINFORCED BIOPOLYMER FILMS

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PROPERTIES AND CHARACTERIZATION OF LIGNIN NANOPARTICLES REINFORCED BIOPOLYMER FILMS

by

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LIST OF SYMBOLS

+	Addition
C–O	Alkoxy
C≡C	Alkynes
α	Alpha
β	Beta
C=O	Carbonyl
C=C	Carboxyl
cm	Centimeter
cP	Centipoise
a*	Color channel of redness-greenness
b*	Color channel of yellowness-blueness
°	Degree
°C	Degree Celcius
°C/min	Degree Celcius per minute
ρ	Density
D	Distance
÷	Division
Dw	Dry weight
eV	electronvolt
ε	Epsilon
=	Equal
T_2	Final thickness
W_2	Final weight
γ	Gamma

GPa	Gigapascal
g	Gram
G	Guaiacyl
h	Hour
O–H	Hydroxyl
T_{onset}	Initial degradation temperature
T_1	Initial thickness
W_1	Initial weight
ι	Iota
κ	Kappa
kg/m^3	Kilogram per cubic meter
kJ/mole	Kilojoule per mole
kV	Kilovolt
λ	Lambda
l	Length
<	Less than
L*	Lightness
m	Mass
T_{max}	Maximum degradation temperature
MPa	Megapascal
C–H	Methyl
mg/°C	Milligram per degree Celcius
mL	Milliliter
mm	millimeter
mm/min	Millimeter per minute
mV	Millivolt
min	Minute

>	More than
×	Multiply
nm	Nanometer
1D	One-dimension
H	p-hydroxyphenyl
%	Percentage
±	Plus-minus
cm^{-1}	Reciprocal centimeter
rpm	Revolutions per minute
m^2/g	Square meter per gram
–	Subtraction
S	Syringyl
θ	Theta
t	Thickness
3D	Three-dimension
~	Tilde
ΔE	Total color difference
T	Transmittance
T_g	Transition temperature
2D	Two-dimension
V	Volume
v/v	Volume per volume
λ	Wavelength
W	Weight
w/w	Weight per weight
wt. %	Weight percentage
0D	Zero-dimension

LIST OF ABBREVIATIONS

AFM	Atomic force microscopy
Al	Aluminum
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
C	Carbon
$(C_2H_5)_2O$	Diethyl ether
C_2H_5OH	Ethanol
$C_3H_8O_3$	Glycerol
C_6H_{12}	Cyclohexane
$C_{24}H_{36}O_{25}S_2^{-2}$	Kappa-carrageenan
Ca	Calcium
CH_3OH	Methanol
CH_4	Methane
Cl	Chlorine
CLP	Colloidal lignin particles
CO	Carbon monoxide
CO_2	Carbon dioxide
Cu	Copper
DLS	Dynamic light scattering
DPPH	1,1-diphenyl-2-picrylhydrazyl
DSC	Differential scanning calorimetry
DTG	Derivative thermogravimetric
EDX	Energy dispersive X-ray
EFB	Empty fruit bunches

EP	Epoxy
FESEM	Field emission scanning electron microscope
FT-IR	Fourier transform infrared
GC-MS	Gas chromatography–mass spectrometry
H_2SO_4	Sulfuric acid
HDPE	High-density polyethylene
HRR	Heat release rate
K	Potassium
KBr	Potassium bromide
KOH	Potassium hydroxide
LNPs	Lignin nanoparticles
MW	Molecular weight
N_2	Nitrogen
Na	Sodium
Na_2S	Sodium sulfide
NaOH	Sodium hydroxide
O	Oxygen
P	Phosphorous
PBAT	poly-(butylene adipate-*co*-terephthalate)
Ph–OH	Phenolic hydroxyl groups
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxy butyrate
PHBHV	Polyhydroxybutyrate-co-3-hydroxy valerate
PHRR	Peak heat release rate
PLA	Polylactic acid
PLLA	Poly(L-lactide)
Pt	Platinum

PU	Polyurethane
PVA	Polyvinyl alcohol
RI	Refractive index
S	Sulfur
Si	Silicon
TEM	Transmission electron microscope
TGA	Thermogravimetric analysis
TEMPO	2,2,6,6-tetramethylpiperidin-1-yl
USEPA	The United States Environmental Protection Agency
UV	Ultraviolet
WVP	Water vapor permeability
WVTR	Water vapor transmission rate
XRD	X-ray diffraction

SIFAT DAN PENCIRIAN FILEM BIOPOLIMER

DIPERKUAT PARTIKEL NANO LIGNIN

ABSTRAK

Kajian ini membentangkan idea baharu, konstruktif, penting, dan kritikal untuk fabrikasi filem biopolimer daripada matriks rumpai laut merah (*Kappaphycus alvarezii*) dengan penggabungan partikel nano lignin. Di peringkat global, pelbagai isu alam sekitar telah pun dikaitkan dengan pencemaran plastik berasaskan petroleum, yang kebanyakannya digunakan oleh aplikasi pembungkusan. Sebaliknya, pembangunan bahan biopolimer yang berasaskan bio dan/atau boleh terurai lazimnya dipersembahkan sebagai alternatif yang mengurangkan, menawarkan sifat mampan dan mesra alam berbanding plastik konvensional yang berasal daripada sumber petroleum. Tetapi, sifat hidrofilik, penghalang air yang lemah, mekanikal yang rendah, dan antimikrob yang lemah filem biopolimer telah menghalang penggunaannya dalam aplikasi pembungkusan. *Kappaphycus alvarezii* tidak terkecuali daripada cabaran filem biopolimer. Dalam kajian ini, partikel nano lignin dengan proses penulenan telah digunakan dalam pembebanan yang berbeza sebagai penambahbaikan dalam matriks *Kappaphycus alvarezii* untuk mengurangkan sifat hidrofilik dan meningkatkan sifat antibakteria matriks dan dibandingkan dengan partikel nano lignin yang tidak ditulenkan. Pengisi nano dijana menggunakan kaedah mesra alam daripada likor hitam tandan kosong kelapa sawit (*Elaeis guineensis*) sisa pemulpaan soda melalui kaedah pemendakan asid. Pemuatan pengisi partikel nano lignin dipelbagaikan daripada 0, 1, 3, 5, dan 7% sebagai peningkatan sifat zarah nano dalam matriks rumpai laut merah menggunakan kaedah tuangan pelarut. Kesan penggabungan kedua-dua jenis partikel nano lignin pada sifat kefungsian filem biopolimer, seperti fizikal, mekanikal, morfologi, struktur, kebasahan, haba, antimikrob, dan biodegradasi, telah dikaji dan didapati dipertingkatkan dengan ketara. Filem biopolimer yang diperkuat dengan partikel nano lignin yang tidak ditulen menunjukkan peningkatan kekuatan tegangan, modulus tegangan, pemanjangan semasa putus, dan sudut pendarjahan masing-masing 53.37%, 57.83%, 16.56%, dan 29.73%. Berbanding dengan partikel nano lignin yang tidak ditulenkan, kesan peningkatan yang unggul pada matriks telah disediakan oleh partikel nano lignin yang telah ditulenkan. Filem biopolimer yang mengandungi 5% partikel nano lignin yang telah ditulenkan mempersembahkan peningkatan optimum dalam hampir semua persembahan muktamad, manakala peningkatan optimum sifat antimikrob diperhatikan daripada filem biopolimer dengan penggabungan 7% partikel nano lignin yang telah ditulenkan. Penggabungan partikel nano lignin yang telah ditulenkan dalam matriks *Kappaphycus alvarezii* menunjukkan peningkatan yang ketara dalam kekuatan tegangan, modulus tegangan, pemanjangan semasa putus, sudut pendarjahan, dan diameter zon perencatan terhadap bakteria, masing-masing sebanyak 36.70 MPa, 343.59 MPa, 44.76%, 97.62°, dan 22.48 mm. Peningkatan ini berkaitan dengan interaksi antara muka yang kuat antara partikel nano lignin dan matriks seperti yang diperhatikan dalam morfologi permukaan selepas analisis mekanikal, menghasilkan keserasian tinggi filem. Filem biopolimer yang direka daripada kajian ini boleh mempunyai kelebihan tambahan dan memberikan kejayaan dalam bahan pembungkusan untuk pelbagai aplikasi.

PROPERTIES AND CHARACTERIZATION OF

LIGNIN NANOPARTICLES REINFORCED BIOPOLYMER FILMS

ABSTRACT

This study presents a new, constructive, essential, and critical idea for the fabrication of biopolymer films from a red seaweed matrix (*Kappaphycus alvarezii*) with the incorporation of lignin nanoparticles (LNPs). Globally, various environmental issues are already attributed to petroleum-based plastic pollution, which is majorly consumed by packaging applications. On the other hand, the development of biopolymer materials that are bio-based and/or degradable is commonly presented as an alleviating alternative, offering sustainable and eco-friendly properties over conventional petroleum-derived plastics. However, the hydrophilicity, poor water barrier, low mechanical, and weak antimicrobial properties of biopolymer films have hindered their utilization in packaging applications. *Kappaphycus alvarezii* is no exception to these challenges of biopolymer films. In this study, lignin nanoparticles (LNPs) with a purification process were used in different loadings as enhancements in a *Kappaphycus alvarezii* matrix to reduce the hydrophilic nature and improve antibacterial properties of the matrix and compared with unpurified LNPs. The nanofiller was generated using an environmentally friendly method from an oil palm (*Elaeis guineensis*) empty fruit bunch (EFB) black liquor of soda pulping waste via acid precipitation technique. The LNPs filler loading was varied from 0, 1, 3, 5, and 7% as nanoparticle properties enhancement in the red seaweed matrix using a solvent casting technique. The effects of the incorporation of both types of LNPs on functional properties of biopolymer films, such as physical, mechanical, morphological, structural, wettability, thermal, antimicrobial, and biodegradability, was studied and found to be remarkably enhanced. Biopolymer films reinforced with unpurified LNPs showed an enhancement of the tensile strength, tensile modulus, elongation at break, and contact angle of 53.37%, 57.83%, 16.56%, and 29.73%, respectively. As compared to unpurified LNPs, a superior enhancement effect on the matrix was provided by purified LNPs. Biopolymer film containing 5% purified LNPs presented the optimum enhancement in almost all of the ultimate performances, while the optimum improvement of antimicrobial behavior was observed from biopolymer film with the incorporation of 7% purified LNPs. The incorporation of purified LNPs in the *Kappaphycus alvarezii* matrix presented a significant improvement in the tensile strength, tensile modulus, elongation at break, contact angle, and inhibition zone diameter against the bacteria of 36.70 MPa, 343.59 MPa, 44.76%, 97.62°, and 22.48 mm, respectively. The enhancement is related to strong interfacial interaction between the LNPs and matrix as observed in the surface morphologies after the mechanical test, resulting in high compatibility of the films. Biopolymer films fabricated from this study could have additional advantages and provide breakthroughs in packaging materials for a wide range of applications.

CHAPTER 1

INTRODUCTION

1.1 Research background

Environmental pollution triggered by petroleum-derived plastics has become a global issue and has garnered much attention from many stakeholders, including researchers (Abdul Khalil et al., 2019; Mujtaba et al., 2023). Production of environmentally sustainable material as an alternative to petroleum-derived materials has attracted scientists and society's foremost concern at large (Abdul Khalil et al., 2017; Ali Qamar et al., 2023). Significant progress has been made in developing biodegradable plastics, primarily from renewable natural resources, to produce biodegradable materials with similar functionality to oil-based polymers. Mani et al. (2023) estimated that 8.3 billion tons of plastic had been produced worldwide from the 1950s to 2022, of which 9% has been recycled, 12% was incinerated, and the remaining 79% was accumulated in landfills or the environment.

Most conventionally used plastics are not biodegradable; thus, it remains in the environment for years. Although plastic materials can be physically broken down into micro-sizes particles (<5 mm) in landfills or marine environments, they enter into food chains or animal bodies, causing various diseases (Mamun et al., 2023). With the acceleration in the production, consumption, and disposal of plastic-based materials worldwide, the future of our planet appears bleak. This phenomenon places an overwhelming burden on earth because of the accumulation of non-renewable natural resource-based plastics in the biosphere, impacting humans, wildlife, and their natural environments (Abdul Khalil et al., 2016; Thew et al., 2023).

The cleaner technology approach using biodegradable resources has been aggressively encouraged to control worldwide pollution. Hence, numerous research has been conducted to develop natural alternatives, with safer, cleaner, and biodegradable, renewable resources as precursor materials instead of petroleum (Ezati et al., 2023; Jafarzadeh et al., 2020). Cleaner production technologies using safe, sustainable, and natural resources help reduce the adverse impact on the earth, water, and air by processes and products of conventional manufacturing industries. Natural alternatives such as starch, cellulose, plants proteins, polylactic acid (PLA), polyhydroxy butyrate (PHB), and bio-based polyamide (bio-PA) all possess unique advantages which are utilized in different applications (Kargarzadeh et al., 2018).

Biopolymer-based materials have been extensively evaluated and used in many applications (Zhao et al., 2019). Still, their commercialization has been restricted due to the poor properties, yet they need to be improved to reach or exceed the petroleum-based ones. The poor mechanical strength and water barrier properties, for example, have always been a challenge associated with bioplastics and natural packaging materials (Hasan, et al., 2019a). However, the use of reinforcement material/s has been proposed to improve the mechanical and water barrier properties. Different organic fillers, which are hydrophobic or hydrophilic reinforcement materials, have been used to improve the properties of biopolymers for industrial applications (Ravindran et al., 2019). In this sense, lignocellulosic biomass is the primary source of organic fillers, mainly cellulose, hemicellulose, and lignin that have been employed in the properties enhancement of the biopolymer films.

To date, plastics have become the widely used products of choice due to their high levels of stability, affordability, and functionality. However, the consumption of synthetic plastic materials derived from raw petrochemicals has a severe impact on environmental pollution since the majority of synthetic plastics are not easily degraded by microbial decomposers (McAdam et al., 2020). Although synthetic plastics can be fragmented into smaller particles in environmental media such as landfills, soil, water, and marine environments, they accumulate in the food-chain system, causing various diseases (Zhu et al., 2023).

Numerous approaches have been devoted to developing the synthesis of biodegradable polymers. One such approach is bioplastics, a rapidly growing class of polymeric compounds that are produced from renewable feedstock and are both environmentally sustainable and functionally similar to synthetic plastics (Kim et al., 2022). Therefore, bioplastics derived from renewable sources have been developed to alleviate the synthetic plastic pollution problem, as the majority of bioplastics can degrade whenever exposed to bioactive environments such as compost and soil (Abdul Khalil et al., 2023). Recent studies reported on biodegradable plastic films fabricated from biomass feedstocks, such as chitosan (Jin et al., 2023), alginate (Bahraminejad et al., 2023), starch (Celletti et al., 2023), and seaweed (Kanagesan et al., 2022). Such prior publications revealed a considerable improvement in the biopolymer films' properties.

Biopolymers are natural polymers derived from plants and animals, which include a variety of polysaccharides, polypeptides, and polynucleotides (Baranwal et al., 2022), while biomonomers are small molecules (i.e., monomeric subunits) that can undergo ex situ chemocatalytic polymerization to produce biobased polymers (Magalhães Júnior et al., 2021). The utilization of biopolymers is not limited to bioplastics but ranges from sustainable production of other materials such as biofuels, bio-implants, and medicinal products (Faizan Muneer et al., 2021).

Seaweed is considered one of the most potential biopolymers. *Kappaphycus alvarezii*, a red seaweed, is an eco-friendly, abundant, sustainable, and low-cost bioresource. In line with the advantages, carrageenan is a natural carbohydrate (polysaccharide) and commercially essential phycocolloid generated from red seaweeds. The extraction and purification process of carrageenan is expensive, time-consuming, and requires abundant chemicals and water during processing (Mudhulkar et al., 2022). To overcome these issues, raw red seaweed was used for the development of biopolymer films since it is cheaper and easier to process than pure carrageenan.

Furthermore, a matrix plays a key role since it can be designed for a specific application if a biopolymer matrix is properly selected. Previous studies found that raw seaweed can form a biopolymer film with adequate ultimate performance (Abdul Khalil et al., 2018a; Uthaya Kumar et al., 2020). However, biopolymer films suffer from several shortcomings, which were encountered as the majority of biopolymer films, are unable to stand if used alone. The hydrophilic nature of seaweed, with its poor water barrier, mechanical, and antimicrobial properties, has limited its use for packaging applications (Lavrič et al., 2021).

To overcome these challenges, either organic or inorganic fillers that are less hydrophilic or hydrophobic are blended with raw seaweed or seaweed-derived polymers to broaden their applications (Hasan, et al., 2019b). The effectiveness of introducing organic fillers to improve the seaweed-based biopolymer film has been previously reported (Uthaya Kumar et al., 2019). Hence, the use of organic fillers derived from solid biowaste is an excellent candidate for reinforcing materials in seaweed films due to their cost-effective, abundant, and eco-friendly nature.

Biofillers can improve the functional properties of the synthesized biopolymer film (Hazrati et al., 2021). In this sense, lignin is the second most abundant bioorganic polymer on earth after cellulose (Baniasadi et al., 2023). Lignin is not naturally available in its isolated form, but it is physically combined with cellulose and hemicellulose. Currently, lignin is being extensively used as a reinforcement material for biopolymer composite production (Agustiany et al., 2022). It is an abundantly available, sustainable, and low-cost polymeric material that enhances polymer composites' strength. Lignin polymer is a by-product available in comparatively large amounts, meaning that the valorization of lignin in composite materials for any high-value application could result in large economic gains. It has been used in various sectors as a main component or additive for the production of new innovative bioplastics products for different applications (Yang et al., 2020a).

A potential breakthrough to improve the functional properties is to strengthen it with a reinforcement agent. Hence, one possible approach for strengthening the *Kappaphycus alvarezii*-based film is to blend it with lignin nanoparticles (LNPs). The valorization of lignin as a reinforcement material in biopolymer matrices is a comparatively new and emerging research field. Extensive ranges of polymers reinforced with lignin have been classified and investigated (Kargarzadeh et al., 2020). The increasing number of researchers is motivated by the desire to overcome all the barriers and challenges associated with biopolymer composites. This has resulted in the production of biopolymers reinforced with various types of lignin for novel biopolymer applications. Since LNPs have high total phenolic content, nano-size distribution, and antimicrobial activity, the incorporation of LNPs in *Kappaphycus alvarezii* biopolymer film is able to provide superior physical, mechanical, antimicrobial, and thermal stability to the prepared biopolymer films.

In the literature, no study has been reported on the incorporated improvement of LNPs in seaweed matrix. It is necessary to study the incorporation of LNPs in *Kappaphycus alvarezii* which can be a promising choice for reinforcing and cost-reducing the biopolymer films since the LNPs were generated from black liquor as industrial waste. The present study is focused on the preparation and characterization of *Kappaphycus alvarezii* biopolymer films reinforced with LNPs. The LNPs were isolated from an oil palm (*Elaeis guineensis*) empty fruit bunch (EFB) of soda pulping waste, which is the most suitable technique for non-wood pulping. The isolation of lignin from industrial waste is a prime contribution to waste valorization, particularly in the pulp and paper industry. In this study, a novel approach has been proposed to enhance the properties of LNPs through a purification process to eliminate natural impurities such as fats, fatty acids, waxes, lipids, and tannins. The present approach was compared with that of the unpurified LNPs towards the characteristics. These two types of LNPs were then used as reinforcing nanofillers in a *Kappaphycus alvarezii* matrix. Several techniques and analysis methods investigated the functional properties of the biopolymer film networks.

1.2 Problem statement

Synthetic polymer from fossil fuels has contributed significantly to industrial and technological development. However, the disposal of synthetic polymers has resulted in severe environmental pollution. The rapidly increasing production of permanent waste generated from synthetic polymers and their effect on the environment is now a global challenge. This is mainly due to their non-biodegradability, difficulty in recycling, and contamination. This has adversely impacted humans, wildlife, and the natural environment of wildlife (Hasan et al., 2019b). Therefore, continuous findings have been made to manage synthetic waste by replacing them with eco-friendly alternatives.

Polymers derived from natural materials are called biopolymers and have been researchers' focus as an alternative to the non-biodegradable polymer. Biopolymers are derived from natural plants, living organisms, and biological materials (Mellinas et al., 2020). Biopolymers are isolated renewable and edible ingredients such as polysaccharides, protein, and lipids. Biopolymers are a suitable alternative source for packaging materials due to their nontoxicity and biodegradability (Priyadarshi et al., 2022). Seaweed is an abundant source of polysaccharide derivatives such as agar, carrageenan, and alginate (Lomartire & Gonçalves, 2022). Seaweed-based biopolymer has been studied by many researchers. Seaweed-based biopolymer films are good oxygen vapor barrier properties, biocompatible, and have low deformability. However, seaweed-based biopolymer film often possesses a relatively poor water barrier, mechanical, and antimicrobial features (Sudhakar et al., 2022).

Seaweed has been incorporated with a hydrophobic nanofiller to reduce its drawbacks for biopolymer film packaging applications. This combination of materials has been reported to improve seaweed film's functional properties (Jafarzadeh et al., 2020). *Kappaphycus alvarezii* is one type of red seaweed, which is fast-growing seaweed and a type of edible material found abundantly in Malaysia, mainly on the east coast of Sabah (Ali et al., 2018). It has been popularly cultivated in tropical areas for its hydrocolloids for 40 years, in particular carrageenan (Ariano et al., 2021). In the application for food packaging, a neat biopolymer film of *Kappaphycus alvarezii* matrix has poor physical and mechanical properties and is relatively brittle (Lomartire et al., 2022). To broaden the application of *Kappaphycus alvarezii*-based materials, the use of bionanofiller as a reinforcing and strengthening agent is necessary to improve the physical, mechanical, and antimicrobial properties (Lisha et al., 2022).

Black liquor is the liquid left over from the pulp mill's pulping operation. It is extremely complex, with high levels of dissolved solids including lignin residues, broken down carbohydrates, and inorganic components, as well as high levels of alkalinity and dissolved solids (Magdeldin & Järvinen, 2020). Although the highly-proposed soda pulping method uses simply sodium hydroxide as the pulping chemical (with or without anthraquinone as a catalyst), the discharge of this spent liquor into downstream without sufficient treatment would undoubtedly result in serious water pollution (Xu et al., 2020). This challenge should be resolved for the pulp and paper sector to progress in the future. Numerous research works have established that soda pulping is best suited for non-wood pulping, particularly oil palm EFB (Jahan et al., 2021).

Since degraded lignin from black liquor can cause problems for aquatic life and the biosphere, it suggested an appropriate approach to treat the black liquor before releasing it into downstream. The isolated lignin from black liquor is widely known to be employed as a filler, additive, or binder for reinforcing agents and adhesives such as biopolymeric filler, industrial binders, agricultural chemicals, concrete additives, and oil well drilling additives (Bajwa et al., 2019). This transforms the lignin-rich black liquor into value-added materials that will contribute to the development of the pulp and paper industry worldwide. Recovery of the black liquor will provide advantages to the pulp and paper sectors in addition to reducing effluent issues (Chen et al., 2023).

The isolation of lignin from industrial waste is a major contribution to waste valorization, particularly in the pulp and paper industry. Since lignin is hydrophobic in nature with thermoplastic behavior that molten when heated and solidifies upon cooling, the incorporation of LNPs in *Kappaphycus alvarezii* to improve its functional properties for food packaging applications is a novel challenge (de S. M. de Freitas et al., 2021). However, the challenge of low miscibility and compatibility with a natural matrix is still limited to be addressed (Isnard et al., 2022). In line with these drawbacks, poor interfacial lignin-matrix bonding induced by the low purity and poor methoxy groups of lignin hinder the compatibility of biopolymer films. Purification of lignin using organic solvents has the potential prospect to solve the challenge. In addition, recent studies have reported on the use of lignin in micro size as a reinforcement or filler in biopolymer matrix, such as starch (Aqlil et al., 2017), protein (Leskinen et al., 2017a), polylactic acid (Iglesias Montes et al., 2019), PHB (Kai et al., 2018), and bio-PA (Muthuraj et al., 2019). Nano-structured lignin as a functional nanofiller in *Kappaphycus alvarezii* is not previously reported in the literature.

In this study, LNPs were used as reinforcement material to improve the compatibility of *Kappaphycus alvarezii* matrix with the nanofiller. Its nanosize and large surface area was applied to increase the compatibility and strength of LNPs-matrix interaction. It has great thermal, various functional groups, and chemical compositions that can provide promising properties in biopolymer film production for food packaging applications (Ház et al., 2019). Therefore, LNPS was used in the present research. Its nanosize contributes to excellent dispersion, high specific surface area, and great distribution (Pang et al., 2020). Instead of using direct unpurified LNPs themselves, this study also used purified LNPs as reinforcement to determine the properties when compared to unpurified ones.

The present study aims to develop and characterize LNPs/*Kappaphycus alvarezii* biopolymer films. The red seaweed was incorporated with unpurified or purified LNPs from the black liquor of soda pulping waste, and their functional properties were characterized. Several techniques and analysis methods observed the physical, mechanical, morphological, structural, wettability, thermal, antimicrobial, and biodegradability properties of fabricated biopolymer films to determine the effect of the purification process of LNPs and different LNPs loadings in biopolymer films structure. The incorporation of LNPs in *Kappaphycus alvarezii* biopolymer film has not been reported in the literature. This study highlights the feasibility of using novel purified LNPs as a reinforcing nanomaterial, and versatile *Kappaphycus alvarezii* as the matrix to improve the functional properties and observe the potential application for food packaging purposes.

1.3 Objectives

This research aims to investigate the potential of LNPs and the organic solvent purification process of LNPs to be employed in excellent-performance *Kappaphycus alvarezii* seaweed biopolymer films. The specific objectives of this present research work are:

1. To evaluate the physico-chemical and thermal properties of *Kappaphycus alvarezii* for biopolymer film fabrication.
2. To determine the effect of the purification process on the lignin nanoparticles (morphological, physical, structural, and thermal properties).
3. To study the effect of different unpurified and purified lignin nanoparticles loadings on the physical, mechanical, morphological, structural, wettability, thermal, antimicrobial, and biodegradability properties of biopolymer films.

1.4 Thesis layout

This entire thesis has been organized into five corresponding chapters as exhibited in Figure 1.1.

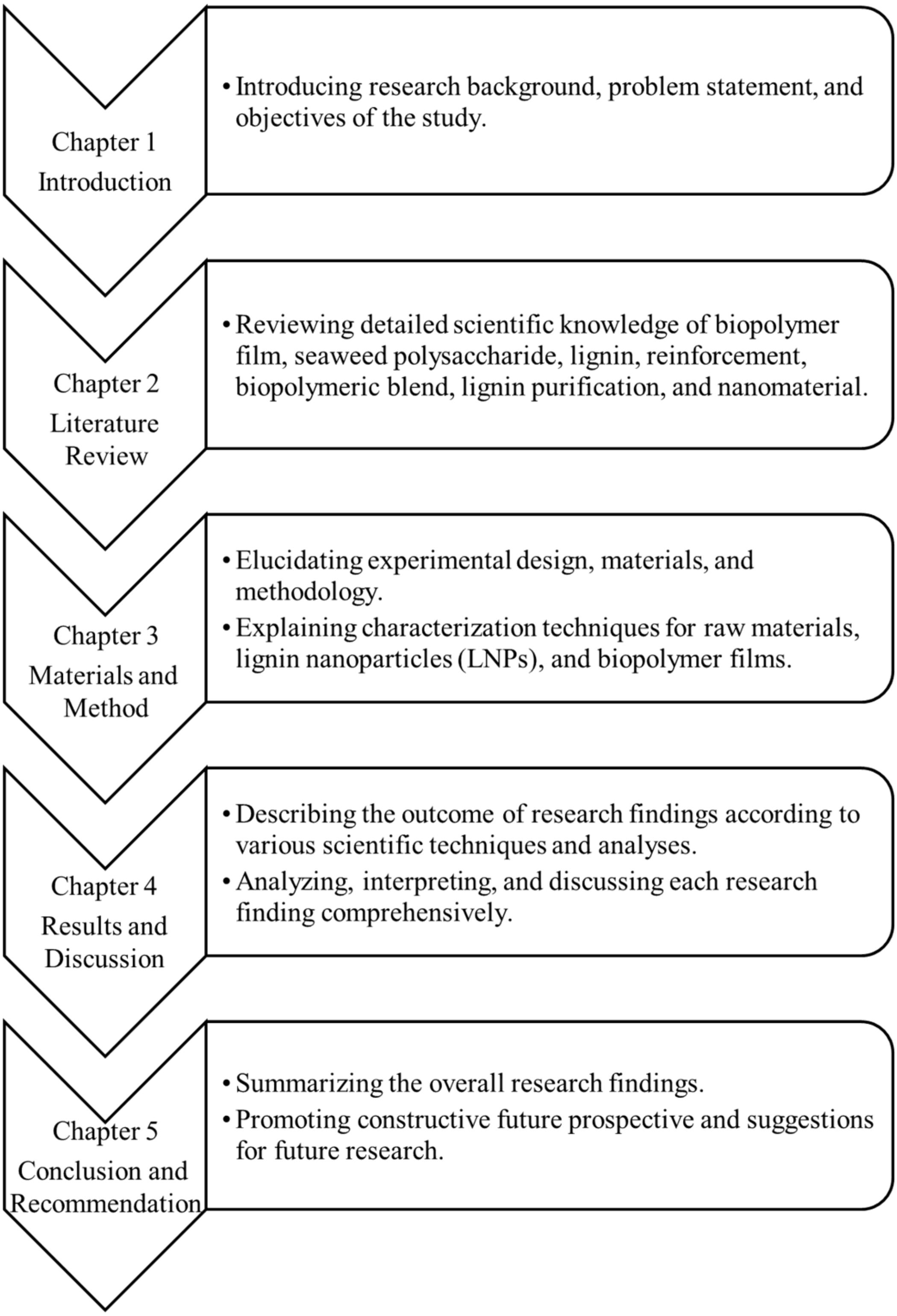

Figure 1.1 Thesis layout.

CHAPTER 2

LITERATURE REVIEW

2.1 Biopolymer film

Biopolymer film is an alternative to synthetic plastic film, which is made out of renewable resources, such as starch, protein, polysaccharide, and cellulose (Abdul Khalil et al., 2018b). This type of material usually consists of a biopolymer matrix (continuous phase) and filler (discontinuous phase) (Yaashikaa et al., 2022). Biopolymer is widely employed in the medical sector, where it is used primarily for drug delivery systems and surgical sutures, but it also has a high potential for utilization as food packaging material (Udayakumar et al., 2021).

Biopolymers can be divided into three major categories that depend on the origin and technique of production: directly isolated from bioresource, synthesized bio-derived monomers, and generated from microorganisms, as displayed in Figure 2.1 (Lisitsyn et al., 2021). Polysaccharides and proteins are the most potential biopolymers in the fabrication of packaging products (Priyadarshi et al., 2022). Throughout its shelf-life, the main function of food packaging is to cover and protect the food product from the surrounding environment, and to preserve the packaged food quality. Hence, biopolymer film acts as a barrier to protect against the migration of oxygen moisture, carbon dioxide, flavors, and aromas from adjacent food and the environment (Jafarzadeh et al., 2022).

Biopolymer film can degrade more facilely than non-renewable petroleum-derived polymer film. Most conventional plastic materials are non-recyclable and non-biodegradable and increase solid waste (Saalah et al., 2020). According to the USEPA, more than 14.5 million tons of plastic packaging and containers were generated in 2018, approximately 30% of which came from plastic bags, wraps, and sacks (Macena et al., 2021). Hence, now more than ever, the packaging and food industries are participating in efforts to decrease the number of food packaging and to utilize biodegradable materials as renewable, sustainable, and eco-friendly substitutes to petroleum-based polymers, both for environmental purposes and cost-effectiveness.

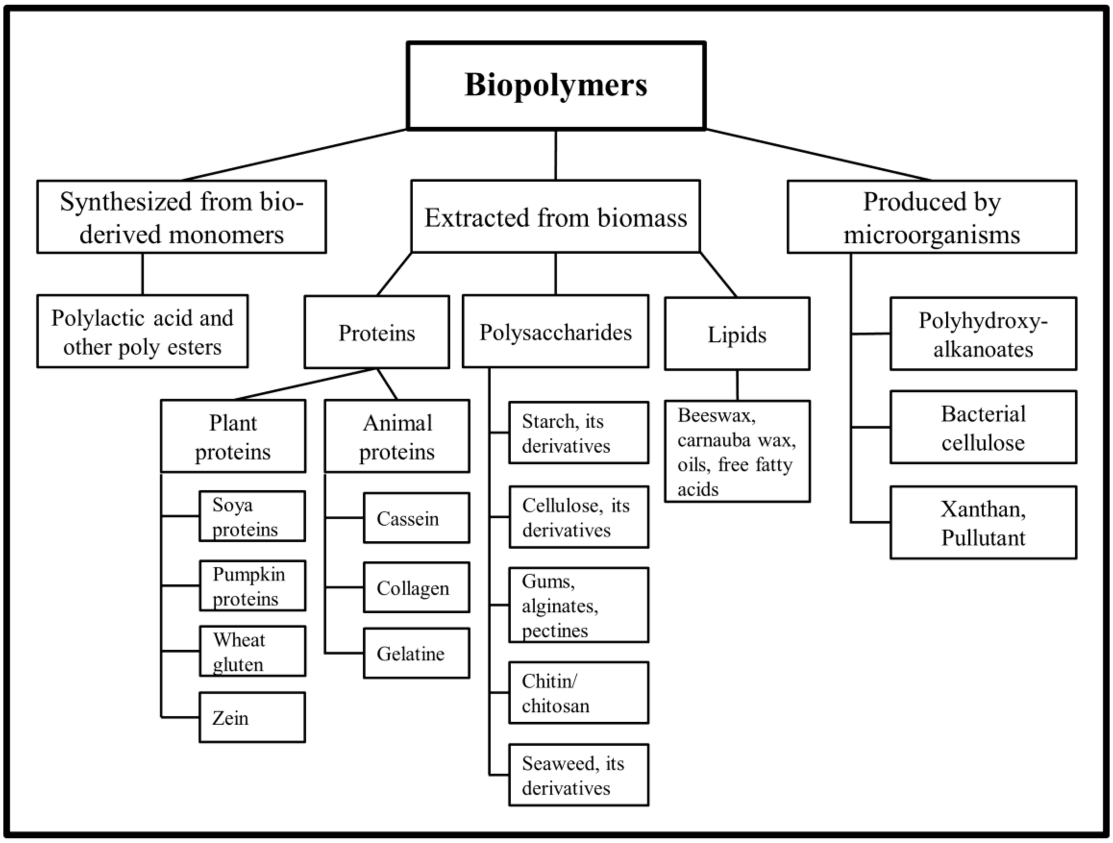

Figure 2.1 Schematic classifications of biopolymer types. Adapted from (Lisitsyn et al., 2021).

Besides its environmental merits, biopolymer film can also enhance the quality of food products. The role of biodegradable packaging in the protection of minimally processed vegetables and fruits by enhancing mechanical handling and endowing a barrier to the gases and moisture properties of food (Alfei et al., 2020). Also, Biopolymer film can be employed as a carrier agent for most additives. It can maintain the integrity of food during physical handling and curtail mechanical damage due to adequate strength and stress transfer properties while functioning as a conveyance for active compounds; antimicrobials and antioxidants (Castro-Rosas et al., 2016). The utilization of biopolymers in packaging and biodegradable films provides the utilization of bioresources which are frequently waste byproducts of the food system, contributing to decreasing pollution of solid waste. For food packaging to function, the functional properties of biopolymer film need to be fulfilled such as the physicomechanical, biochemical, absence of toxics, microbial stability, and safety (Lionetto & Esposito Corcione, 2021).

2.1.1 Biopolymer film from polysaccharide

Matrix plays a vital role as a continuous phase in biopolymer film. Recently, the polysaccharide-based matrix is popular since it has renewable and sustainable characteristics towards the environment features compared to the fossil-based polymer. Biopolymer films are naturally formed during the development cycles of all organisms (Yaashikaa et al., 2022). The two most popular polysaccharides are cellulose and starch which are extensively explored renewable biopolymers for many applications since they are low-price, abundant, sustainable, and environmentally-friendly (Muhammad et al., 2021). Carrageenan, agar, alginate, and chitosan are polysaccharides from the marine. They have been investigated in the production of biopolymer films for packaging applications due to their great film-forming behavior (Fathiraja et al., 2022).

Polysaccharide is a polymeric carbohydrate molecule that is composed of a long chain of monosaccharide molecules. It can be produced from plants, animals, bacteria, and fungi, and has various structures with unique and many biological features and functions (Zhang et al., 2022). Also, it is frequently one of the prime structural components in animal and plant exoskeletons (Malliappan et al., 2022). Its derivatives include fructose, glucose, mannose, and galactose which combined multiple molecules of sugar through glycosidic bonds (Du et al., 2022). Typically, this carbohydrate can be formed either branched or linear, consisting of amorphous, semi-crystalline, and merely one type of homopolysaccharides (homoglycans) or more than one type of heteropolysaccharides (heteroglycans) (Silva & Fabi, 2022). Starch and cellulose are examples of homopolysaccharides, while carrageenan, alginate, and agar are examples of heteropolysaccharides. The majority of polysaccharides perform their function in aqueous media since they are mostly water soluble and form colloid solutions (Yang et al., 2020b).

Since polysaccharide contains multiple hydroxyl groups (O–H), it has a high affinity towards water molecules (hydrophilic feature). This contributes to a stable hydrogen bonding formation among molecules of polysaccharide (Dong et al., 2023). Moreover, Gheribi et al. (2019) reported that polysaccharide possesses good film-forming, physical, gas barrier, and mechanical properties than other biopolymers; hence, the polysaccharide has been extensively employed for food packaging purposes. Polysaccharide exhibits excellent features including high rigidity and low deformability, biocompatible, edible, biodegradable, non-toxic, renewable, abundant, low-cost, immunogenic, non-carcinogenic, and non-antigenic (Eulálio et al., 2020). In comparison with other biopolymer materials like protein and lipids, the polysaccharide is more stable because it is not irreversibly denatured by heating (Mellinas et al., 2020).

With sundry characteristics of a high level of chirality, branched or linear structure, and high molecular weight, leads polysaccharide to a potential matrix for sustainable and eco-friendly production of biopolymer film (Xiao et al., 2022). However, pure biopolymer film suffers some drawbacks in thermal, moisture barrier, and mechanical properties comparatively with those conventional polymer films. This phenomenon hinders the application and functionality of the biopolymer film (Basumatary et al., 2020). Consequently, many attempts have been devoted to developing techniques to improve the functional properties of biopolymer films with the incorporation of filler into polysaccharide-based matrices (Ezati et al., 2022). Cunha & Grenha (2016) reported that the gelation of polysaccharide solutions can be developed by coil-to-helix conformational progression followed by the aggregation among ordered helices, as illustrated in Figure 2.2.

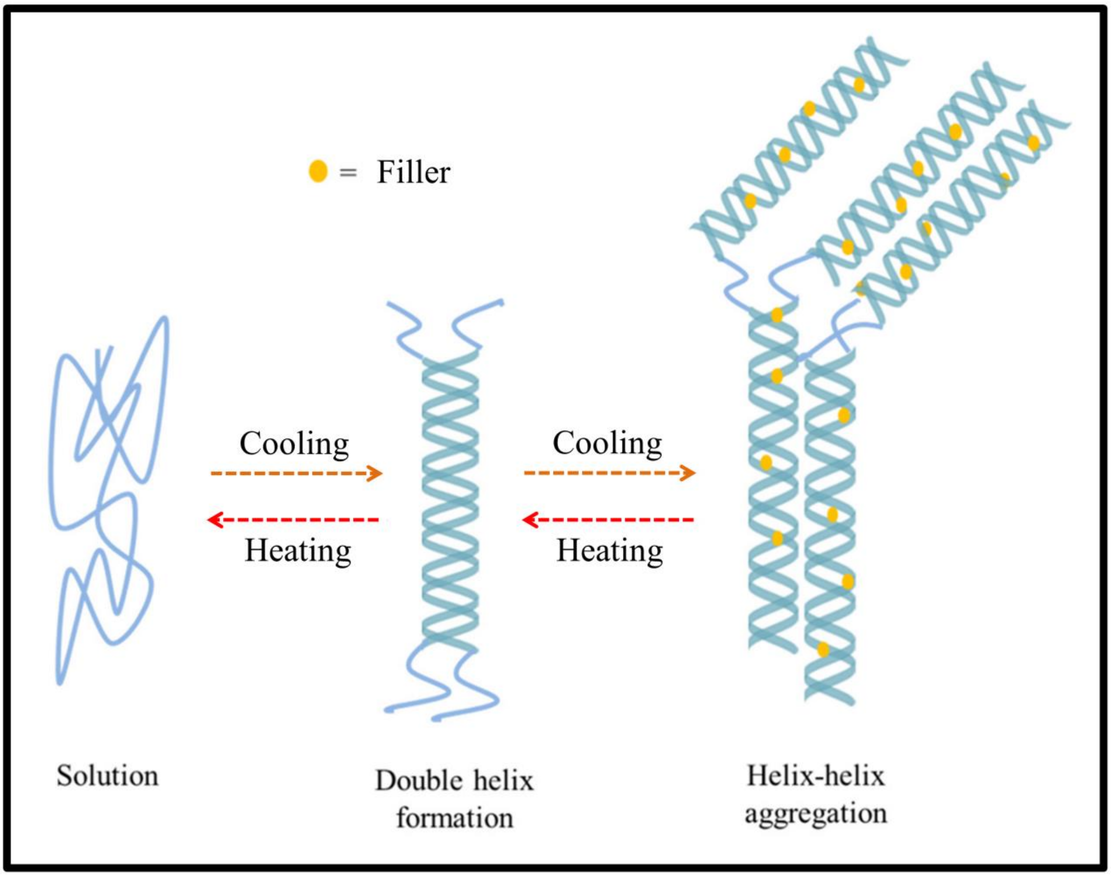

Figure 2.2 Mechanism of polysaccharide gel formation with the incorporation of filler. Adapted from Cunha & Grenha (2016).

2.2 Seaweed polysaccharide for fabrication of biopolymer film

Seaweed or also known as marine macroalgae is a plant-like organism, which commonly lives in saline water environments or marine. It grows by attaching to a hard substrate or rock in the coastal area, sub-tidal region, estuaries, and shallow marine water up to a depth where 0.01% of available photosynthetic light is present (Sarker et al., 2021). Seaweed can be easily grown in a natural setting, is widely available, adaptable to a variety of locales, affordable, and produces all year round. Seaweed-based materials have recently become a leading research material, especially for fabricating biopolymer composite films. It is globally used in a variety of applications, including those related to pharmaceuticals, food, agriculture, and other end-users (van den Burg et al., 2021).

According to the morphology and color of its pigments, the classification of seaweed includes red (Rhodophyceae), green (Chlorophyceae), and brown (Phaeophyceae) seaweeds. Red seaweed is exclusively found in the warmer ocean (tropical regions), green seaweed usually grows in fresh water (lake and river), marine sediments, ocean surface, and even in the terrestrial area, while brown seaweed can be found on rocky intertidal (Ross et al., 2022). Seaweeds are beneficial for human health as they contain large amounts of vitamins and minerals. They are frequently employed in herbal medicine and as a source of nourishment for humans (particularly red and brown seaweeds). Seaweeds can be consumed in pastries, soups, raw salads, dinners, and sauces since they are edible and packed with healthy nutrients.

Seaweed also contains carbohydrates, lipids, dietary fiber, and protein. Its high amount of carbohydrates has supported the industrial application of seaweed as a hydrocolloid (seaweed derivative) source. Table 2.1 lists the seaweed-derived hydrocolloids such as carrageenan, agar, and alginate which are used in the fields of biotechnology, medicine, microbiology, food technology, and bioplastic industry. When dispersed in water, these hydrocolloids are characterized by their ability to create gels and viscous dispersions that are determined as long chain hydrophilic polymers or polysaccharides. They are generally used to adjust the functional characteristics of aqueous solutions as gelling or thickening agents. In addition, seaweeds are well known for their sulfated polysaccharides, namely phycocolloids. They are crucial components of both the intercellular matrix and the cell walls. Hence, these sulfated polysaccharides are appealing in the application of biopolymer films because of their film-forming ability and good mechanical characteristics (Perera et al., 2021).

Table 2.1 The availability of polysaccharides in red, green, and brown seaweeds (Abdul Khalil et al., 2017b).

Polysaccharides	Red seaweed	Green seaweed	Brown seaweed
Carrageenan	√	–	–
Alginate	–	–	√
Agar	√	–	–
Cellulose	√	√	√
Floridean starch (α-1,4-bindingglucan)	√	–	–
Mannan	√	–	–
Fucoidan (sulfated fucose)	–	–	√
Mannitol	–	–	√
Laminarin(β-1, 3 glucan)	–	–	√
Sargassan	–	–	√
Porphyran	√	–	–
Sulfated galactans	√	√	–
Xylans	√	√	–
Sulfuric acid polysaccharides	–	√	–

2.2.1 Kappaphycus alvarezii

Kappaphycus alvarezii, also known as *Eucheuma cottonii*, is one of the red seaweed species (Rhodophyceae). The red seaweed can be found in lower intertidal to upper subtidal regions in Indonesia, Malaysia, Phillipines, Tanzania, Madagascar, Mexico, Kenya, Kiribati, Fiji, and Brazil (Kasim & Mustafa, 2017). It has been cultivated in more than 20 countries in the tropical zones for over 40 years primarily for commercial production and demand of hydrocolloids, namely carrageenan since it has low-cost maintenance and a high growth rate (Valderrama et al., 2015). Carrageenan, a hydrocolloid, mainly contains magnesium, niacin sodium, potassium, phosphorus, iron, and calcium salts of sulfated esters of galactose and 3,6-anhydro-galactose copolymers (Hentati et al., 2020). Three main forms of carrageenans are commercially employed; kappa (κ), iota (ι), and lambda (λ). They vary from each other in the degree of gelling solubility and sulfation characteristics (Mokhtari et al., 2021).

In Malaysia, *Kappaphycus alvarezii* has been initially cultivated since the year 1978 in Sabah, East Malaysia (Tan et al., 2022a). For the production of semi-refined carrageenan, two industries have been established in Tawau and Semporna, Sabah, Malaysia. Thenceforth, the red seaweed has been an industrially important bioresource in Malaysia since it contains mainly kappa-carrageenan and a small amount ($\leq 10\%$) of iota-carrageenan. At present, it is the most cultivated species in the country, especially on the east coast of Sabah (Tan et al., 2022b).

Besides its production to produce pudding, soup, and salad, it also serves as a potential bioresource due to its high growth rate, high efficiency in carbon dioxide (CO_2) capture, and high yield per area of cultivation (Guo et al., 2022a). *Kappaphycus alvarezii* is rich in proteins, bioactive substances, lipids, vitamins, minerals, and polyphenols possessing antiviral, antifungal, antimicrobial, and other pharmaceutical characteristics. Mandal et al. (2023) reported that the main chemical component in the red seaweed is kappa carrageenan of 74%. Hence it is mainly utilized for kappa-carrageenan extraction.

2.2.2 Physico-chemical properties of *Kappaphycus alvarezii*

The physical properties of raw *Kappaphycus alvarezii* are still paucity because the majority of previous studies are devoted to investigating the properties of the biopolymer film as the final product than the raw matrix material. Nevertheless, a study carried out by Jumaidin et al. (2017) found that the moisture content of *Kappaphycus alvarezii* was 1.13%. They revealed that the low moisture content was probably due to the initial heating of the red seaweed before the analysis.

In Malaysia, the visual appearance of *Kappaphycus alvarezii* is mostly shiny green to yellow range in color, as exhibited in Figure 2.3. It morphologically shows a bushy and spiny crop with numerous irregular smooth layer branches. Its cell wall consists of two layers, namely inner and outer cell walls. The former wall contains a fibrillar skeleton of sulfated polysaccharides, while the latter wall is an amorphous embedding matrix by phospholipid and cellulose fibers (Lisha et al., 2022). The polysaccharides can be extracted to isolate kappa-carrageenan through extraction technique.

In the chemical properties of raw *Kappaphycus alvarezii*, the chemical compositions include carbohydrate, ash, protein, lipid, minerals, and sulfated groups (Table 2.2). Carbohydrate is the main constituent in *Kappaphycus alvarezii* (dry weight) of 65.20%. The long-chain sulfated polysaccharides (galactons groups), cellulose, and hemicellulose contribute to a large carbohydrate fraction in the red seaweed, which make up the *Kappaphycus alvarezii*'s cell wall (Tan et al., 2020a).

Figure 2.3 Visual image of raw *Kappaphycus alvarezii*. Adapted from (Ali et al., 2014).

Although there have been reports of about 15 distinct forms of carrageenan, the three isomers of carrageenans that are most significant to the industry are the kappa, iota, and lambda carrageenans. The chemi cal structure of kappa-carrageenan is displayed in Figure 2.4. Kappa-carrageenan has firm and robust gelling behaviors, lambda-carrageenan has no gelling polysaccharide owing to the absence of helical formation, and iota-carrageenan has good elasticity features. The sulfate ester group of 3,6-anhydro-galactose unit affects the carrageenan properties (Boukhatem et al., 2021). However, *Kappaphycus alvarezii* contains a large proportion of kappa-carrageenan and a small fraction of iota-carrageenan.

Table 2.2 Chemical composition of *Kappaphycus alvarezii* (Abdul Khalil et al., 2018b).

Chemical compositions	Unit	Value
Carbohydrate	%	65.20
Ash	%	11.57
Protein	%	3.40
Lipid	%	1.10

Concerning the hydroxyl and sulfate groups, carrageenan dissolves in boiling water (Adam et al., 2020). Moreover, Firdayanti et al. (2023) reported that carrageenan is soluble at above 80 °C. It has a melting point between 50 and 70 °C and a gelling point between 30 and 50 °C. Its thermo-reversible qualities lead carrageenan to gel again as it cools down, despite liquefying when heated to the melting point (Cheng et al., 2022). Since it contains fewer sulfates groups and the existence of 3,6-anhydro-galactose unit, which is mostly found in the species of *Kappaphycus alvarezii*, kappa-carrageenan is less hydrophilic and soluble than the other three isomers.

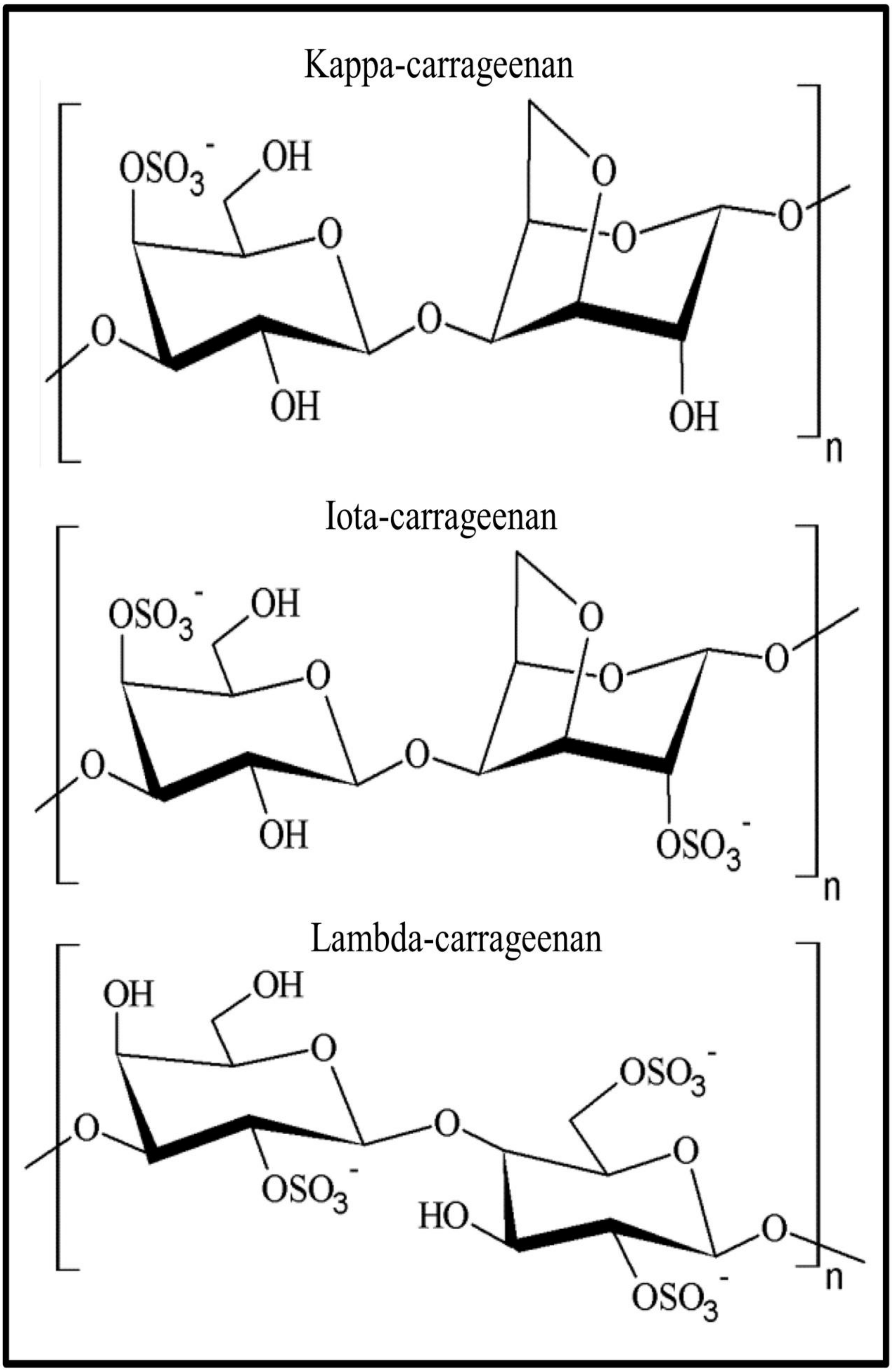

Figure 2.4 Chemical structure of kappa-carrageenan, iota-carrageenan, and lambda carrageenan. Adapted from Cunha & Grenha (2016).

Ash content is the second highest constituent in the red seaweed, which is 11.57%. The *Kappaphycus alvarezii* has a large number of macrominerals including magnesium (Mg), calcium (Ca), and sodium (Na) as well as some trace constituents like molybdenum (Mb), iron (Fe), copper (Cu), and zinc (Zn), contributing to the high proportion of ash content. The third largest component in the red seaweed is the protein of 3.40%, which enriches with many amino acids. The functional characteristics of protein such as gelation and solubility are pivotal to the ultimate properties of the film- and gel-formings potency (Momen et al., 2021). Meanwhile, lipid (1.10%) is the lowest percentage of the chemical composition of the red seaweed. This constituent usually is able to improve the water vapor permeability of biopolymer film (Karimi-Khorrami et al., 2022).

2.2.3 The potential application of *Kappaphycus alvarezii* as a matrix for biopolymer film

The potential of seaweed can be elaborated with three major functions; agriculture, pharmaceutical, and food (Lomartire et al., 2021). It has a promising foliar spray to reduce pesticides in agriculture, tissue engineering, drug delivery, and source of drugs used in cancer chemotherapy, and antioxidant in nutraceutical and functional food applications (Sugumaran et al., 2022). In recent decades, studies on the socio-economic, biodiversity, and application of seaweed keeps a progressive increase over terrestrial crops. Annual production of dry seaweed in Malaysia reaches 900,000 metric tons (Hussin & Khoso, 2021).

Seaweed and its derivatives have been investigated as potential precursors that offer many benefits since it is able to generate higher yields and easily cultivated than terrestrial plants. The notable advantage of seaweed is unnecessary to compete with plant crops since it does not require valuable land. Moreover, seaweed is not contaminated with fertilizers or chemicals that reduce the negative impact on the food