

**PREPARATION OF A STABILIZED NANO-
LIGNIN EMULSIFIER FROM OIL PALM EMPTY
FRUIT BUNCH**

SITI HAJAR BINTI SEKERI

UNIVERSITI SAINS MALAYSIA

2022

**PREPARATION OF A STABILIZED NANO-
LIGNIN EMULSIFIER FROM OIL PALM EMPTY
FRUIT BUNCH**

by

SITI HAJAR BINTI SEKERI

**Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science**

Januari 2022

ACKNOWLEDGEMENT

First and foremost, I would like to express my deep sense of gratitude full of blessings to Allah SWT for giving me this golden opportunity and give a hand for me endlessly to finish my journey in Master of Science (Chemistry). I am very honoured to show my gratitude to my main supervisor, Associate Professor Dr Mohamad Nasir Mohamad Ibrahim to help me pleasantly in numerous consultations, utmost guidance, advice, support, and the new knowledge and not forgotten to my co-supervisor Dr Muhammad Bisyrul Hafi Othman for his help throughout this research. The development of this thesis, "Preparation of a Stabilized Nano-Lignin Emulsifier from Oil Palm Empty Fruit Bunch", can finally be used and applied to all community network for a brighter and better future of this outstanding university.

Not to mention, I would like to express my appreciation to the School of Chemical Sciences, Universiti Sains Malaysia (USM), for the financial support of this study through providing the Universiti Sains Malaysia Grant research grant (1001/PKIMIA/8011070). In particular to my friend, Ms Fatin Silmi, my colleagues, Asim Ali and Taqi-uddeen, the science officers and laboratory assistance at the School of Chemical Sciences, and Science and Engineering Research Centre (SERC) for all of the splendid help and patience in order guide me to finish my research's study.

Last but not least, a special dedication to my parents, a tower of my strength, Mr Sekeri Ab Ghani and Mrs Halijah Daud for the countless support and duas. I would like to extend my uttermost appreciation to my siblings Mrs Siti Sarah, Mr Mohammad Faiz, and Ms Siti Sufiah for the tremendous love given along with the continuous support received.

TABLE OF CONTENTS

ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	vii
LIST OF FIGURES	ix
LIST OF SYMBOLS	xii
LIST OF ABBREVIATIONS	xiv
ABSTRAK	xv
ABSTRACT	xvii
CHAPTER 1 INTRODUCTION	1
1.1 Overview	1
1.2 Lignin as an emulsifier.....	1
1.3 Problem Statement	4
1.4 Research Objectives	5
1.5 Scope of Research	6
CHAPTER 2 LITERATURE REVIEW	8
2.1 Emulsion	8
2.2 Classifications of emulsifier.....	13
2.3 Sources of emulsifier.....	17
2.4 Lignin as a plant-based emulsifier	20
2.4.1 Lignin extracted from oil palm	25
2.5 Factors affecting the stability of the emulsion	28
2.5.1 Particle size	28
2.5.2 Concentration	37
2.5.3 Changes in pH.....	39

2.6	Kinetics of thermal degradation and shelf-life prediction of NSL as an emulsifier.....	42
2.6.1	Flynn-Wall-Ozawa (F-W-O) method.....	46
2.6.2	Kissinger method	46
2.6.3	Shelf-life prediction	47
CHAPTER 3	49
3.1	Overview	49
3.2	Materials.....	50
3.3	Extraction of lignin via soda pulping	52
3.4	Purification of soda lignin.....	54
3.5	Preparation of nano-sized lignin	55
3.6	Optimization of nano-sized lignin.....	56
3.6.1	Transmission electron microscopy (TEM)	56
3.6.2	Extreme high-resolution field emission scanning electron microscope (XHR-FESEM)	56
3.6.3	Zeta potential.....	56
3.7	Physicochemical characterizations of SL and optimized NSL series	57
3.7.1	Fourier Transform infrared (FT-IR) spectroscopy	57
3.7.2	Ultraviolet-visible (UV-Vis) spectra.....	57
3.7.3	Thermogravimetric analysis (TGA).....	58
3.7.4	Differential scanning calorimetry (DSC) analysis	58
3.8	Soda lignin and nano-sized lignin as emulsifiers.....	58
3.9	Preparation of emulsions.....	60
3.10	Characterizations of Newly Develop Emulsifiers.....	61
3.10.1	Interfacial tension	61
3.10.2	Cross-polarized microscopy	62
3.10.3	Rheological properties.....	62
3.11	Kinetics of thermal degradation	62

3.12	Toxicological study	63
CHAPTER 4 RESULTS.....		64
4.1	Overview	64
4.2	Optimization of NSL as an emulsifier	64
4.2.1	Transmission electron microscopy (TEM)	65
4.2.2	Extreme high-resolution field emission scanning electron microscope (XHR-FESEM)	68
4.2.3	Zeta potential analysis.....	70
4.3	Physicochemical characterizations of soda lignin.....	73
4.3.1	Fourier Transform infrared (FT-IR) spectroscopy	73
4.3.2	Ultraviolet-visible (UV-Vis) spectroscopy	75
4.3.3	Thermogravimetric analysis (TGA).....	77
4.3.4	Differential scanning calorimetry (DSC) analysis	79
4.4	Preliminary step in formulating the optimum condition of emulsion via.....	81
4.4.1	Effect of emulsifier dosage	82
4.4.2	Effect of water-oil-ratio (WOR).....	84
4.4.3	Effect of stirring intensity	86
4.4.4	Optimized emulsion condition	88
4.5	Characterizations of the stability of emulsion.....	91
4.5.1	Cross-polarized microscopy (CPM).....	91
4.5.2	Interfacial tension (IFT)	93
4.5.3	Rheological study.....	95
4.6	Kinetic of thermal degradation and shelf-life prediction	97
4.6.1	Flynn-Wall-Ozawa (F-W-O) method.....	100
4.6.2	Kissinger method	102
4.6.3	Shelf-life prediction	104
4.7	Toxicological study	107

CHAPTER 5	114
5.1 Conclusions	114
5.2 Future Recommendations	117
REFERENCES.....	118
APPENDICES	
LIST OF PUBLICATIONS AND PATENT	

LIST OF TABLES

	Page
Table 2.1	Summarized the type of emulsifier surfactants..... 16
Table 2.2	Lignin used as an emulsifier. 23
Table 2.3	Lignin extracted from different sources and used as an emulsifier 26
Table 2.4	Different chemical methods to produce NSL 32
Table 2.5	Different physical methods to produce NSL 35
Table 2.6	List of lignin with different concentration and their performances..... 38
Table 2.7	List of LCMs with pH modification and their performances..... 41
Table 2.8	Summarized the activation energy of lignin from the previous studies at varied method..... 45
Table 3.1	Chemical reagents and solvents used in research study 52
Table 3.2	The formation of NSL series at varied shear speed of homogenizer and stirring time. 55
Table 3.3	The detailed preparation of emulsifiers at various concentration..... 59
Table 3.4	Preliminary step in formulating the optimum condition of emulsion using Taguchi method..... 60
Table 3.5	Trial number based on the optimum condition of emulsion using Taguchi method 60
Table 4.1	The stability behaviour of NSL series at varied shear speed homogenizing and time stirring 72
Table 4.2	The information on glass transition temperature (T_g) and a maximum thermal decomposition temperature DTG_{max} of all samples 81
Table 4.3	The details of trial number based on the optimum condition of emulsion using Taguchi method..... 88
Table 4.4	Average effects of factor for each levels. 89
Table 4.5	Estimate of the optimum condition of design..... 89

Table 4.6	IFT value of SL, NSL 10, NSL 11, NSL 12, and CE.	94
Table 4.7	Result of TG traces of NSL 12 in nitrogen atmosphere.	99
Table 4.8	Activation energy of NSL 12 at conversion, $\alpha < 30$ using F-W-O method.	101
Table 4.9	Value of activation energy, E_a of NSL 12 using F-W-O and Kissinger method at varied conversions.	103
Table 4.10	Estimation values of shelf-life of NSL 12 based on mass loss of 5-20 % at various temperatures.	105
Table 4.11	Onset and reversibility toxicity test in administered animals (female rat) using NSL 12.	111
Table 4.12	Effect of acute (single dose) administration of the test sample on neurobehavioral activities.	112

LIST OF FIGURES

	Page
Figure 2.1	The type of emulsions 8
Figure 2.2	Gibbs free energy of emulsification produced (a) spontaneously (b) unspontaneous [38] 10
Figure 2.3	Type of instability of emulsion 12
Figure 2.4	Type of emulsifier surfactants 13
Figure 2.5	Proposed lignin structure. Reprinted (adapted) with permission from [97]. Copyright 2010 American Chemical Society 20
Figure 2.6	The major phenylpropanoid monomers and the most common inter-unit linkage of lignin 21
Figure 2.7	Biomass and products from oil palm tree 25
Figure 2.8	Surface tension toward the surfactant concentration 37
Figure 3.1	Research workflow of preparation of a stabilized NSL emulsifier from OPEFB 51
Figure 3.2	SL extraction process from OPEFB (a) OPEFB fibers, (b) extraction process via soda pulping, (c) formation of black liquor after filtration from the pulp, (d) precipitation process of lignin, (e) after centrifugation of precipitate lignin, and (f) dry lignin..... 53
Figure 3.3	Purification process of soda lignin via Soxhlet extraction 54
Figure 3.4	Steps of preparing the emulsifier 59
Figure 4.1	TEM micrographs of NSL series at different shear speed and stirring time of homogenizer 66
Figure 4.2	Histogram of the size distribution of NSL series at different shear speed and stirring time of homogenizer 67
Figure 4.3	The morphology of SL by SEM 68
Figure 4.4	SEM image of NSL series at different shear speed and stirring time of homogenizer..... 69
Figure 4.5	Zeta potential graphs of SL..... 70

Figure 4.6	Zeta potential curves of NSL series at different shear speed and stirring time of homogenizer.....	71
Figure 4.7	FT-IR spectrum of SL, NSL 10, NSL 11, and NSL 12	74
Figure 4.8	UV-vis spectra of SL, NSL 10, NSL 11, and NSL 12.....	76
Figure 4.9	(a) Thermogravimetric (TG) curves and (b) derivative thermogravimetric (DTG) curves of SL and NSL series	77
Figure 4.10	DSC curve of SL and NSL series	80
Figure 4.11	The percentage of creaming index of SL, NSL 10, NSL 11, and NSL 12 for 7 days (a) at dosage 1 g (b) at dosage 1.5 g (c) at dosage 2 g	83
Figure 4.12	The visual observation of emulsion at different WOR in (a) SL (b) NSL 10, (c) NSL 11, and (d) NSL 12.	85
Figure 4.13	The percentage of creaming index for 7 days storage of (a) SL, (b) NSL 10, (c) NSL 11, and (d) NSL 12.	87
Figure 4.14	Showing the visual observation of emulsion: (a) emulsions produced right after the process and (b) after 30 days.	90
Figure 4.15	CPM images of (a) SL, (b) NSL 10, (c) NSL 11, (d) NSL 12, and (e) CE	92
Figure 4.16	Pendant drop image of interfacial tension analysis for: (a) SL, (b) NSL 10, (c) NSL 12 and (d) CE.....	93
Figure 4.17	Rheograms of shear rate against viscosity with the different temperature at (a) 25°C, (b) 45°C, (c) 65 °C, and (d) 85 °C.....	96
Figure 4.18	(a) Thermogravimetric (TG) curves and (b) derivative thermogravimetric (DTG) curves of NSL 12 at different heating rate (β).	98
Figure 4.19	Graph of (a) $\log \beta$ versus $1000/T$ following the F-W-O method for NSL 12 with mass loss ranging from 10 % to 90 % of conversion ($\alpha \times 10^{-3}$) and (b) E_a obtained by using F-W-O method for NSL 12 with mass loss from 10 % to 90 % conversion in nitrogen.....	100
Figure 4.20	Graph of (a) $\ln (\beta/T_p^2)$ versus $1000/T$ following the Kissinger method of NSL12 with mass loss ranging from 10 % to 90 % of conversion ($\alpha \times 10^{-3}$) and (b) E_a obtained by using Kissinger method for NSL 12 with mass loss from 10 % to 90 % conversion in nitrogen.....	102

Figure 4.21	Shelf-life of NSL 12 (a) by month at temperature 25-100 °C and (b) by days at temperature 55-100 °C in nitrogen atmosphere	106
Figure 4.22	Cellular morphology and photomicrographic images of human endothelial cells (ea.hy926) of NSL12 at (a) low concentration and (b) high concentration.	108
Figure 4.23	Effect of the test sample on the proliferation of ea.hy926 cells.	109
Figure 4.24	(a) Effect of LNP12* on cellular viability of ea.hy926 cells, (b) Non-linear regression curve of the anti-proliferation effect of the sample to estimate its IC ₅₀ value.....	110

LIST OF SYMBOLS

α	Conversion
A	Pre-exponential factor (min^{-1})
β	Heating rate (K min^{-1})
$^{\circ}\text{C}$	Degree celsius
CI	Creaming index
C_p	Heat Capacity ($\text{J } ^{\circ}\text{C}^{-1}$)
$d\alpha/dt$	Rate decomposition of polymer at rate
E_a	Kinetic activation energy (kJ mol^{-1})
$f(\alpha)$	Differential expression of kinetic function
g	Gram
h	Hour
ΔH	Enthalpy
k	Constant rate
kg	Kilogram
L	Litre
μm	Micrometer
mL	Millilitre
mg	Milligram
mV	Millivolts
mN/m	MilliNewtons per meter
min	Minute
M_o	Initial mass of sample
M_t	Mass of sample at time
M_f	Final mass of sample

nm	Nanometer
R	Gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$)
RPM	Revolutions per minute
t	Time
T	Temperature
T_g	Glass transition temperature
T_m	Melting Temperature
v/v	Volume per volume
w/v	Weight per volume
Υ	Gamma

LIST OF ABBREVIATIONS

OPEFB	Oil palm empty fruit bunch
OPB	Oil palm biomass
O/W	Oil-in-water
W/O	Water-in-oil
SL	Soda lignin
NP	Nano-sized particle
NSL	Nano-sized lignin
HLB	Hydrophilic-lipophilic balance
FTIR	Fourier Transform infrared
F-W-O	Flynn-Wall-Ozawa
CE	Commercial emulsifier
CMC	Critical micelle concentration
CPM	Cross-polarized microscopy
DSC	Differential scanning calorimeter
DTG	Derivatized thermogravimetry
DDTG	Second derivate thermogravimetric
SEM	Scanning electron microscope analysis
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
IFT	Interfacial tension
UV-Vis	Ultraviolet-visible
XHR-FESEM	Extreme high resolution field emission scanning electron microscope

PENYEDIAAN PENGEMULSI NANO-LIGNIN YANG STABIL DARI TANDAN KOSONG BUAH KELAPA SAWIT

ABSTRAK

Dalam projek ini, suatu kajian dijalankan untuk menentukan keberkesanan lignin terekstrak daripada biomas kelapa sawit (*Elaeis guineensis*) sebagai sebagai agen pengemulsi air-dalam-minyak (W/O). Bagi mencapai tujuan ini, soda lignin (SL) telah diekstrakkan melalui proses pempulpaan soda dan satu siri zarah lignin dalam saiz nanometer (NSL) telah disediakan dengan menggunakan penghomogen pada tiga kelajuan ricih yang berbeza iaitu 10400 rpm (NSL 10), 11400 rpm (NSL 11) dan 12400 rpm (NSL 12) selama satu jam. Semua sampel yang tersedia dicirikan dengan teknik spektroskopi transformasi Fourier inframerah (FTIR), ultralembayung dan cahaya nampak (UV-Vis), analisis termogravimetri (TGA), kalorimetri pengimbasan berbeza (DSC), penganalisis keupayaan zeta, mikroskop elektron transmisi (TEM) dan mikroskop elektron pengimbas pelepasan medan resolusi sangat tinggi (XHR-FESEM). Hasil keputusan FT-IR menunjukkan bahawa tiada perubahan yang nyata berlaku dalam spektrum semua sampel selain itu kestabilan yang baik telah ditunjukkan pada lengkung TGA dan telah disokong oleh analisis DSC. Peratusan indeks krim dan pemerhatian visual daripada semua sampel menunjukkan NSL 12 pada dos 2 g paling stabil antara semua sampel untuk mengstabilkan pengemulsi W/O pada nisbah air-minyak 70:30 pada intensiti kacauan sebanyak 10400 rpm. Selain itu, hasil ketegangan antara muka (IFT) dan sifat reologi menunjukkan bahawa NSL 12 terbukti lebih stabil daripada produk komersial yang lain. Oleh itu, NSL 12 telah menjalani analisis selanjutnya untuk meramalkan jangka hayat menggunakan kinetik

degradasi termal. Kaedah Flynn-Wall-Ozawa (F-W-O) dan Kissinger telah digunakan dalam kinetika degradasi termal untuk menentukan tenaga pengaktifan (E_a) mendapati kedua-dua kaedah mematuhi antara satu sama lain. Nilai E_a NSL 12 telah menunjukkan kestabilan yang lebih baik dan jangka hayat yang lebih lama (sekitar 20 bulan) pada suhu di bawah 35 °C. NSL 12 juga telah terbukti selamat dalam kedua-dua kajian toksikologi, *in vitro* dan *in vivo* pada kepekatan di bawah 2000 mg kg⁻¹.

PREPARATION OF A STABILIZED NANO-LIGNIN EMULSIFIER FROM OIL PALM EMPTY FRUIT BUNCH

ABSTRACT

A study was carried out to determine the effectiveness of lignin, extracted from oil palm (*Elaeis guineensis*) biomass as a water-in-oil (W/O) emulsifier. To achieve this goal, soda lignin (SL) was extracted via soda pulping process and a series of nanosized soda lignin (NSL) was prepared using a homogenizer at three different speeds i.e., 10400 rpm (NSL 10), 11400 rpm (NSL 11), and 12400 rpm (NSL 12) for one hour. All prepared samples were characterized by Fourier Transform infrared spectroscopy (FT-IR), ultraviolet-visible (UV-Vis) spectroscopy, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), zeta potential analyzer, transmission electron microscope (TEM), and extreme high-resolution field emission scanning electron microscope (XHR-FESEM). The result of FT-IR showed that there is no prominent change occurred in spectra of all samples while good stability was reflected by TGA curves and supported by DSC analysis. The percentage of creaming index and visual observations of all samples demonstrated that NSL 12 at dosage 2 g was found to be the most stable among all samples to stabilized W/O emulsion at a ratio of 30:70 (WOR) with 10400 rpm of stirring intensity. Furthermore, the results of interfacial tension (IFT) and rheological properties indicated that NSL 12 was proven to be more stable than the commercial product. Therefore, NSL 12 was further analysed for shelf-life prediction using kinetic thermal degradation study. Flynn-Wall-Ozawa and Kissinger methods were used in kinetics of thermal degradation to determine the activation energy (E_a) and found that both methods are adhered to each

other. The E_a value of NSL 12 demonstrated better stability and longer shelf-life (around 20 months) at a temperature below 35 °C. NSL 12 showed to be safe both, *in vitro* and *in vivo* toxicological methods at a concentration below 2000 mg kg⁻¹.

CHAPTER 1

INTRODUCTION

1.1 Overview

Biomass is considered as the most logical carbon-based feedstock sources which could be obtained from living biological organisms such as plants, animals, and microorganisms [1]. Lignocellulose is the most common form of biomass [2] which are composed of 40–50% cellulose, 25–30% hemicellulose, 15–20% lignin, and traces of pectin, nitrogen compounds, and inorganic ingredients [3]. The research introduced in this dissertation is aiming of lignin to be used as an emulsifier where the characteristic of lignin amorphous polyphenolic compound is fitting to stabilize the emulsion. Reducing the lignin particle size to nanometer size will increase the stability of the emulsion where it gives a good-affinity to lower the surface and interfacial tension. The lignin in nanometer size was classified as nano-lignin and planned to be used as food emulsifier in the water-in-oil emulsion. This chapter contains background information, the goal of this research, problem statement, research objectives, scope of research study, and the contribution from this research. At the end of this chapter, the planning of this thesis and summary of each chapter are additionally added.

1.2 Lignin as an emulsifier

Generally, emulsion is made up of two immiscible liquids such as oil into water, which are dispersed into one and another [4]. There are two main emulsion types: the oil-in-water (O/W) emulsion and the water-in-oil (W/O) emulsion. The O/W emulsion is defined as the oil droplets dispersed and encapsulated into the water phase. Whereas the W/O emulsion system act completely opposite from O/W emulsion, where the water droplets are dispersed and encapsulated into the oil phase. The detail

of emulsions was discussed in Chapter 2, Section 2.1. In food industry, O/W emulsions are usually used in milk, mayonnaises, creamers, ice cream, etc, while W/O emulsions are applied in the production of butter, margarine, other fat-based spreads, etc [5]. This research mainly focuses on the development of W/O emulsion for food applications.

In an emulsion, stability is the main factor in measuring the performance of the emulsion. The difference between hydrophobic and hydrophilic content in oil and water is the force one needs to overcome to get a stable emulsion. In addition, the droplets tend to aggregate with their neighbour. The aggregated droplets that bound each other and create more than enough mass for gravity to influence the stability. The gravitational forces will separate emulsion at a certain time and a stable emulsion will take longer the time for the emulsion to separate [6]. Instability of emulsion occurs when the disperse phase leads to physical instability such as creaming, sedimentation, flocculation, or/and coalescence. There are many methods to improve and maintain the stability of emulsion and one of them is by using emulsifier. Emulsifier is a surface-active agent that works to reduce the attractive forces occurs between the oil and water the interface [7]. The application of emulsifiers has been used in industries as a foaming agent, wetting agent, dispersant, emulsifier, antistatic agent, etc [8].

A good emulsifier can adsorb the droplets of the disperse during the integration period. This adsorption protects the droplets from interacting with other droplets stopping the aggregation [9]. Theoretically, an emulsifier is known as amphiphilic, consist of hydrophilic and hydrophobic parts and has the ability to lower the interfacial tension between oil and water [5,10]. The emulsifiers are formulated either from natural sources (animal-based and plant-based) or synthetic chemicals. The protein from natural sources is extracted and then used as emulsifiers [11] while synthetic

emulsifier or/and stabilizing agents is made up from bio-based alkyls, petroleum-derived, fatty acid or fat [12]. Meanwhile, bio-based emulsifiers from biomass have been widely studied and drew interest by the industry due to the sustainable materials as raw materials for producing some high-value products [13-14]. Biomass is considered as the most logical carbon-based feedstock sources which could be obtained from living biological organisms such as plants, animals, and microorganisms [1].

The rapid growth of the oil palm plantation industry in Malaysia has resulted in abundant of empty bunches of oil palm (OPEFB). OPEFB biomass consist of cellulose, hemicellulose, and lignin. Lignin is a phenolic polymer present in large amounts in the cell wall of plants, especially in woody tissues and connects together with cellulose and hemicellulose. Lignin is insoluble in water and stable in nature. In contrast to other biopolymers, lignin is a network polymer that results from the dehydrogenative radical polymerisation of monolignols (e.g. *p*-coumaryl-, coniferyl- and sinapyl-alcohols), which are connected via carbon-carbon and ether linkages [15-16]. Strong reactive sites of phenolic (highly branched polyphenolic), aliphatic hydroxyl groups make lignin have a good affinity to polar and nonpolar phases [17]. The relatively of high hydrophobicity of lignin can enable it for acting as a emulsifier in food emulsions [18]. Including emulsifier, the functionality of lignin can be used to reduce the interfacial tension between oil-water interface [19-20]. The preparation of lignin in nanometer size (less than 100 nm) which is classified as nano-sized lignin (NSL) can provide a new approach for a high-value-added application of lignin-based products. Nanoparticles (NP) have a large surface area for a given volume and they often behave in a way different to micro-sized particles [21].

1.3 Problem Statement

In general, there are numerous kinds of synthetic and natural emulsifiers that can be utilized in the food industry, including proteins, polysaccharides, phospholipids, and surfactants. Many of food products are stabilized using emulsifiers where the sources come from natural-based (animals and plants) or synthetic-based. However, this industry has been interested since there is a demand to change emulsifiers from animal-based or synthetic to natural plant-based [11,22]. The consumer perceived fears (e.g. diseases), religious inhibitions (Muslim, Hindu etc) [23], and dietary and lifestyle awareness correlated with consuming synthetic-based and animal-based by-products [24]. Thus, the industry is approaching towards reformulating their products to replace synthetic or animal-based by products with more label-friendly natural alternatives which is plant-based products [25]. As veganism and natural product consumers increased, they are starting to replace animal-based and avoid synthetic ingredients in their meals and food products [26]. In particular, manufacturers often want to make new products entirely from natural ingredients so that they can make “all-natural” claims on their labels [27]. Therefore, recent interest in finding natural alternatives to animal-based or synthetic emulsifiers has since led researchers to focus on plant-based and bio-based emulsifier.

Plant-based emulsifier such as soy lecithin is predominantly used in food industry. In Malaysia, soy is not grown commercially and therefore lecithin has been imported and marketed at high prices. The rapid growth of the palm oil industry in Malaysia accumulated oil palm biomass (OPB) waste which generates up to 100 million tons per year. Yearly, around one million tons of fresh fruit bunches have been used in the production of palm oil and only produce 10 % of the oil while the remaining 90% is considered as biomass such as oil palm stems, fronds, leaves, palm kernel shell,

and empty fruit bunches (EFB) [28-29]. These OPB are naturally decompose on the ground and used for soil fertilization. Lignin of lignocellulosic biomass is the second most abundant natural polymer and found around 17-21% in OPEFB [30-31]. Lignin has high hydrophobicity and special affinity to polar and nonpolar phases which may enable to be used as emulsifier [18]. Therefore, this is the best approach to extract lignin from OPEFB and utilize as a bio-based emulsifier.

Recently, the interest in the use of particles in nanometer size or called as nano-sized particle (NP) to stabilize emulsions has gained much attention in research. Past studies reported that the approach methods that have been used to produce NP is through either chemical or physical (mechanical) methods [32-33]. The preferred method for this study is via physical method which is a simple method and less chemical consumptions. NP have been reported to have a better tendency to adsorb at the interface and reduce surface tension of emulsion [34]. However, emulsion stability can be achieved if the NP are stable enough to be used as emulsifiers. The instability of emulsion can affect the food quality, shorten the food shelf-life and will become a toxic [35]. Thus, the preparation of a stabilized NP is indeed important to improve the emulsifier performance and emulsion stability.

1.4 Research Objectives

The present study the properties of nano-sized lignin as emulsifier to stabilize W/O emulsion derived from OPEFB biomass. Therefore, research objectives are presented as below:

1. To mechanically prepare nano-sized lignin from OPEFB and investigate the performance of nano-sized lignin using complementary analyses to study stability of emulsifier on emulsion.

2. To determine the thermal stability of the optimized nano-sized lignin as emulsifier using non-isothermal kinetic degradation
3. To analyze the toxicological properties of optimized nano-lignin as food emulsifier via *in vitro* and *in vivo*.

1.5 Scope of Research

The scope of this study is divided into four stages in order to fulfil the aim of the research objectives. The first stage of this study is to prepare a series of nano-sized lignin (NSL) from the OPEFB by using a homogenizer at different speeds. Three types of NSL are prepared and the particles size are checked via TEM. The second stage of this study is identifying and comparing the zeta potential values between soda lignin (SL) and a series of NSLs. Further, the most optimum dosage of NSL is chosen and applied in the W/O emulsion as the emulsifier. A comparison study on zeta potential values and the dosage that affected the W/O emulsion performance is presented. The third stage of this study is to produce the ideal ratio of water and oil with NSL as the emulsifier to obtain the most stable emulsion. The creaming and separation of emulsion are tested and observed by measuring the creaming index percentage (CI %). The most stable ratio is chosen for further characterizations steps. The fourth stage of this study is to investigate the toxicity of the NSL so that it is suitable to be applied in the food industry. Detail explanation of the scopes study are as follow:

Stage 1:

- To produce NSLs by using a homogenizer at different speeds.
- To characterize the prepared NSLs using FT-IR, UV-Vis, TEM, SEM, TGA and DSC

- To investigate the stability of NSL in the suspension by using zeta potential technique.

Stage 2

- To identify the critical micelle concentration (CMC) affected on dosage of NSLs by applying it into W/O emulsion.
- To correlate and observe the applied dosage of NSLs on emulsion via visual observation and measurement of creaming index.

Stage 3

- To determine the ratio of water and oil that can produce a stable emulsion.
- To examine the morphology, surface tension and interfacial tension behaviour of SL, selected NSL and commercial emulsifier.
- To investigate the rheological properties of emulsions at different temperatures.

Stage 4

- To interpret TGA data and analyse the kinetic thermodynamic stability of emulsion.
- To predict the NSL shelf-life at various temperatures.
- To examine the toxicity of the NSL both *in vitro* and *in vivo* modes.
- To identify the safe dosage of NSL to apply in emulsion.

CHAPTER 2

LITERATURE REVIEW

2.1 Emulsion

Emulsions can be labelled based on the dispersed phase. The type of emulsions can be categories either simple emulsions or complex emulsions as presented in Figure 2.1. The simple emulsions consists of W/O and O/W where W/O occurs when water is in the dispersed phase, while the O/W comprises oil as the dispersed phase. Generally, a dispersed phase is a suspended phase that contributes to a smaller volume, percentage-wise, than the other phase. For example, a W/O type of emulsion is a small volume of water droplets scattered inside a much bigger volume of oil. Multiple emulsions or called double emulsion are known as complex emulsions comprised of tiny droplets dispersed in a larger droplet while suspended in another phase. It can be O/W/O or W/O/W and the primary application is to prolong the release of hydrophilic drugs [36].

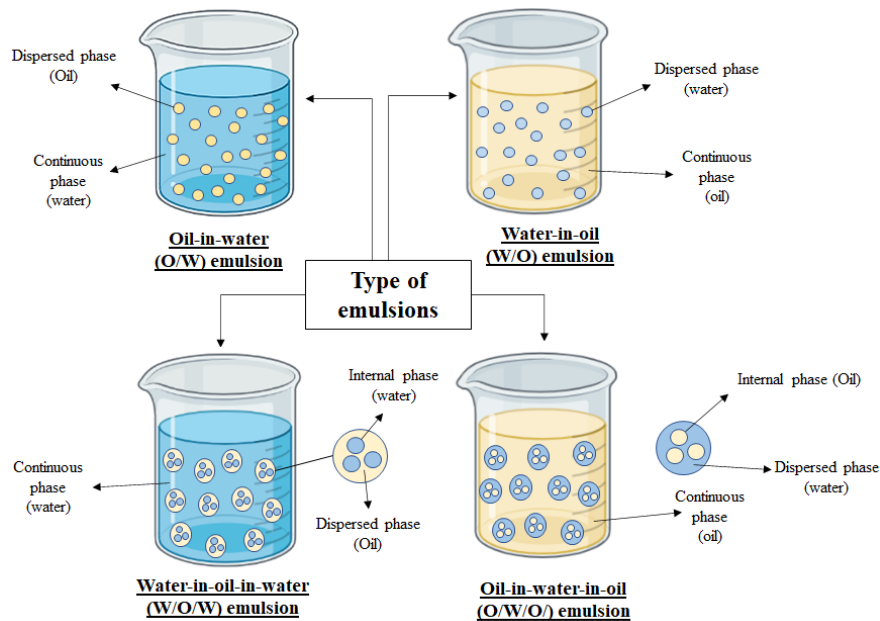
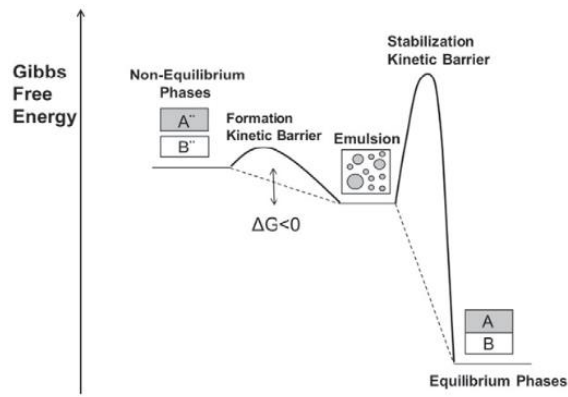


Figure 2.1 The type of emulsions

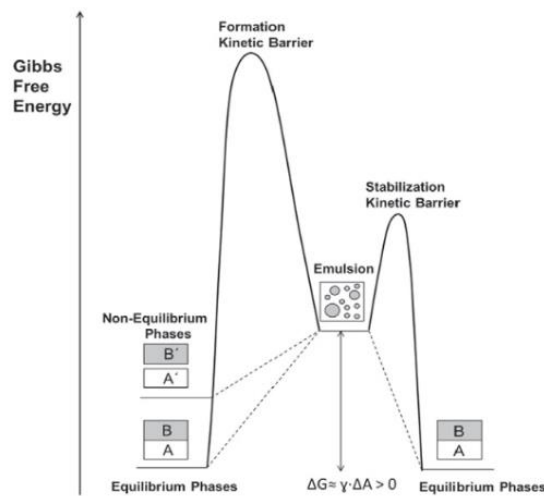
The boundary that connecting the two phases is called as an interface. These natural forces make it hard to break the droplets of the dispersed phase into much smaller droplets, thus separating the two phases. The accumulation of these forces that separate the two phases is known as interfacial tension. The emulsion stability can be achieved once the interfacial tension is reduced and can be accomplished by using an emulsifier. Emulsifiers work by allowing the dispersed phase droplets to produce small droplets and prevent them from coalescence [9]. Since the emulsifier is built with hydrophobic and hydrophilic, it will align itself so that both are embedded in their appropriate phase. Emulsions are generally obtained by dispersing one fluid into another immiscible fluid via high-energy shearing (i.e., hand shaking, stirring, vortexing, high-shear mixing, homogenizing, or ultrasonication). The emulsification process follows the Gibbs free energy change equation Eq. (2.1) [37], as below:

$$\Delta G = \Delta H - T\Delta S \quad \text{Eq. (2.1)}$$

Where ΔG is the free energy change, ΔH is the enthalpy energy, ΔS is the conformational entropy change, and T is the temperature. In an emulsion, the term $T\Delta S$ represents the entropy that correlates with the dispersion of the droplets. More droplets that are dispersed will create a positive value. Since ΔH embodies the energy required to expand the interface during the emulsification, and lower ΔH value indicates more emulsion stability. Then a value of $T\Delta S$ that exceeds the ΔH means that the emulsification process is spontaneous (Figure 2.2 (a)) since $\Delta G < 0$ ($\Delta H < T\Delta S$). The fact is that the ΔG often positive indicates that the emulsification process is rarely spontaneous, as indicated in Figure 2.2 (b). Hence the requirement of energy is enough to achieve emulsion stability.



(a)



(b)

Figure 2.2 Gibbs free energy of emulsification produced (a) spontaneously (b) unspontaneous [38]

In emulsion technologies, stability is the key in any application [39]. As oil and water are generally un-mixable, an emulsifier additive was added to help the two phases mix by establishing a barrier that protects the dispersed phase from separating. Generally, the function of emulsifiers should: (a) rapidly adsorb to the oil droplet surfaces during homogenization to reduce the interfacial tension and facilitate droplet disruption; (b) form a stable protective layer around the oil droplets that prevents them from aggregating during manufacture, transport, and storage; (c) easy to use, and available in a reliable quantity and quality; and (d) be economically viable [40]. It should be noted that the stabilization against demulsification can be accomplished by

including a very polar emulsifier for either W/O or O/W emulsions. An emulsifier will adsorb the interfacial tension between the two phases as the concentration of the emulsifier influence the value of interfacial tension based on the Gibbs isotherm shown in Eq. (2.2) below:

$$\Gamma = -\frac{1}{RT} \frac{\partial \gamma}{\partial \ln c} \quad \text{Eq. (2.2)}$$

The Γ is the excess interfacial concentration of emulsifier (mmol m^{-2}) measured from the interfacial tension, γ (mN m^{-1}) against emulsifier concentration, C . Thus, increasing the emulsifier concentration will also increase the surface pressure.

An emulsifier that produces high stability has excellent value in the open market. The efficiency of the emulsifiers plays a big part in adsorbing kinetics of the droplet generation by reducing the interfacial tensions of the surface, thus stabilized the emulsion process [41]. The polar and non-polar parts of the emulsifier reside in the aqueous phase and organic phase, respectively [42]. The surface of suspended droplet solution must be adsorbed immediately by the emulsifier to prevent the instability of emulsion e.g., coalescing droplets with another droplets. There are a few types of instability phenomena that occur in emulsion systems such as sedimentation, flocculation, coalescence, and Ostwald ripening, as shown in Figure 2.3

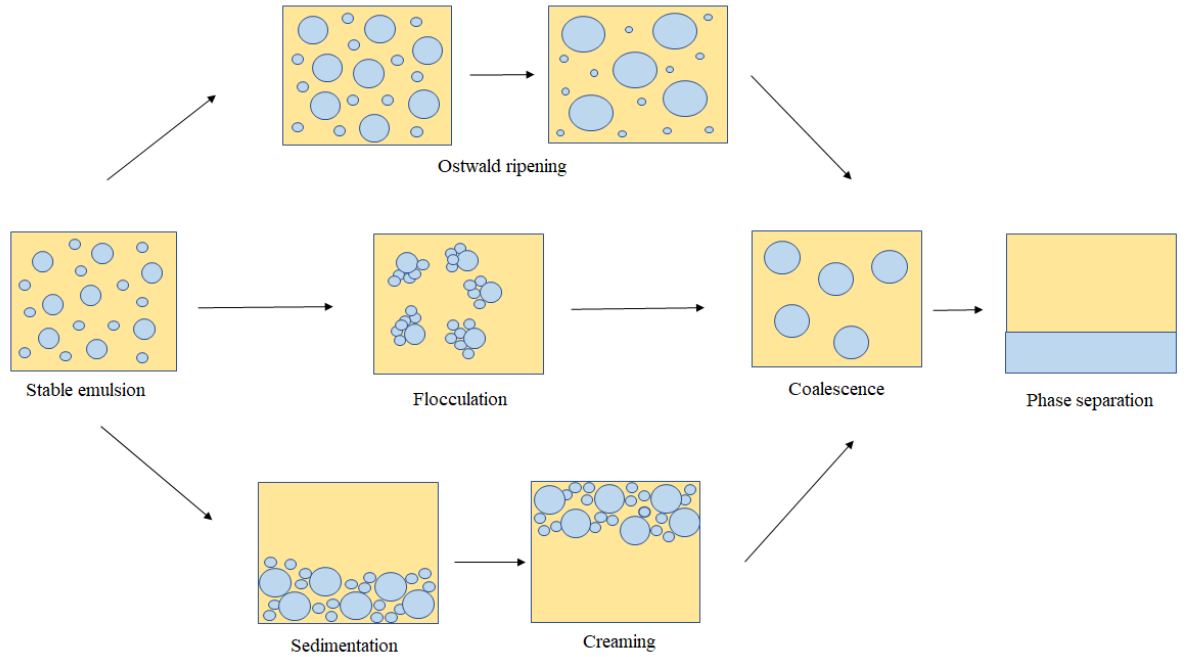


Figure 2.3 Type of instability of emulsion

In emulsification process, Van Der Waals forces (attractive forces) and electrostatic and steric forces (repulsive force) are involved during the collisions of particle droplets. The flocculation of emulsion instability occurs when the small droplets have a weak Van Der Waals force to repulse between them, hence the attractive force will take place and merged small droplets into larger droplets [43-44]. The Ostwald ripening in emulsion instability is connected to the movement and changes in small droplet size, wherein the small droplets will disperse and be deposited into larger droplets to turn into large bulk droplets [44]. Meanwhile, the creaming and sedimentation of emulsions is correlated to the gravitational forces and densities of oil and water droplets. It formed when the gravitational forces of the droplets are exceeding the Brownian motion and will lead the movement of larger droplets either upward or downward, depending on the type of emulsion, respectively [44].

2.2 Classifications of emulsifier

Generally, emulsifier surfactant is known as amphiphilic whereas it consists of hydrophilic (polar) and hydrophobic (non-polar) [10]. Ideally, the polar part consists of heteroatoms i.e., nitrogen, oxygen, or sulfur, while for nonpolar group contains alicyclic, aliphatic, or aromatic hydrocarbons [45]. Berton Carabin and Schroen reported that proteins mostly stabilize the amphiphilic from biopolymers as food emulsifiers [46]. There are four types of emulsifier surfactant that work to stabilize the emulsion system: 1) emulsifier with charge in the aqueous system (e.g., anions and cations), 2) solubility, 3) hydrophilic and lipophilic balance, and 4) colloidal particle. Figure 2.4 shows a type of surfactant consisting of various hydrophobic and hydrophilic charges that function individually when in an aqueous system, which are anionic, cationic, non-ionic, and amphoteric surfactant.

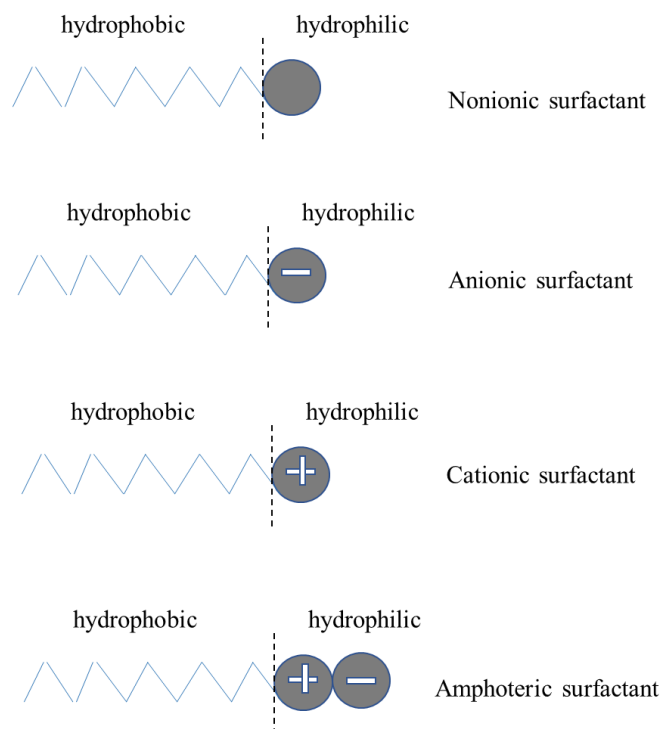


Figure 2.4 Type of emulsifier surfactants

Anionic surfactant is association of anions molecule (carries a negative charge) or hydrophilic group in aqueous solutions with a small positive counterion [47]. The low cost of anionic surfactants such as carboxylates, sulfonated, sulfated, and phosphates have been widely used in cosmetic, personal care products, food, also oil and gas industry [48]. In contrast to anionic surfactant, cationic surfactant carries a positive charge and rarely been used due to its high production cost [47]. Non-ionic surfactant consists of non-ionic charge of a hydrophilic head and undissociated with any ions in an aqueous solution. The common hydrophilic of non-ionic surfactants are lecithin, monoglyceride of long-chain fatty acid, polyoxyethylenated alkylphenol, and polyoxyethylenated alcohol [49]. In reverse to non-ionic surfactant, amphoteric or zwitterionic surfactant have both anions and cations of hydrophilic group in the surface-active portion e.g. sulfobetaine and amino acid [47].

Emulsion stabilized by balancing the index value of hydrophilic and lipophilic groups is called as hydrophilic-lipophilic balance (HLB). Index value is calculated based on the ratio of the weight percentage of molecule hydrophilic to the hydrophobic and ranges from 0-20 [50]. The index values is depending on the molecular structure of the emulsifiers or the experimental and observation of emulsions [51]. The low HLB value indicates W/O emulsion as it is more soluble in oil, while the high HLB value is referring to O/W emulsion as it is attracted to water (water-soluble) [52]. Additionally, the preferable HBL values for W/O emulsions are in the range 3-6 while for O/W emulsions are around 8-18. In food application (as food humectant or wetting agent), the most desirable of HBL values are between 7-9 [53]. Propylene glycol monostearate (PGMS), glycerol esters, polyglycerol esters, propylene glycol fatty acid esters are the example of emulsifier resulted in good value of HBL for O/W emulsions whereas

lecithin, proteins, hydrocolloids, phospholipids are emulsifier for W/O emulsion [51,54].

The colloidal particle is related to the Pickering emulsion where it physically stabilized by solid colloidal particles locating at oil-water interface [55]. The particles are able to irreversibly attach to the oil-water interface which more efficient stabilization than surfactant adsorption and requires high energy for adsorption process [46]. Despite from that, Pickering emulsion only required low dosage of emulsifiers to exhibit long-term stability [56]. According to the previous study, solid particles stabilized emulsion by forming a dense layer between the water-oil interface and the water-oil droplets are coated with emulsifier to prevent from coalescence and flocculation [57].

The mechanism of emulsifier works on stabilizing the O/W emulsion by the strong interaction of the emulsifier with water [51]. The emulsifier will form micelle via self-assembly to absorb the interfaces between oil and water in emulsion [47]. As a result, the free energy of the emulsion system is lowered as well as the surface tension between oil and water [46]. Starch granules, chitin nanocrystals, solid lipid nanoparticles, whey protein microgel particles, flavonoid particles, cellulose, and lignin are the example of natural-based Pickering emulsifiers that have been used in a wide range of fields such as biomedicine, food, fine chemical synthesis, cosmetics [58-59]. Table 2.1 summarized the emulsifiers from all categories that mentioned above.

Table 2.1 Summarized the type of emulsifier surfactants

Classification of emulsifier	Description	Advantages	Disadvantages
Charge in the aqueous system	Emulsifiers with charges of the hydrophilic group (positive or negative charge)	<ul style="list-style-type: none"> • Have better stability, stable at high temperature [48,60,61]. 	<ul style="list-style-type: none"> • Non-ionic surfactants less toxic than ionic surfactants [62]. • Cationic surfactant cannot apply as food additives due to toxicity issues [60]. • Zwitterionic surfactants require high cost of producing [60].
Solubility	Depending on the preferential of emulsifier solubility (in continuous phase)	<ul style="list-style-type: none"> • Good in aggregations and associations of the hydrophilic groups (form micelles) [63]. 	<ul style="list-style-type: none"> • Inconsistency of stability (depending on chain length and branching) [64]. • Only selected amphiphilic can promote longer hydrocarbon chain [49].
Hydrophilic and lipophilic balance (HLB)	Depending on index value of hydrophilic and lipophilic balance (HLB)	<ul style="list-style-type: none"> • Type of surfactant assigned based on HLB index value [60]. • Wide applied in the industries [60]. 	<ul style="list-style-type: none"> • Differences chemical groups may have the same HLB number but different wetting characteristics [60] • the actual size of the hydrophilic or lipophilic group hard be measured [65].

Pickering Emulsion	Stabilized emulsion by solid colloidal particles	<ul style="list-style-type: none"> • High resistance to coalescence and easy to control coagulation [66]. • High ability to disperse high-discontinuous-phase [67]. • Environment-friendly, recyclable, and ultra-stability [68]. 	<ul style="list-style-type: none"> • Consist of large emulsion droplets that can promote to creaming or sedimentation [46]. • The stability of emulsion decreased at low concentration of solid particles [69].
--------------------	--	--	---

2.3 Sources of emulsifier

In food industries, emulsifier is used to improve the quality and sensory of food including the flavour, texture, and mouthfeel [70]. Usually, the emulsifiers is made up from either natural sources (animal-based and plant-based) or synthetic. In animal-based emulsifier, protein from such as casein, porcine, fish, chicken, or other insects is extracted and used as a food stabilizer of emulsifier [71]. These animals' protein can be found from their organs include muscles, eggs, milk, silk proteins and others. Tissues from their meat proteins contain proteins that can be used to assemble protein-based encapsulation and delivery systems to stabilize the emulsion [72]. Also, the amino acids from the protein can act as emulsifiers where they attached to the polar (water molecules) and non-polar (oil/fat) and mingle in a continuous system.

Egg protein is one of common food emulsifier. Egg lecithin is a good emulsifier and has been widely used in food such as mayonnaise, pastries, salad dressing, and others [50]. Anton et al. reported that the egg yolk consist of natural supramolecular assemblies of lipids and proteins where the interactions between proteins and phospholipids emulsified the emulsion [73]. Ovalbumin from egg white

also can be used as emulsifier where its contains both of hydrophobic and hydrophilic groups to stabilize the emulsion [74].

Synthetic emulsifiers begin to be used in the second half of the twentieth century before widely used in industry [75]. Synthetic emulsifier surfactant is made up from bio-based alkyls or petroleum-derived or polyol and fatty acid or fat [12]. Tween (polysorbate), Span (sorbitan), sucrose esters, fatty alcohol ethoylates are the example of common synthetic emulsifiers that usually used in industries [24]. Recent studies were nominated that Tween 80 [24] is the most stable emulsifier compared to lecithin [76], and it has been used in several studies as a food emulsifier [77-78]. Meanwhile, other synthetic emulsifiers such as mono and di-glycerides are made from chemical formulation by the blending edible oil and glycerine. As a result, synthetic emulsifier derives from fatty acid i.e., propylene glycol ester, polysorbates, polyglycerol esters, sucrose esters, sorbitan esters, and stearyl lactylates. However, the usage of synthetic emulsifier especially in food industry can cause a long-term effects due to slow degradation process [79-80]. Partridge et al. reported that the synthetic food emulsifier can contribute to health problems, such as metabolic syndrome, gut problems, diabetes, obesity, and bowel diseases [81].

Plant-based emulsifier can be obtained from a variety of sources or their waste products e.g., soybean and sunflowers, gum Arabic, peas, coconuts, zein (corn), cellulose and lignin (plant biomass), etc [82-83]. Plant proteins in their entirety or as partial substitutes have been employed in various food formulations. Lecithin from soybean is widely used in food emulsion. Its surface activity and structure formation, make them suitable to act as emulsifier. Lecithin from soy protein has been used as Pickering emulsion and its properties has successfully improved the emulsion stability

[84]. However, the soy protein must be carefully extracted and purified to prevent excessive protein denaturation and aggregation which lead to loss of functionality [72].

The pea protein has well-balanced of amino acid and has been extracted by Zhang et al. [85], Burger et al. [11], and Kutzil et al. [86] as an alternative emulsifier to soy proteins to stabilize emulsion. However, the stabilization of emulsion using pea protein is still low compared to soy protein due to its less solubility and weak electrostatic repulsion and structural flexibility [86]. Gum arabic is natural polysaccharide-based emulsifier in the food industry [87], particularly in beverage emulsions [88]. However, the major disadvantage of gum arabic is that a relatively high emulsifier-to-oil ratio is required to form stable emulsions [89-90]. Other studies have shown that polysaccharides isolated from basil seed were good emulsifiers, with the surface activity being attributed to the presence of protein moieties and non-polar groups on the carbohydrate backbone [91]. Corn fiber (zein) has also been shown to have good emulsifying properties also attributed to the presence of protein moieties [92].

Commonly, biomass cover all agricultural waste from the chopped tree trunks, thrown-away husks, and the seeds to even the waste generated from animals, such as manure, shells, and decay bodies [93]. Apart from agriculture waste, waste generated from forest industries such as wood processing, pulp industries, and paper mills contributes significantly to the abundance of lignocellulosic biomass in the world. The studies on naturally-occurring polymeric surfactants from biomass have been centered on polysaccharides derivatives [94]. The various polymer types and the characteristic property profiles distinguishing polymeric surfactants have been discussed recently. Polymers seem attractive in many fields because of their good performance in lowering

surface tension. Despite the fact that lignins from black liquors don't have the chemical structure of conventional amphiphilic molecules, they exhibit surface activity [95]. It is hypothesized that lignin can act as emulsifier where it adsorbs at the oil-water interfaces forming a condensed, viscoelastic surface or interfacial film.

2.4 Lignin as a plant-based emulsifier

Lignin is a complex chemical structure consists of high molecular weight, polydispersity, and heterogeneous material due to the breaking process of its intermolecular bond [96]. Lignins as presented in Figure 2.5 contain several functional chemical groups, such as hydroxyl (phenolic or alcoholic), methoxyl, carbonyl and carboxyl, in various amounts, depending on origin and the applied isolation process [34].

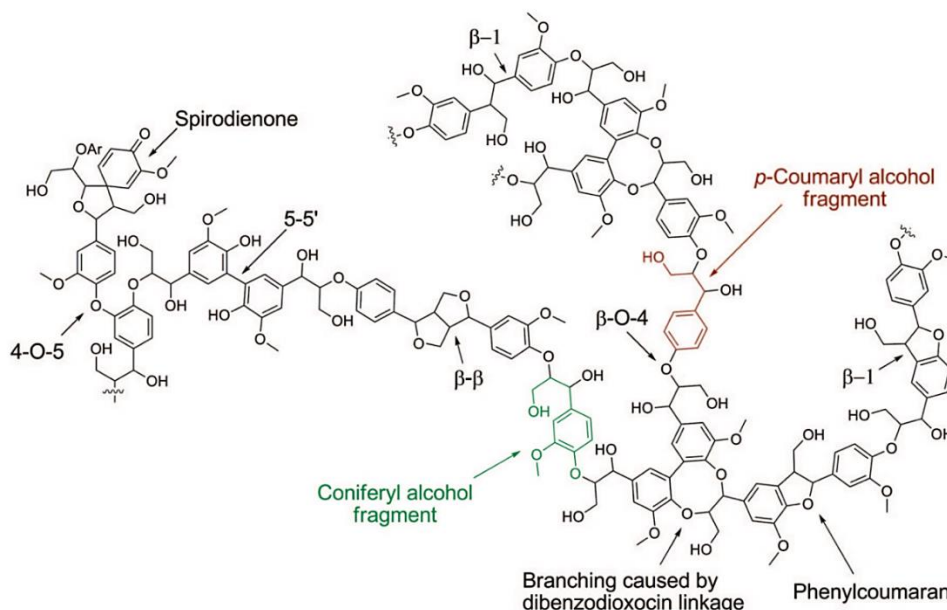


Figure 2.5 Proposed lignin structure. Reprinted (adapted) with permission from [97]. Copyright 2010 American Chemical Society

Lignin springs up from an enzyme-mediated dehydrogenates polymerization and made up from three major phenylpropanoid monomers, shown in Figure 2.6. The lignin monomers consists of p-coumaryl (no methoxy groups on the romantic ring),

coniferyl (one methoxy group on the aromatic ring), and sinapyl (two methoxy groups on the aromatic ring) alcohol [15]. Harley reported that softwood lignins contain mostly G units with traces of H units, while for hardwood lignin contain mostly G and S units and low of H units [98]. Lignin consists of strong reactive sites of phenolic (highly branched polyphenolic), aliphatic hydroxyl groups [17]. These monolignols resulted from dehydrogenative radical polymerisation lead and linked together with ether and carbon-carbon linkage (between aliphatic and aromatic bond) [99-100].

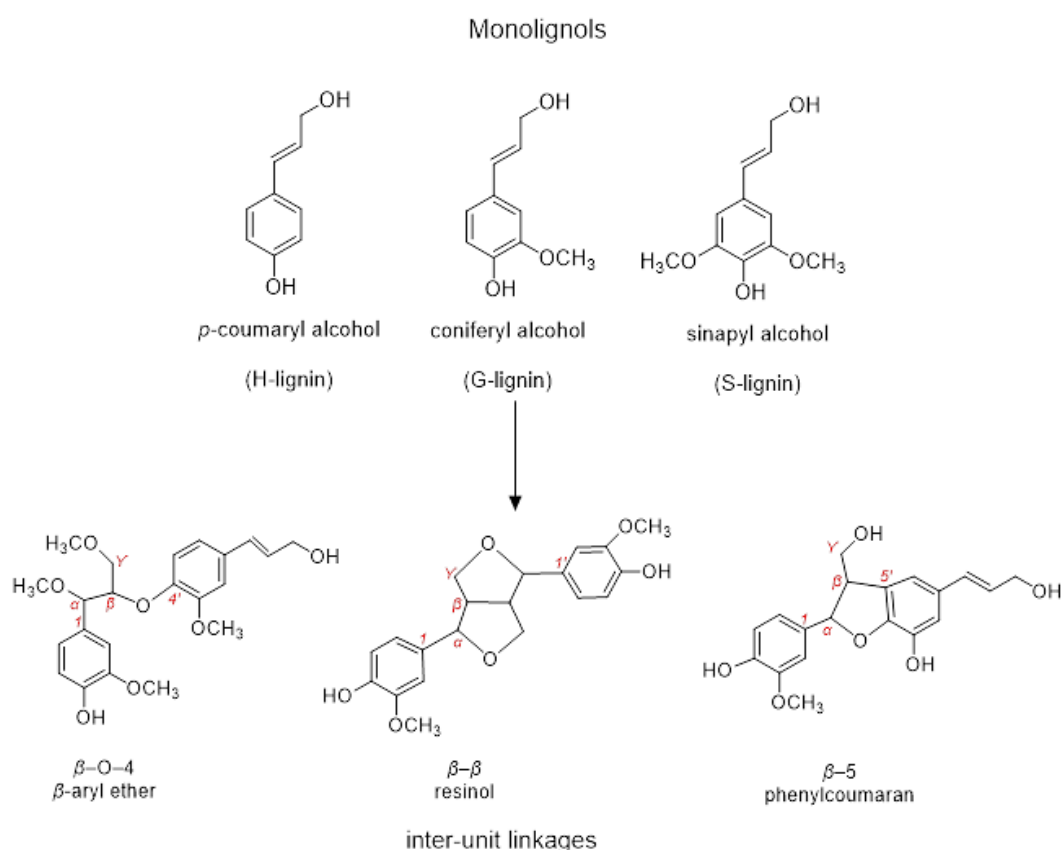


Figure 2.6 The major phenylpropanoid monomers and the most common inter-unit linkage of lignin

The guaiacyl group in the lignin or also known as the methoxyl group is a hydrophilic group that will react strongly with water [101] and this is indeed a great aspect. The structure and characteristic of lignin strongly depend on the types of plants (specific parts and growth conditions) and pulping process. Lignin is usually insoluble

in all solvents and can only be degraded by physical or chemical treatments. Based on its properties, it can be promising to be used as an emulsifier in food emulsion [18].

Currently, natural emulsifiers were extracted from either plant-based or animal-based as mentioned under the Section 2.3. Intriguingly, lignin act as polymeric amphiphiles to stabilize the emulsions as the lignin derivatives were reported to have a high affinity towards proteins and it works as carrier immobilized enzyme [17]. The affinity of lignin drives the particles to the absorption of the water-oil interface [102]. The complex structure of lignin governs the structure of the protein and was found as amphiphilic particles which suited as a stabilizer that can reduced the interfacial between oil and water [103].

Lignin was reported to stabilize the emulsion by increasing the mechanical, steric, and electrostatic stabilization from the absorption of the polymer layer on the droplets [104]. Dissociation of the phenolic hydroxyl group in the lignin surface establishes the lignin condition from neutral to alkaline [103]. Lignin is extensively used as an emulsifier and came from a variety of sources. Table 2.2 presented the overviews of the previous study that used lignin as an emulsifier.

Table 2.2 Lignin used as an emulsifier.

Type of lignin	Main finding	Emulsion droplets	Remarks	Ref
Cationic lignin modified with tetraethylene pentaamine and formaldehyde	Preparation of bitumen (70 wt% contained of bitumen) emulsions.	-	Emulsions is more viscous and sensitive to shear rate as pH decreased. Modified lignin soluble in acidic solution at pH ranging from 1 to 5, with concentration about 2 wt%.	[105]
Polymer-grafted NSL	Produced a stable O/W Pickering emulsions with 0.1 wt% concentration (pH 7).	~ 100 nm	The interfacial tension was found to decrease with decreasing graft density and increasing salinity.	[55]
Amphoteric lignin (absorbed with SiO ₂ nanoparticles via electrostatic interaction)	Stabilized pH-responsive O/W Pickering emulsions.	20-40 μm	The emulsion stable at pH range of 3-4 and instable at pH above than 4.	[68]
Lignin-coated polystyrene	Stabilized pH-responsive O/W Pickering emulsions.	20–58.9 μm	The emulsion stable at pH range of 3-4 and instable at pH range 5-9	[106]
Carboxymethylated lignins	Stabilized O/W (kerosene-in-water, 30:70 of ratio) emulsion	1–100 μm	low surface activity at concentration 3% and pH 7. and a high affinity with the oil phase at low pH or high salt concentration in the aqueous phase	[107]
Kraft and soda lignin	Stabilized O/W (kerosene-in-water at ratio 30/70)	-	emulsions at concentration 1% and pH 2	[95]

Calcium lignosulfonate and kraft lignin.	Stabilized O/W emulsions at low concentration, 0.05 % w/w	0.717 μm	The emulsion stable at pH to 7.0.	[108]
Cationic colloidal lignin	Stabilized Pickering emulsions (toluene-in-water) at concentration 4%	259 nm	The emulsion stable at pH 2 to 6.	[103]
Kraft lignin with tannic acid	Stabilized O/W (cyclohexane-in-water)	100-500 nm	Although the optimum condition is at pH 11 but it reported that with the lower pH of 3 had a higher interfacial tension, it showed the greatest stability against phase separation	[109]
