COMPARISON STUDY BETWEEN ENZYMATIC HYDROLYSIS & ACIDOLYSIS LIGNINS ISOLATED FROM OIL PALM EMPTY FRUIT BUNCH PULPS

H’NG YIN YING

UNIVERSITI SAINS MALAYSIA
2013

COMPARISON STUDY BETWEEN ENZYMATIC HYDROLYSIS & ACIDOLYSIS LIGNINS ISOLATED FROM OIL PALM EMPTY FRUIT BUNCH PULPS

By

H’NG YIN YING

Thesis submitted in fulfillment of the requirements for the
Degree of Master of Science

JULY 2013

ACKNOWLEDGEMENTS

Firstly, I would like to express my deepest appreciation to my supervisor, Dr. Leh Chue Peng for her helpful advice and constructive guidance throughout the study. I am thankful to my field supervisor, Prof. Hiroshi Ohi for his knowledge that I acquired from his comments and suggestions.

Besides, I would like to thank the lab assistance. They are Mr. Abu Mangsur Mat Sari, Mr. Raja Khairul Azmi and Mr. Azli Sufryzal Bunizan who helping me to deal with the laboratory machines and handling all the chemicals required along the investigation. A same time, staffs in PPTI, USM are also never hesitate provide assistance in all the aspects.

Furthermore, not forgotten is RU Grant 811088 of USM which providing me with all the financial resources included facilities which was needed for my project. I feel thankful because without the financial resources, all the instruments are not possible available and hence my investigation will not be so efficient.

Apart from that, I would like to thank my course mates, Mr. Goh Soh Wei, Mr. Goh Chee Yong who are being so helpful and supportive throughout the project. Everyone was given their co-operation to take turn to use the facilities and help each other. Tolerance and advices from them are much appreciated.

Lastly, I would like to apologize for any inconvenience and mistakes which I made throughout my project to anyone who directly or indirectly involve in my project. Thank you very much.

H’ng Yin Ying

2013

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS AND SYMBOLS

AA	Active alkali
AQ ($C_{14}H_8O_2$)	Anthraquinone
CEL	Celluloytic enzyme lignin
CHN	Carbon hydrogen nitrogen
ClO_2	Chlorine dioxide
^{13}CNMR	Carbon 13 nuclear magnetic resonance
DMSO	Dimethyl sulfoxide
EFB	Oil palm empty fruit bunch
FID	Flame ionization detector
FTIR	Fourier transform infrared spectroscopy
G	Guaiacol
GPC	Gel permeation chromatography
HCl	Hydrochloric acid
H_2O	Water
HNMR	Proton nuclear magnetic resonance
KBr	Potassium bromide
KI	Potassium iodide
LCC	Lignin carbohydrate compound
MWL	Milled wood lignin
N_2	Nitrogen
nC_{20}	n- eicosane
NaOH	Sodium hydroxide
Na_2S	Sodium sulfide
NMR	Nuclear magnetic resonance
O_2	Oxygen

OD	Oven-dry weight
OH^-	Hydroxide anions
OPF	Oil palm frond
OPT	Oil palm trunk
PyGCMS	Pyrolysis gas chromatography mass spectrometry
S	Syringol
S^{2-}	Sulfide
SO_3^{2-}	Sulfite
THF	Tetrahydrofuran
TIC	Total ion chromatograms
UV	Ultraviolet

KAJIAN PERBANDINGAN ANTARA LIGNIN HIDROLISIS ENZIM & ACIDOLISIS YANG DIASINGKAN DARIPADA PULPA TANDAN BUAH KOSONG KELAPA SAWIT

ABSTRAK

Kajian asas terhadap pulpa kertas yang dihasilkan daripada serabut tandan buah kosong (EFB) kelapa sawit (*Elaeis guineensis*) telah dijalankan demi mendapatkan pemahaman yang lebih mendalam terhadap struktur baki lignin. Baki lignin telah diasingkan daripada pulpa kraft, pulpa soda, pulpa kraft-anthraquinone (AQ) dan pulpa soda-anthraquinone (AQ). Dalam kajian ini, dua kaedah telah digunakan untuk mengasingkan baki lignin: hidrolisis asid dalam dioxane-air 82:18 (v/v) dengan 0.1M asid hidroklorik (HCl) di bawah hidrolisis refluks dan enzimatik dengan mengunakan enzim selulotik komersial dalam penimbal asetat dengan pH 4.5 pada suhu 45°C dengan goncangan yang berterusan. Masa yang diperlukan unuk prosedur hidrolisis enzimatik adalah lebih lama daripada prosedur hidrolisis asid. Maka, dalam eksperimen ini, struktur baki lignin daripada dua kaedah ini telah dibandingkan. Prosedur hidrolisis menghasilkan lignin yang lebih banyak berbanding dengan hidrolisis asid. Kebanyakan baki lignin enzimatik tidak dapat melarut dalam pelarut ujian yang biasa seperti tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), aseton, kloroform dan sebagainya sedangkan baki lignin asidolisis dapat melarut dengan mudah dalam semua pelarut ujian. FTIR menghasilkan jalur yang hampir sama bagi kedua-dua lignin yang terasing seperti bahagian OH, bahagian CH, unit S, unit G dan sebagainya. Selain itu, analisis CHN menunjukkan peratusan nitrogen yang lebih tinggi bagi baki lignin enzimatik. Spektroskopi UV menunjukkan setiap baki lignin yang terasing mengandungi jenis kumpulan hidroksil fenolik yang berlainan. ^{l}HNMR menunjukkan isyarat yang terhad bagi kedua-dua lignin yang terasing atas sebab kepekatan yang rendah. Tambahan lagi, PyGCMS dijalankan untuk menguji perubahan dalam baki lignin. Asid lemak rantai panjang telah ditemui dalam baki lignin yang dipautkan bersama oleh ikatan ester. Nisbah S/G turut diperoleh daripada kiraan program PyGCMS.

COMPARISON STUDY BETWEEN ENZYMATIC HYDROLYSIS & ACIDOLYSIS LIGNINS ISOLATED FROM OIL PALM EMPTY FRUIT BUNCH PULPS

ABSTRACT

Fundamental research on paper pulp produced from oil palm (*Elaeis guineensis*) empty fruit bunch (EFB) fiber was carried out in this study to gain better understanding of the residual lignin's structures. Residual lignin was isolated from EFB chemical pulps which are kraft pulp, soda pulp, kraft-anthraquinone (AQ) pulp and soda-anthraquinone (AQ) pulp. In this study, there are two methods used for the isolation of residual lignin: acidic hydrolysis in dioxane-water 82:18 (v/v) with 0.1M hydrochloric acid (HCl) under reflux and enzymatic hydrolysis by using commercial cellulolytic enzymes in an acetate buffer with pH 4.5 at 45°C under continuous shaking. The time required for enzymatic hydrolysis procedure is much greater than the acidic hydrolysis procedure. Hence, in this experiment, the structures of residual lignin by these two methods are compared. Results showed that the enzymatic hydrolysis procedure gave lignin with higher yield than acidic hydrolysis did. Most of the enzymatic residual lignin could not dissolve in the common testing solvents such as tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), acetone, chloroform and so on whereas the acidolysis residual lignin able to dissolve easily in all the testing solvents. Fourier transform infrared spectroscopy (FTIR) absorption gave almost similar band for both isolated lignin such as OH stretch, CH stretch, S unit, G unit and so on. Besides that, carbon hydrogen nitrogen (CHN) elemental analysis showed higher percentage of nitrogen for the enzymatic residual lignin compared to the acidolysis residual lignin. UV spectroscopy showed that each isolated residual lignin contained different type of phenolic hydroxyl group. Proton nuclear magnetic resonance (^{1}HNMR) showed limited signal and information for both isolated lignin due to low concentration. Furthermore, pyrolysis-gas chromatography mass spectrometry (PyGCMS) was carried out to examine the changes in the residual lignin. Long chain fatty acid was found in the residual lignin which linked together by ester bond. S/G ratio was also obtained from the calculation of PyGCMS programs.

CHAPTER 1 INTRODUCTION

1.1 GENERAL

Deforestation issues have always been widely debated worldwide, but a proper solution had yet to be discovered. Therefore, the use of non-wood lignocellulosic biomass is highly encouraged as an alternative to wood. Non-wood lignocellulosic biomasses are more commonly categorized as annual crops and agro-wastes such as kenaf, bamboo, oil palm empty fruit bunches (EFB) and others.

Non-wood lignocellulosic materials such as oil palm EFB biomass are abundantly available as a by-product from the oil palm industry. The chemical and physical properties of EFB have been well examined, and it has also been utilized as a raw material for various fibrous-based products, including pulp and paper. Although various types of unbleached and bleached EFB pulps have been produced, the full understanding of the EFB, residual lignin structure still has not been identified.

Lignin is a highly-polymerized organic substance in plant materials consisting primarily of phenyl propane units linked together in three dimension structures. It is found mostly in the middle lamellae between plant cell walls. The middle lamellae hold fibers together and its existence affects the mechanical strength properties. Lignin is a hydrophobic compound which is insoluble in most solvents (Smook, 1992).

During acidic hydrolysis, lignin is isolated by refluxing the pulp under an inert atmosphere (nitrogen) with acidic dioxane-water and then recovering the lignin from the solution (Gellerstedt, 1994). The yield of residual lignin is low but results in very high purity and has been proposed that some modification to the lignin structure happen. Enzymatic hydrolysis has been introduced since 1981 (Yamaki, 1981). Lignin is isolated by using the enzyme cellulase. Therefore, no structural changes will occur for the isolated residual lignin. Carbohydrates were dissolved by the enzyme cellulase, leaving behind solid residues known as residual lignin. This method gives a high yield of residual lignin but with low purity.

There are several lignin reactions during alkaline pulping. For example, lignin will undergo degradation or fragmentation, which involves the cleavage of β-O-4 ether bonds of non-phenolic lignin structure by hydroxide ions. These will cause the hydrophilicity of lignin to increase due to the generation of new phenolic hydroxyl groups. Besides that, lignin will also undergo condensations, which were caused by the formation of C-C linkages between fragmented lignin and also the formation of covalent bonds between lignin & carbohydrate.

In both soda and kraft pulping process, anthraquinone (AQ) was used as a pulping additive to increase the delignification and to decrease the carbohydrate degradation. Furthermore, AQ will also increase the pulp yield because of the stabilization of the carbohydrate content in the pulp. AQ works by going through a cycle which leads to the reduction of lignin and the oxidation of reducing end group of the cellulose from an aldehyde to a carboxylic acid (Holton, 1977).

1.2 JUSTIFICATION

Previously, studies on the residual lignin structure mostly concentrated on softwood and hardwood kraft pulps (Tong et al., 2000; Jaaskelainen et al., 2003). In order to gain a better understanding of non-wood residual lignin structure for future applications, similar studies cannot be neglected for non-wood pulps. In this study, the effect of kraft and soda pulping on the residual lignin structure of non-wood oil palm EFB pulp is examined. Comparison between the different pulping methods is necessary because soda pulping is more suitable for EFB fibers, and EFB soda pulps are easier to be bleached than kraft pulps. As the prehydolysed EFB pulps show much lower kappa number than non-prehydroysed pulps even after 1 stage of oxygen delignification. The effect of hydrolysis on EFB fiber, especially lignin structure (functional group) should be identified. The two major isolated methods commonly used for the isolation of residual lignin, which is acidic hydrolysis and enzymatic hydrolysis. As acid hydrolysis method gave changes on the isolated lignin. Enzymatic hydrolysis which gives fewer changes on the isolated lignin structure will be used in this study.

1.3 OBJECTIVE

This research was carried out:

- To characterize the structure of lignin isolated from oil palm EFB fiber and its chemical pulps with and without prehydrolysis.
- To study the effect of pulping and isolation methods on lignin’s structure.
- To determine the effect of prehydrolysis on residual lignin structure after different pulping methods.

CHAPTER 2 LITERATURE REVIEW

2.1 EMPTY FRUIT BUNCH (EFB)

Oil Palm is cultivated as a source of oil where it is originated in Malaysia, Indonesia and Thailand. In Malaysia, oil palm is one of the most popular commercial crops, which had been double up planted area compared with rubber. Being one of the biggest producers and exporters of oil palm, Malaysia is facing the problems on replanting operations and environmental issues. Oil palm industry left an enormous amount of lignocellulosic materials such as EFB, which is renewable and low cost to extract into fibers form. However, 0.1% of oil content usually is found in EFB fiber, and its colour stability is low due to the lignin content. Hence, EFB fiber has been studied extensively by using various types of pulping processes in the pulp and paper productions.

Table 2-1 Composition of EFB

Biomass	EFB
Cellulose %	35-40
Hemicelluloses %	20-25
Lignin %	25-30
Extractive and others %	8-10

2.2 PULPING

Pulping referred to the process which wood or non wood fibrous raw material is reduced to a fibrous mass (Casey, 1981). Generally, it is meant by the bonds are systematically rupture within the wood or non wood structure to constituent fiber's structure. The task can be accomplished by mechanically, thermally, chemically or combinations of treatments. Existing commercial processes are broadly classified as mechanical, chemical or semi chemical (Smook, 1992).

2.2.1 Chemical pulping

Chemical pulping mainly referred on the chemical reactants and heat energy to soften and dissolve the lignin in the raw material (Sixta, 2006). Based on the principle, fiber is liberated by dissolving the lignin from a middle lamella, which is freed undamaged and without any mechanical action, or with only a small amount of it (Ince, 2004, Kamarudin et al., 1999).

An ideal chemical pulping process is ensured each fiber receiving the same amount of chemical treatment at the same time and temperature. The chemicals and energy must be transported uniformly throughout each of the reaction sides to middle lamella. In practice, chemical pulping process is removed almost the lignin in EFB fibers. Besides that, the process also degraded or dissolved certain amount of hemicellulose and cellulose in EFB fibers. Hence, the yield of pulp is low relatively compared to mechanical pulping process. The yield of pulp usually is 40% to 50% of the original raw materials (Smook, 1992). The most common chemical pulping processes are soda pulping and kraft pulping (known as sulfate pulping). Both soda and kraft pulping is categorized under alkaline pulping. The alkaline pulping process commonly using NaOH (soda pulping), SO_3^{2-} (sulfite pulping), and alkaline S^{2-} (kraft pulping) as cooking liquors (Ibrahim et al., 2011).

2.2.1.1 Soda pulping

Soda process is the first recognized chemical pulping method. In 1850s, Watt (England) and Burgess found that using strongly alkaline solution of sodium hydroxide (NaOH) to delignify wood chips. At that time, costs of sodium hydroxide are expensive. Soon, it replaced by the sulfite process in 1867. Even it has been replaced by the sulfite process; it is still important and performs well in non-wood pulping such as EFB fibers. Furthermore, soda pulping also known as an environmentally friendly method (Khristova et al., 2002). Sodium hydroxide (NaOH) is used as a chemical to degrade and dissolve the lignin in the soda pulping. Wood can be swell better in the medium of NaOH in order to make the cell wall more accessible to the pulping process. Besides that, the generated hydroxide ion (OH-) from NaOH will react with lignin and degrade it into smaller fragments (Ibrahim, 2000). The strength of the soda pulp is lower compared to the kraft pulps. Soda pulping usually produces pulp with lower yield and brighter colors, which contents some bleaching chemicals (Francis *et al.*, 2006; Labid *et al*., 2008). The lower yield of soda pulp is due to the degradation of lignin, extractive, holocellulose, alpha cellulose. However, these drawbacks can be overcome with adding anthraquinone (AQ). With the addition of AQ, it will increase the process rate, yield, and pulp strength properties.

2.2.1.2 Kraft pulping

Kraft pulping process is also known as sulfate pulping process (Sarkanen and Ludwig, 1971). A mixture of sodium hydroxide (NaOH) and sodium sulfide (Na_2S) are used as a chemical to degrade and dissolve the lignin in the kraft pulping (Sixta, 2006). This mixture can generate an equilibrium condition for the pulp (Sarkanen and Ludwig, 1971). Kraft pulping currently dominant in the lignin removal process because of it superior properties which mainly because of the ability on chemical recovery site and strong pulp strength (Walker, 2006, Sixta, 2006). Although kraft pulping gave superior pulp properties and low carbohydrate lost compared to soda pulping, but it outcome's waste will cause environmental pollution. Besides that, the kraft pulp also gave darker colour and caused a difficulty on bleaching. Furthermore, kraft pulping process will also be releasing some malodorous gases (methyl mercaptan and other sulfur compounds) during the chemical recovery process (Johansson *et al.*, 1987).

2.2.2 Hydrolysis pretreatment

The hydrolysis pretreatment process is carried out with water under temperature of 165°C for 1 hour. The process will break down the structure of cellulose, hemicellulose, and lignn for further process. Prehydrolysis is normally used for the production of dissolving pulp, which will result the pulp with lower kappa number (lignin) and lower hemicellulose content. In previous studies, it is found that prehydrolyzed EFB fiber is darker with similar or slightly higher lignin content than non-prehydrolyzed EFB fiber before applying chemical pulping process. However, after pulping, the prehydrolyzed EFB pulps show lower kappa number (6-7) in comparison to the non-prehydrolyzed EFB pulp (12-13). During the pulping process, the prehydrolyzed fiber is easier to be delignified could be caused by the following phenomena during prehydrolysis state:

- Degradation of lignin into lower molecular weight
- Cleavage of Lignin-Carbohydrates Compounds (LCC)
- Changes of lignin’s side chains

Cleavage of Lignin-Carbohydrates Compounds will increase the solubility of lignin. Lower molecular weight is due to the cleavage of β-O-4 glycosidic linkages in various polysaccharides. When in the steam condition, carbohydrate will convert into furfural and hydroxymethylfurfural which is suggested as lignin degradation (Li *et al*., 2005; Tanahashi, 1990).

2.2.3 Anthraquinone (AQ)

Anthraquinone (AQ) (Figure 2-1) is an aromatic organic compound with a formula of $C_{14}H_8O_2$ (McKelvey and Malcolm, 1978). As a catalyst of pulping process, AQ must be stable to hot and strong alkali conditions. Besides that, AQ also must be zero toxicity, no environmental effects and cost low (Blain, 1993).

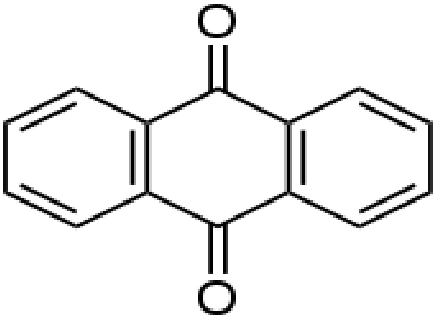


Figure 2-1 Anthraquinone (AQ) chemical structure (Blain, 1993)

AQ act as a pulping additive to:

- Increase delignification (Goyal 1997)
- Decrease and stabilized carbohydrate degradation (Ghosh *et al*., 1977; Smook, 1992)
- Increase pulp yield (Holton, 1977)
- Obtain low kappa number pulp which maintaining ideal viscosity (Minja *et al*., 1997)

Furthermore, Figure 2.2 shown that AQ is work by going through a cycle which leads to the reduction of lignin and the oxidation on reducing end group cellulose from an aldehyde to carboxylic acid (Blain, 1993).

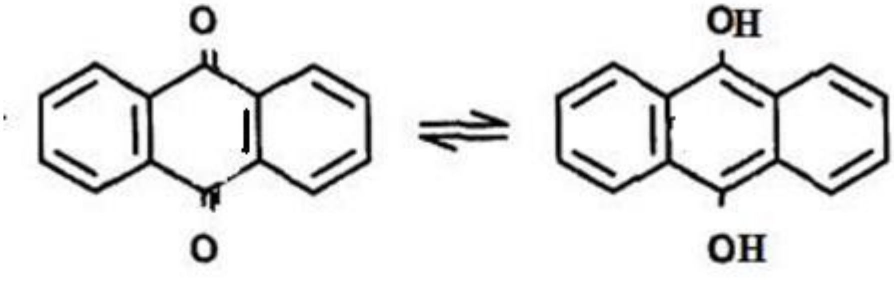


Figure 2-2 Anthraquinone reduction and oxidation of reducing end of cellulose (Blain, 1993)

2.3 LIGNIN ISOLATION METHOD

Mostly, the research aimed on improvement at pulping and bleaching process, which required an understanding of the structure changes residual lignin. Currently, the most common used isolation methods are acidolysis and enzymatic hydrolysis. Both methods have their own limitations that need to be considered. Hence, a new combined isolation method was introduced to improve the output of lignin.

2.3.1 Lignin isolation methods by Acidic Dioxane-water hydrolysis of pulp

During acidolysis, the lignin is isolated by refluxing the pulp under nitrogen atmosphere with 82:18 (v/v) acidic dioxane–water solutions (Gellerstedt et al. 1994). The yield of lignin is lower but with high purity. There are some structural changes in residual lignin such as cleavage of a-aryl, a-alkyl ether and b-aryl ether bonds in benzyl alcohol during the isolation process.

The drawbacks of acidic hydrolysis isolation method:

- Strong acidic conditions are needed to extract the lignin from pulp fibers.
- The acidic hydrolysis conditions expected may cause some structural changes and modifications and towards the lignin. Example:
    i. Cleavage of some α-aryl and α-alkyl ethers as well as β-aryl ethers in benzyl alcohol units. The cleavage of the aryl ether bonds would increase and showed a higher amount of phenolic hydroxyl groups compared to the actual amount which present in the wood or pulp (Gierer and Wannstrom, 1986).
    ii. Condensation reactions between the residual lignin under acidic conditions are possible for various kinds of pulp except in Kraft pulp. This is because a low amount of residual benzyl alcohol and benzyl ether structures in the lignin after a completed Kraft pulping. Hence, the tendency to form a condensed lignin could be low also (Gellerstedt *et al*., 1994). Besides that, the kraft pulp residual

lignin from the acidolysis treatment was compared using flow through and batch reactors. It was found that the structure of kraft pulp residual lignin isolated from a flow-through process was similar to the batch process. Therefore, addition evidence showed that the structure of residual lignin in Kraft pulps is not significantly during the acidolysis treatment (Jiang *et al*., 1987).

- The yield is lower compared to the enzymatic isolation method (40-60%). Increasing the yield of residual lignin is dependent on the use of different isolation conditions. Example:
    i. Higher acid concentration
    ii. Longer reaction time

It was suggested that optimum isolation conditions were necessary to produce better yield of residual lignin.

2.3.2 Lignin isolation method by enzymatic hydrolysis of pulp

Enzymatic hydrolysis has been used for lignin isolation since 1981 (Yamaski et al. 1981). In this method, the carbohydrates are digested and dissolved by cellulase enzymes, and the solid residue contains the residual lignin. Enzymatic hydrolysis retains the linkages between lignin and carbohydrates. Therefore, the origin structures of the residual lignin can be studied. This isolation method gives better lignin yields, but it contains some carbohydrates and protein residues from the enzymes itself (Jääskelä *et al*., 2001; Lachenal *et al*., 1995).

The drawbacks of enzymatic hydrolysis method:

- The residual lignin was found contains carbohydrates that cannot be removed by prolonged and repetitive enzymatic treatments or by purification methods that are commonly used. These caused by the limited ability of enzymes to hydrolyze lignin-carbohydrate complex linkages.
- The residual lignin was found contains some protein impurities, which originating from the enzymes used during the enzymatic hydrolysis. Therefore, these protein impurities need to be removed by purification (Hortling *et al*., 1990).
- Time required for the enzymatic hydrolysis procedure is much longer than the acidolysis isolation method.

The carbohydrate and protein impurities from the enzymatic hydrolysis method will complicate the subsequent of analysis on the lignin structure. However, structural of this residual lignin is considered chemically unchanged.

2.3.3 Lignin isolation method by enzymatic combined with acidic hydrolysis of pulp.

Hence, a new isolation method was established due to the limitations of acidolyis and enzymatic isolation methods (Argyropoulos et al. 2000). Initially, enzymatic isolation method aimed to digest most of the carbohydrates, which present on the surface of fibers by using cellulotic enzyme. Besides that, it also exposes a fresh cellulosic fibers surface. The inability of further carbohydrate's degradation is due to the strongly bonding between the remaining carbohydrates with lignin (Wang J et al. 1997). Then, the residual lignin is further treated with a mild acidolysis treatment (Wu and Argyropoulos, 2003). This acidolysis treatment is to cleave the remaining lignin–carbohydrate covalent bonds. This new isolation method will provide lignin with about 70% yield and low amounts of impurities.

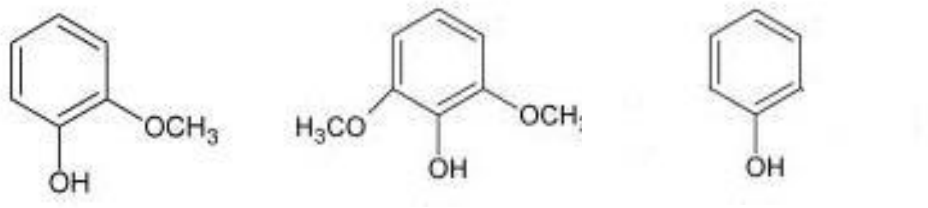
2.4 LIGNIN

Lignin is the polymeric organic substance in the plant world. Its principal role is to form the middle lamella, the intercellular materials which cement the fibers together. Its existence increased the mechanical strength properties, and a macromolecular formed by random coupling. It hydrophobic properties cause the cell wall doesn't swell (Smook, 1992).

Table 2-2 Lignin content found is the different type of raw materials.

	Lignin Content %
Hardwood	20-25
Softwood	25-30
Non-wood (EFB)	30

Lignin mostly known as 3 dimensional of phenylpropane units, which consist of syrinyl, guaiacyl and p-hydroxyphenyl (Grima-Pettenati and Goffner, 1999). Usually hardwood and non wood (EFB) is found to have both S unit and G unit whereas only the G unit is found in the softwood.



(a) (b) (c)

Figure 2-3 Basic lignin elements: G unit (a); S unit (b); p-hydroxyphenyl (c) (Grima-Pettenati and Goffner, 1999)

Basically, syringaldehyde and vanillin are produced from S unit and G unit respectively. The molar ratio of the S unit and G unit is called S/G ratio. It is to know the solubility of lignin during alkaline cooking. S unit lignin decomposed faster than the G unit lignin while alkaline cooking (T. Akiyama et al., 2005).

2.4.1 Major lignin reaction in alkaline pulping

In the soda process, sodium hydroxide is the major pulping chemical whereas in the sulfate process, a mixture of sodium hydroxide and sodium sulfide is used for the delignification process. Between these 2 pulping processes, sulfate pulps produced an outstanding strength pulp. The term's kraft and sulfate pulping are interchangeable.

Delignification is necessary in paper industry because the lignin portion of pulp contributed both chromophoric and leucochromophoric structures. Hence, delignification has been proposed as a responsible for the colour of native lignin and pulps (Johansson, 2000). Overall delignification process consists of a rapid phase (bulk delignification) and a slow phase (residual delignification). Rapid phase removed most of the lignin very fast in the beginning of the pulping process while the slow phase removed the remaining lignin very slowly until the end of the pulping process.

The bulk delignification is caused by rapid degradation or fragmentation of lignin through rupture of bonding by thermal homolysis (Kleinert, 1965; Kleinert 1966). The free radicals formed may also undergo secondary reaction such as:

- Condensation
- Grafting
- Termination
- Radical transfer

2.4.1.1 Lignin degradation/ fragmentation

i. Cleavage of ether bonds

The cleavage of β-O-4 ether bonds probably is one of the most important single reactions of alkaline delignification. This reaction involves the cleavage of β-O-4 ether bonds from non-phenolic lignin structure by hydroxide ions. Cleavage of the β-O-4 ether bonds will increase the hydrophilicity of lignin and generate some phenolic hydroxyl groups. The mechanism of cleavage involved a nucleophilic attack of the neighboring hydroxyl group on the β carbon atom. Therefore, resulting the formation of epoxide and simultaneous removal of the aryl ether substituent as phenoxide ions (Gierer and Kunze 1961; Gierer et al. 1962).

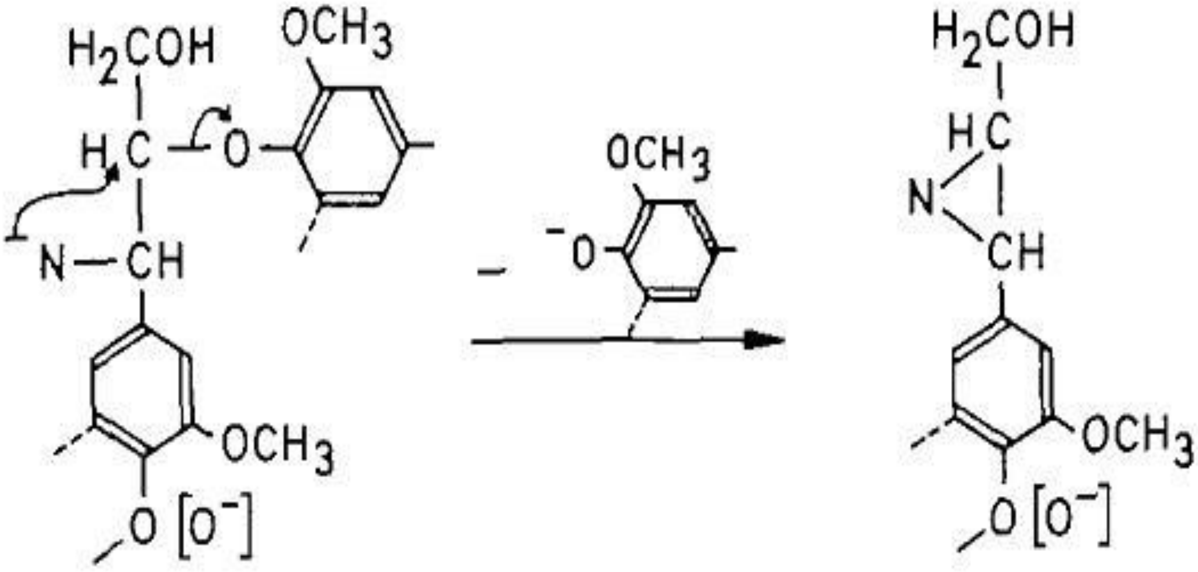


Figure 2-4 Reaction of β-O-4 ether bonds (J. Gierer and I. Kunze 1961; J. Gierer et al. 1962)

ii. Cleavage of carbon-carbon bonds

In contrast to the extensive lignin fragmentation resulting from the rupture of inters unitary ether linkages, the effect of carbon-carbon bond cleavage is relatively minor in this respect (Gierer 1980). Carbon-carbon fragmentation of the propanoid side chain may occur in a variety of laboratory treatment and technical processes. Besides that, C-C fragmentation reaction also depending on:

- Type of substituent on the propanoid side chain
- Reagents involved in the treatment
- Reaction conditions (acidity or alkalinity. temperature)

2.4.1.2 **Lignin condensation**

i. Formation of C-C linkages between fragmented lignin

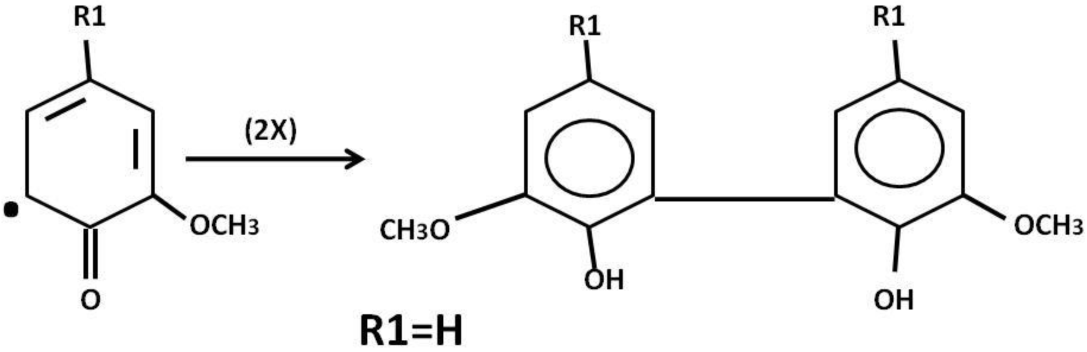


Figure 2-5 Reaction of C-C linkages

Oxidants which are free radicals (e.g., ClO_2 and O_2) or, which are convertible into such species (e.g., conversion of peroxides to OH-) abstract hydrogen atoms from the phenolic hydroxyl group to generate phenoxy radicals. Coupling of the proper mesomeric forms of such radicals ultimately leads to diphenyl structures, as is shown by Figure 2-5. Either or both aromatic rings in such structures may undergo further oxidation, yielding quinonoid and carboxylic acid derivatives analogous in structure to those produced from monomeric units.

ii. Formation of covalent bonds between lignin & carbohydrate

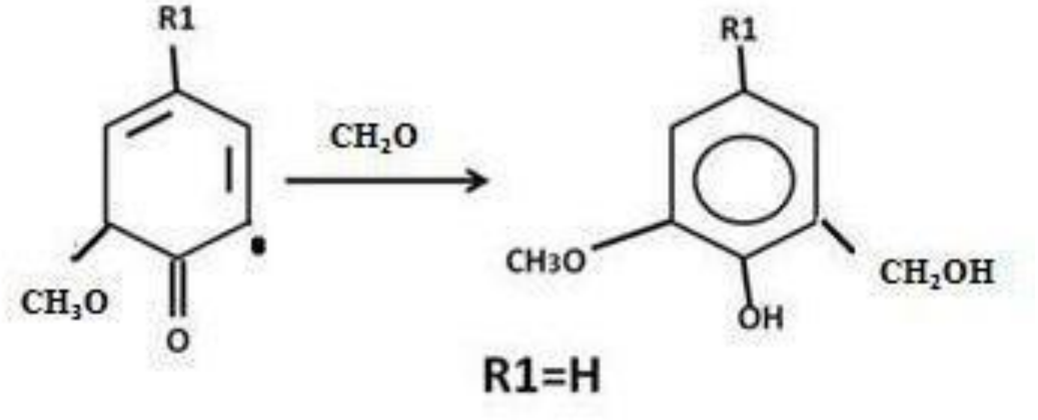


Figure 2-6 Reactions of covalent bonds

In alkaline media, phenolic units may react with formaldehyde and hence forming methylol derivatives that will condense among themselves or react with another phenol, as shown by Firgure 2-6. This formaldehyde condensation reaction forms the basis for using technical lignin in the production of adhesive.

2.4.2 Model of lignin

Over the years, various hypothetical models of lignin molecular structures have been proposed based on:

- Random coupling theory
- Available data on the types and distributions of inter-units or linkages

The actual lignin structure is very complex. These existing lignin models neither imply any particular sequence of monomeric units nor reflect the actual network of monomers in the lignin macromolecule. Further details on the polymerization process of lignin have not been completely established. The coupling model in the monolignols reacts endwise with the growing polymer under simple chemical control, which produces random linkages. Hence, the lignin structure is not absolutely defined or determined (Boerjan *et al.*, 2003). Although the template replication models are proposed in these recent years, but there is still some lacking experimental demonstration on showing the bonding formation in a lignin polymerization process. Polymerization process is believed to be under the control of biochemical. Endwise polymerization is guided by proteinaceous sites on the template that stipulates linkage types and its configurations (Davin and Louis, 2003; Chen and Sarkanen, 2003).

Lignin distribution is quantified by using ultraviolet light (UV) microscopy. Strong or weak portion of lignin can be visualized by using interference microscopy and confocal laser scanning microscopy (Donaldson *et al*., 2001). Lignin is polymerized in a matrix of concentrated polysaccharide gel, which surrounded by water molecules in dynamic conditions. Synthesis using model compounds suggested that construction of the lignin polymer will be affected by the pre-existing polysaccharide gel in the cell wall (Barakat *et al*., 2007). Thus, the structure of lignin is highly affected by environmental factors during biosynthesis. The fundamental unit structure of lignin is now well-identified by the research work of lignin chemists (Sarkanen and Ludwig, 1971) but the polymeric structure of lignin has not been fully elucidated.

Statistical method is applied to determine the higher order of lignin structure, which constructed linking with those possible unit compounds by using a computational means (Glasser and Glasser, 1974; Glasser and Glasser, 1974; Glasser and Glasser, 1976). However, this method has its own limitations since the constructed structure is just an assumed model. It is widely accepted that lignin is not a constitutionally defined compound, but it is a physically and chemically heterogeneous material consisting of representative phenylpropane structures. It may also be appropriate that the diversity of structural combinations is unlimited because the various factors influencing the biosynthetic process of lignin.

Therefore, it is worth considering the structural moiety of various lignins. It is important to realize the amount of functional groups in the lignin (Balakshin *et al.*, 2008). According to all the previous studies, lignin mostly will have a linkage with carbohydrates (Koshijima *et al.*, 1998). This indicates that contaminants of fragments from carbohydrate are unavoidable in isolated lignin because of the presence of lignin-carbohydrate complex (LCC).

2.4.2.1 Lignin-Carbohydrate Complex (LCC)

Basically, a typical water-soluble lignin component in wood is called lignin-carbohydrate complex (LCC). The lignin and hemicellulose are linked together by benzylic ether, benzylic ester, and glycosidic bonds to form LCC.

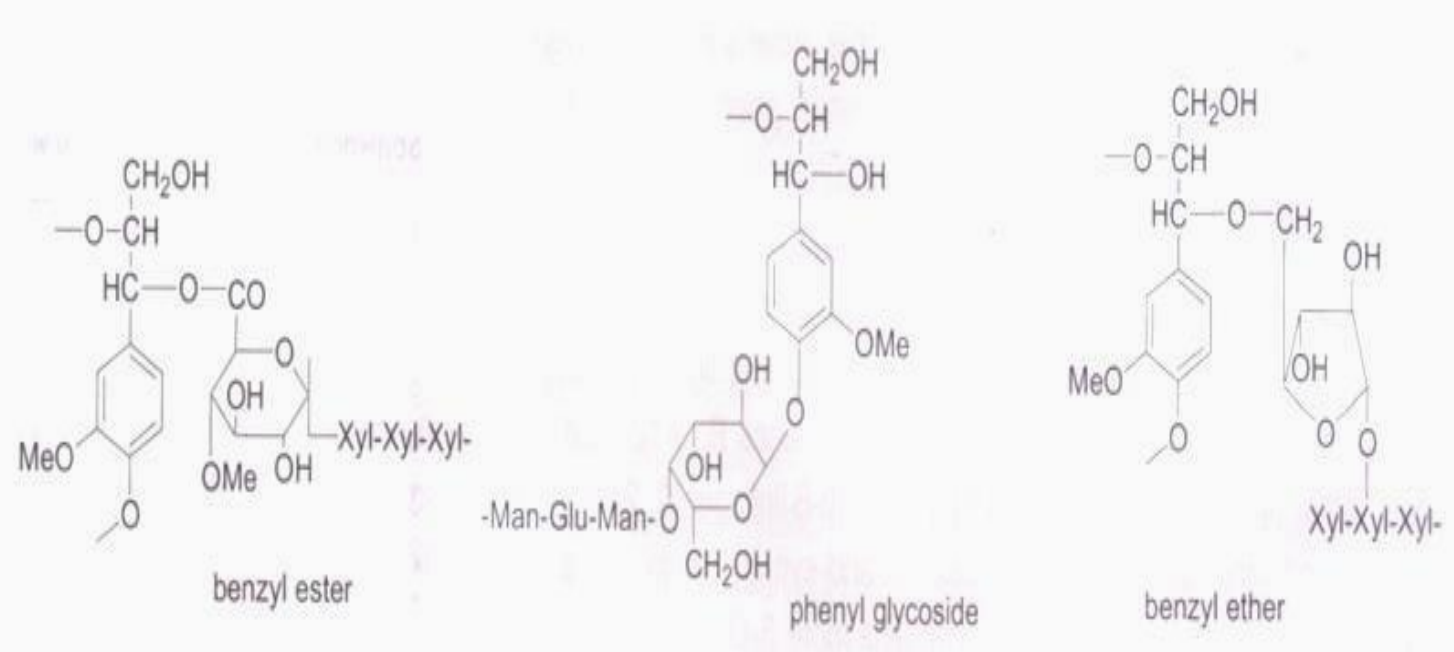


Figure 2-7 Examples of linkages between lignin and carbohydrate in LCC

It is commonly assumed that lignin polymer is bound covalently to polysaccharides to form lignin-carbohydrate complexes (LCCs) (Bjorkman, 1957; Lai and Sarkanen, 1957; Eriksson *et al*., 1980). Main reason of resisting the delignification during kraft pulping and bleaching is probably due to the lignin carbohydrate covalent linkages. From the previous experiments on LCCs, those bonding are existing between lignin and hemicellulose.

These proposed linkages’s type is classified into the following groups:

- Ether linkage of the hydroxyl group at the (z-position of the lignin side chain with alcoholic hydroxyl of sugar residue (Freudenberg, 1965)
- Ester linkage of the alcoholic OH of lignin with the carboxylic group of uronic acid (Yaku *et al*., 1976)
- Hemiacetal or acetal linkage of the carbonyl group located at β-position of lignin with carbohydrates (Bolker and Sommerville, 1963)
- Glycoside linkage with the primary alcoholic OH at the γ-position of the phenylpropane unit (Enoki and Koshijima, 1978)
- Glycosidic linkage at the phenolic OH of lignins (Hayashi, 1961; Smelstorius, 1974)
- Ester's linkage of the carboxylic group of the cinnamic acid unit in lignin with the alcoholic OH of carbohydrates (Kato *et al*., 1983; Lain *et al*., 1992; Iiyama and Stone, 1994)

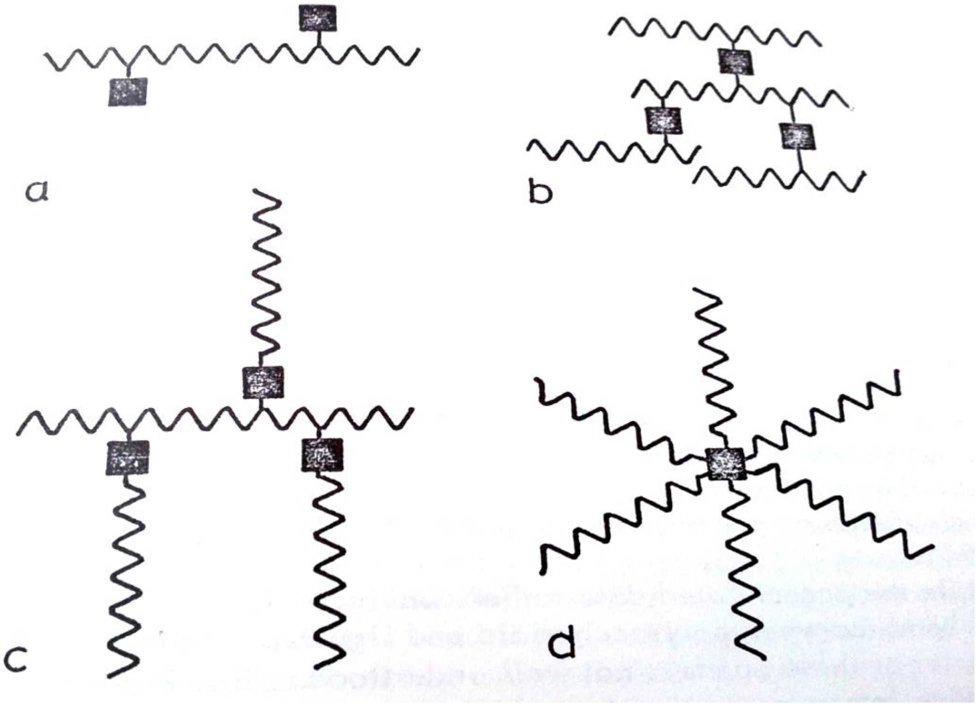


Figure 2-8 Some of the possible structure for lignin-hemicellulose complex

Most of the LCC bonds stated above were obtained from degradation analysis in acid hydrolysis and alkaline hydrolysis conditions. However, it is impossible to fully depolymerize or degrade LCCs into their constituent units quantitatively by these common methods. Therefore, it is inevitable that many undesirable modifications occur during their degradation reactions. Hence, a very small significant evident to this linkage has been obtained (Sipilfi and Brunow, 1991).

Carbon-13 nuclear magnetic resonance (^{13}C-NMR) has proven to be a powerful method for elucidating the detailed chemical features of LCC because it reflects the nature of chemical bonding between lignin and carbohydrates (Watanabe *et al*., 1989; Lewis *et al*., 1989). However, observation of the LCC bonds by ^{13}C-NMR is difficult because the strong signals arising from carbohydrates in LCC overlap with those from lignin.

2.4.2.2 Condensed lignin

Condensed lignin structure is the term used to describe lignin with an aryl or alkyl substituent at the C5 or C6 position on the aromatic ring which has some condensed-type structures such as 5-5, α-5, α-1 and 4-O-5.

Evidence for the formation of condensed structures during Kraft pulping has also been suggested (Kringstad and Morck, 1983; Gellerstedt and Robert, 1987; Gierer *et al*., 1976). Condensed structures are stable under Kraft delignification conditions and believed to be present in the residual lignin of Kraft pulp, which caused the residual lignin to be not reactive. Condensation reactions may occur during cooking condition, and uncondensed structures are expected to undergo solvolysis at a higher rate than the “primary” condensed structures. Therefore, the residual lignins contain much higher concentrations of condensed structures.

2.5 FUNCTIONAL GROUP OF RESIDUAL LIGNIN

Although lignin is one of the most abundant natural macromolecules, and it is available at exceedingly low cost but it has received a relatively low attention as a polymer in the market. Indeed, in those days standard polymer text often does not even acknowledge its existence as one of the important natural polymers (Flory, 1953; Tanford, 1961).

Lignin should be regarded as a family of the 3 dimensional polymers because it is:

- Spherical in solution
- Containing a variety type of functional group and site
- Capable for a surprising selection of modifying reactions

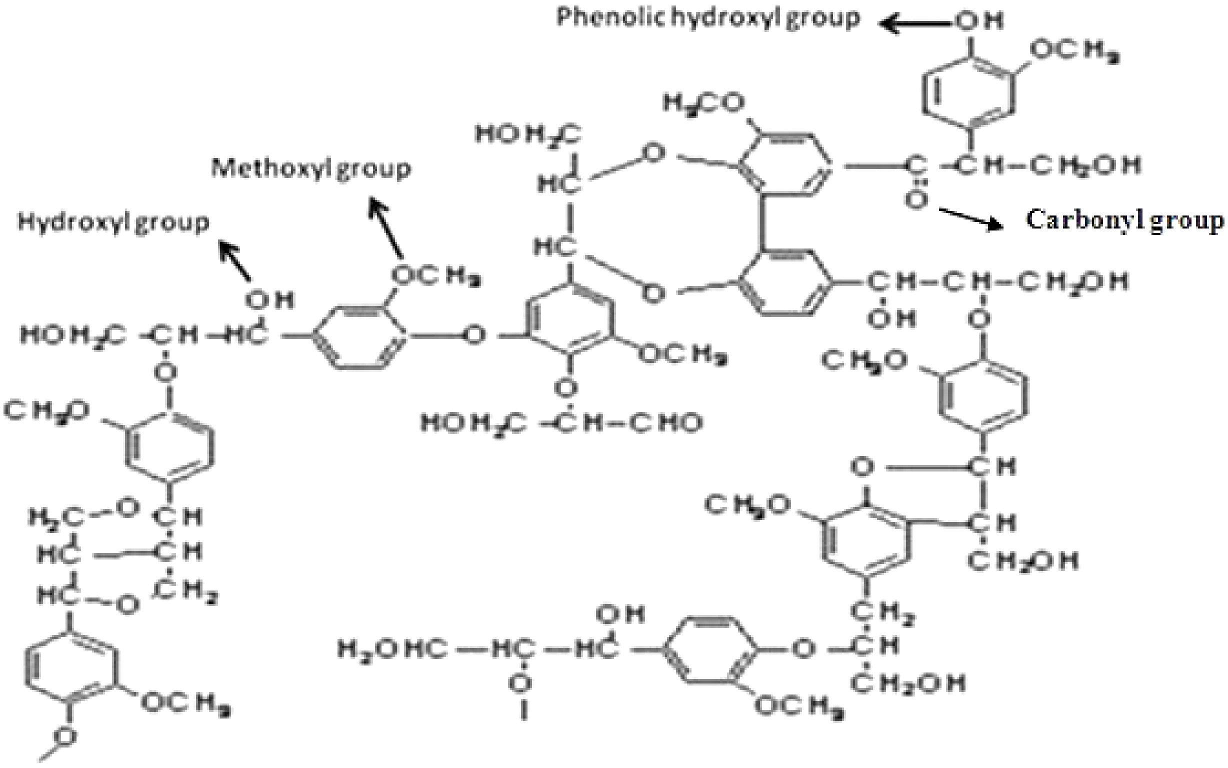


Figure 2-9 Common functional group found in residual lignin

2.5.1 Phenolic hydroxyl group

The phenolic hydroxyl group is an important functional group in lignin that influences pulping and bleaching chemistry. During kraft delignification, the cleavage of ß-*O*-4 linkages creates new phenolic groups. Therefore, the residual lignin has a greater quantity of phenolic groups (Gierer, 1980).

Throughout the reactivity, the most important functionality in lignin is the free phenolic group. Most of the chemical reactions in lignin usually occurred between these phenolic phenylpropane units. Phenolic groups perform well in the dissolution of lignin during pulping process by improving the lignin's solubility in an alkaline medium. Besides that, the phenolic groups also involved in the formation of reactive quinone methide intermediates (Gellerstedt *et al.*, 1988).