Characterization of Lignin in Pulps Obtained from Oil Palm Empty Fruit Bunch by Pyrolysis-Gas Chromatography-Mass Spectrometry

November 2016

Yin Ying H'NG

Characterization of Lignin in Pulps Obtained from Oil Palm Empty Fruit Bunch by Pyrolysis-Gas Chromatography-Mass Spectrometry

A Dissertation Submitted to

The Graduate School of Life and Environmental Sciences,

The University of Tsukuba

In Partial Fulfillment on the Requirements

For the Degree of Doctor of Philosophy in Bioresource Engineering

(Doctoral Program in Appropriate Technology and Sciences for

Sustainable Development)

Yin Ying H'NG

Table of Contents

List of figures and tables

Chapter 1   Introduction

Chapter 2 Characterization of Syringyl and Guaiacyl Lignins in Thermomechanical Pulp from Oil Palm Empty Fruit Bunch by Pyrolysis-Gas Chromatography-Mass Spectrometry Using Ion Intensity Calibration

Chapter 3 Characterization of Residual Lignin Obtained by the Enzymatic Hydrolysis of Oil Palm Empty Fruit Bunch Pulps

Abbreviation

Abbreviation	Description
AA	Active Alkali
AQ	Anthraquinone
CHN	Elemental Analysis Carbon, Hydrogen and Nitrogen
EFB	Empty Fruit Bunch
FID	Flame Ionization Detector
FPU	Filter Paper Unit
FTIR	Fourier Transform Infrared Spectroscopy
G	Guaiacyl
GPC	Gel Permeation Chromatography
HPLC	High Performance Liquid Chromatography
IS	Internal Standard
LCC	Lignin Carbohydrate Complex
MDF	Medium Density Fiberboard
MI	Main Ion Intensity
NaOH	Sodium Hydroxide
Na_2O	Sodium Oxide
NBO	Nitrobenzene Oxidation
NMR	Nuclear Magnetic Resonance
OH	Hydroxyl Group
PH	Prehydrolysis
Py-GC/MS	Pyrolysis-Gas Chromatography-Mass Spectrometry

S	Syringyl
S_a	Syringaldehyde
TI	Total Ion Intensity
TIC	Total Ion Chromatography
UV	Ultraviolet Spectroscopy
V_a	Vanillin

Chapter 1   Introduction

1.1   Empty Fruit Bunch

In Malaysia, oil palm (Elaeis guineensis) is one of the most popular commercial crops, with a planting area that is double that of rubber. Additionally, oil palm is a source of crude oil in Malaysia, Indonesia, and Thailand. After harvesting and removing the oil seeds, large amounts of lignocellulosic materials such as empty fruit bunches (EFB) remain; these materials are renewable and extraction of their fibers is inexpensive (Fig. 1.1). However, one of the oil components (palmitic acid) is found in EFB fiber (Harsono et al., 2016). EFB is composed of cellulose (30–50%), hemicellulose (15–35%), lignin (20–30%), extractive, fatty acid, and other materials (8–10%) (Yunos et al., 2015).

Deforestation issues have been widely debated worldwide. Therefore, non-wood lignocellulosic biomass is highly encouraged as an alternative to wood. Hence, using EFB for paperboard production has been strongly promoted. The development of EFB pulps began in the West (Metro Knight Sdn. Bhd., Johor) and East (Borneo Advance Pulp and Paper Sdn. Bhd., Sabah) of Malaysia in 2007 (www.ecofuture.com.my/metro-knight.htm; www.etawau.com/OilPalm/PulpPaperMill.htm). Currently, EFB fibers are studied extensively using various types of pulping (chemical or mechanical) processes (Harsono et al., 2016; Mulyantara et al., 2016) for pulp and paper production.
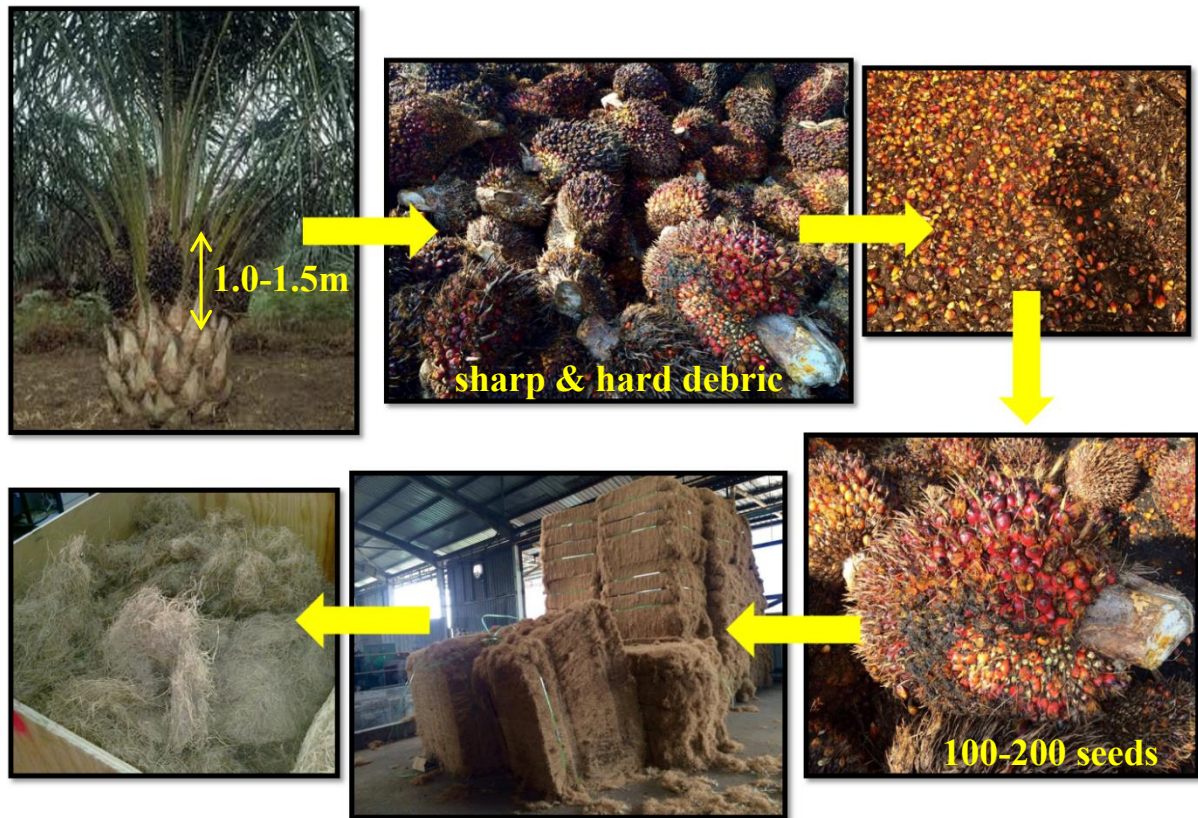
Fig. 1.1 Scheme of oil palm (*Elaeis guineensis*) empty fruit bunch.

1.2   Pulping Processes

Cooking or pulping refers to the process of converting wood or non-wood fibrous raw material to a fibrous mass (Casey, 1981). The structures bonding within wood or non-wood to constituent fibers are systematically ruptured. These tasks can be accomplished by mechanical, thermal, chemical, or combination treatments (Smook, 1992).

1.2.1   Chemical Cooking

Chemical reactants and heat energy are required in chemical cooking to soften and dissolve lignin (Sixta, 2006). Throughout the cooking process, fiber is liberated by dissolving the lignin from the middle lamella, which can be accomplished without mechanical action (Ince 2004; Kamarudin et al., 1999). The optimal conditions for the chemical cooking process result in each fiber receiving the same amount of chemical treatment at the same time and temperature. The chemicals and energy must be uniform throughout each reaction side from the middle lamella. The chemical cooking process removes most of the lignin from the raw materials. Additionally, the process also degrades or dissolves certain amounts of hemicellulose and cellulose in raw materials. This results lowers the yield of pulp by approximately 40–50% (Smook, 1992; Wan Rosli et al. 1998) compared to the mechanical pulping process which reaches nearly 95% yield. The most common chemical cooking processes are soda cooking and kraft cooking. Both methods are categorized as alkaline cooking processes (Ibrahim et al., 2011). The alkaline cooking process commonly uses sodium hydroxide (NaOH) for soda cooking, whereas a mixture of NaOH and sodium sulfide (Na2S) is used for kraft cooking (Ibrahim et al., 2011; Sixta, 2006).

1.2.1.1 Soda Cooking

In 1850s, it was first recognized that using a strongly alkaline solution of NaOH can delignify wood chips, but this chemical is costly. Therefore, the sulfite process was introduced in 1867. Furthermore, soda cooking is an environmentally friendly method (Khristova et al., 2002). Wan Rosli et al. (2003) reported that the optimum cooking parameters for obtaining EFB soda cooking pulps were 161°C for 100 min with an alkali level of 26.1%.

An alkaline solution of NaOH is used to degrade and dissolve the lignin during soda cooking. Wood shows greater swelling in alkali solution, making the cell wall more accessible to the coking process. Additionally, the generated hydroxide ion (OH-) from NaOH reacts with lignin and degrades it into smaller fragments. Soda cooking typically produces pulp with lower yield and brighter colors (Francis et al., 2006; Labid et al., 2008). The lower yield of soda pulp results from the degradation of lignin, extractives, holocellulose, and alpha-cellulose. However, these limitations can be overcome by adding anthraquinone (AQ) to obtain a higher pulp yield and viscosity (Akgul et al., 2009).

1.2.1.2 Prehydrolysis Followed by Soda Cooking

Prehydrolysis is currently carried out with water (steam) at 165°C for 1 h. This process breaks down the structure of cellulose, hemicellulose, and lignin before the cooking process. Prehydrolysis is used to produce dissolving pulp because pulp with a lower kappa number (lignin) and lower hemicellulose content is generated. In the cooking process, the prehydrolyzed fiber is more easily delignified because lignin is degraded into lower molecular weight forms and lignin-carbohydrate complexes (LCC) are cleaved during the prehydrolysis process. Cleavage of LCC improves lignin solubility. The molecular weight is lower because of the cleavage of β-O-4 linkages in various polysaccharides. (Li et al., 2005; Tanahashi 1990).

Wan Rosli et al. (2004) stated that prehydrolysis had positive effects on soda pulp qualities such as a high α-cellulose content, low kappa number, and low ash content.

1.2.1.3 Anthraquinone Addition to Soda Cooking

AQ is an aromatic organic compound with a formula of $C_{14}H_8O_2$, and can be industrially synthesized through a Diels-Alder reaction of naphtoquinone and butadiene. AQ acts as a catalyst in the alkaline cooking process (Fig. 1.2) (Ohi, 1994). Additionally, AQ is non-toxic, has no environmental effects, and is inexpensive (Blain, 1993). Based on previous studies, with the addition of AQ can increase delignification rate (Goyal, 1997), stabilize carbohydrate degradation (Ghosh et al., 1977; Smook, 1992), increase pulp yield (Holton, 1977; Nomura and Nakamura 1978), and give low kappa number pulps that maintain good viscosity (Minja et al., 1997). Furthermore, Fig. 1.3 shows that AQ functions by a cycle that leads to the reduction of lignin and oxidation at the reducing-end group cellulose (Ohi, 1994). Therefore, a combination of prehydrolysis treatment and soda-AQ cooking of EFB is recommended (Harsono et al., 2016).
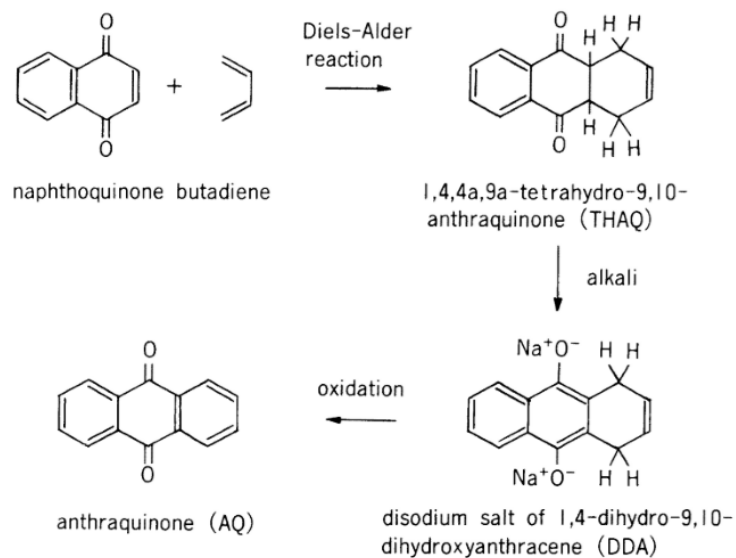
Fig. 1.2 Utilization of anthraquinone (AQ) as a catalyst during alkaline cooking process

(Ohi, 1994).
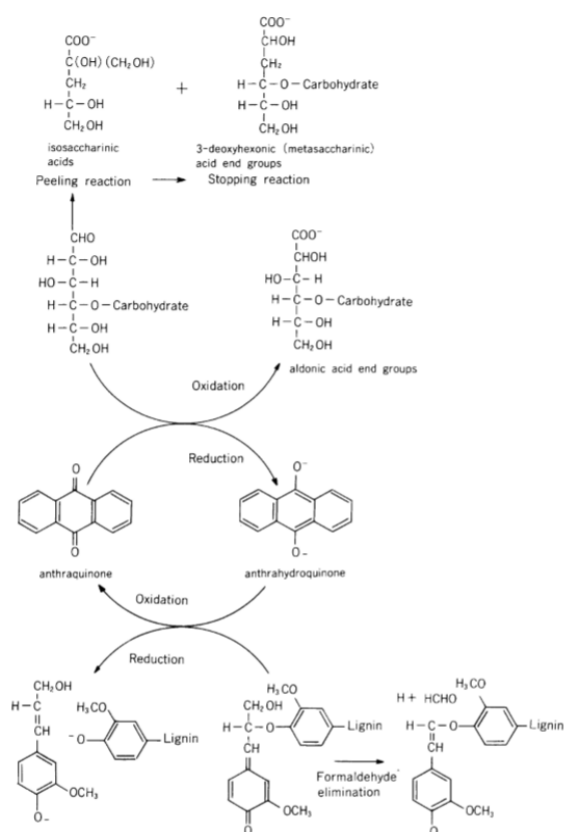
Fig. 1.3 Mechanism of anthraquinone (AQ) against carbohydrates and lignins (Ohi, 1994).

6

1.2.2 Mechanical Pulping

Mechanical pulping requires mechanical energy to separate fibers from wood through grinding action. Mechanical pulping results in minimal lignin removal, and the quality of the paper produced is not as high as the paper from chemical pulps because the removal of significant amounts of lignin is limited.

The advantages of mechanical pulping are a high pulp yield, low operation cost, and strongly absorbent paper. Disadvantages include low strength, low permanence, high tendency of turning yellowish over time, and containing shives or bundles. Therefore, most mechanical pulp is used for lower-grade paper products. There are four main types of mechanical pulping: stone ground wood pulping, refiner mechanical pulping, chemi-thermomechanical pulping, and thermomechanical pulping.

Thermomechanical pulping is conducted similar to refiner mechanical pulping, but under higher temperature and pressure. These new mechanical pulping methods can overcome the disadvantages without compromising the advantages of mechanical pulping by using two revolving disks and heat. Lignin can be softened by heat, allowing for greater separation of the cellulose fibers and less fiber damage. Thermomechanical pulping generates the highest-grade mechanical pulp but also uses high-energy intensity because steam is used. This process also produces a darker pulp that is costlier than bleach. However, thermomechanical pulping is the most common mechanical process currently in use (Gorski *et al*. 2010).

1.3 Methods for Isolation and Analysis of Lignin

The most commonly used lignin-isolation methods are acidolysis and enzymatic hydrolysis, for which the structural changes to residual lignin in the pulps are well-understood. Both methods have limitations that must be considered. Lignin is traditionally isolated by acidolysis (Gellerstedt et al., 1994); however, its structure is modified or changed because of the breakdown of some linkages under the isolation conditions (Lundquist, 1992). In contrast, by using enzymatic isolation methods, unaltered residual lignin can be obtained (Hortling et al., 1990).

1.3.1 Enzymatic Hydrolysis

Enzymatic hydrolysis has been used for lignin isolation since 1981 (Yamasaki *et al*. 1981). During the hydrolysis process, carbohydrates are hydrolyzed and dissolved by cellulase enzymes, and the solid residue remaining is known as residual lignin. Enzymatic hydrolysis retains the linkages between lignin and carbohydrates while the original structures of the residual lignin can be obtained. This isolation method gives better lignin yields, but the product contains some carbohydrates, protein residues from the enzymes, and other contaminations (Jääskelä *et al*., 2001; Lachenal *et al*., 1995).

There are some limitations of enzymatic hydrolysis methods; for example, the residual lignin obtained contains carbohydrates that cannot be removed by prolonged and repetitive enzymatic treatments or by common purification methods. This is because of the limited ability of enzymes to hydrolyze lignin-carbohydrate complex linkages. Additionally, the residual lignin contains some protein impurities, which originate from the enzymes used for enzymatic hydrolysis. Therefore, these protein impurities must be removed by purification (Hortling *et al*., 1990). Furthermore, the time required for the enzymatic hydrolysis procedure is much longer than that for the acidolysis isolation method. In addition, these carbohydrate and protein

impurities from the enzymatic hydrolysis method complicate subsequent analyses of the lignin structure. However, the lignin structure is considered chemically unchanged.

1.3.2 Nitrobenzene Oxidation

Numerous previous studies have characterized the structure of lignin from several raw materials (Takayama et al. 1997; Ibarra et al., 2005; Zier et al., 1997; Kuroda et al., 2002a, b; Gonzalez-Vila et al., 1999; Del Rio et al., 2001); the most common method used to determine syringyl (S) and guaiacyl (G) compound yields is nitrobenzene oxidation under alkaline conditions. Nitrobenzene oxidation (NBO) was introduced by Freudenberg to confirm the aromatic nature of lignin (Chen, 1992). During NBO, lignin is oxidatively cleaved to form aromatic carbonyl compounds such as syringaldehyde (Sa) and vanillin (Va) as the main products (Schultz et al., 1986). The composition and quantity of the aromatic aldehydes obtained represents the structure of uncondensed lignin because condensed lignins typically do not give aromatic aldehydes except dehydrodivanillin. These procedures require long sample preparation time and consume many chemical reagents. Thus, a faster, safer, and more economical procedure such as pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) must be developed.

1.4 Lignin

Lignin is a polymeric organic substance (Rowell, 2005) that is the second-most abundant component after cellulose and deposits present predominantly in the walls of secondarily thickened cells of plants (Vanholme et at., 2010). Its principal role is to form the middle lamella, which are the intercellular materials that cement the fibers together (Hatfield and Vermerris, 1991). Its existence increases the mechanical strength properties, and a macromolecule is formed by random coupling. The hydrophobic properties of the cell wall

prevent the cell from swelling (Smook, 1992). In the papermaking industry, lignin is an undesirable component in the chemical composition of wood into bleached pulp (Campbell and Sederoff, 1996; Rodrigues et al., 1999). There is approximately 20–25% lignin in hardwood and 25–30% in softwood and non-wood. As a major component of plant cell walls, lignin has been studied in the field of papermaking for biorefinery in recent years (Li et al., 2008; Studer et al., 2011).

The three-dimensional polymers of lignin contain phenylpropane units, which consist of syringyl (S), guaiacyl (G), and para-hydroxyphenyl groups (Fig. 1.4) (Grima-Pettenati and Goffner, 1999). These components are biosynthesized by the polymerization of the three monolignols p-coumaryl, coniferyl and sinapyl alcohols (Boerjan et al., 2003; Ralph et al., 2004). Typically, hardwood and non-wood contain both S-unit and G-unit, whereas only the G-unit is found in softwood. Syringaldehyde (Sa) and vanillin (Va or Ga) in Fig. 1.5 produced by NBO are from the S-unit and G-unit, respectively. The molar ratio of the S-unit to G-unit is known as the S/G molar ratio. The high S/G molar ratio confers higher solubility to lignin during alkaline cooking; Akiyama et al. (2005) noted that S-unit lignin decomposed faster than G-unit lignin during alkaline cooking.
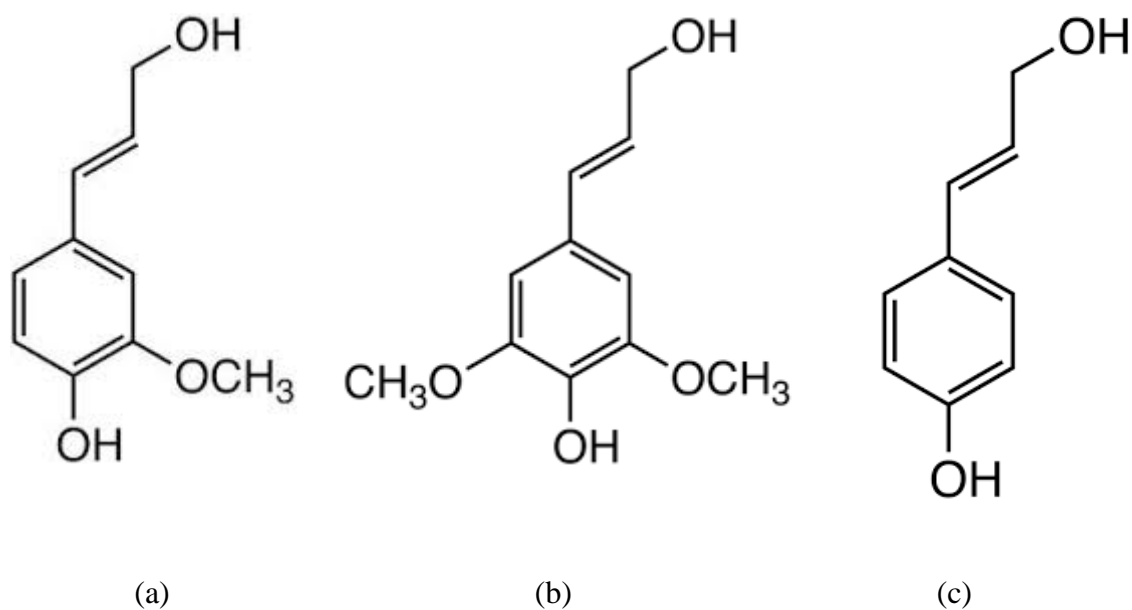
Fig. 1.4 Basic lignin elements: G unit (a); S unit (b); *para*-hydroxyphenyl (c) (Grima-

Pettenati and Goffner, 1999).
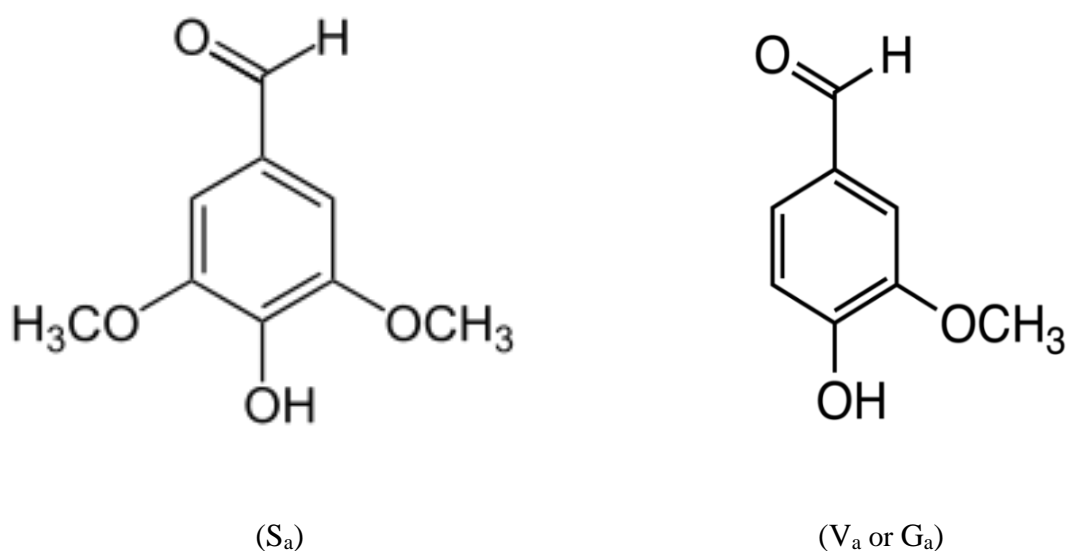
Fig. 1.5 Structures of syringaldehyde (S_a) and vanillin (V_a or G_a) as the main lignin products

by nitrobenzene oxidation (NBO).

1.4.1 Lignin-Carbohydrate Complex

Lignin and hemicellulose are considered to be linked together by benzylic ether, benzylic ester, and glycosidic bonds to form the lignin-carbohydrate complex (LCC) as shown in Fig. 1.6. It is thought that the lignin polymer is bound covalently to polysaccharides to form LCCs (Bjorkman, 1957; Lai and Sarkanen, 1957; Eriksson et al., 1980). Delignification during alkaline pulping and bleaching process is prevented likely because of the lignin carbohydrate covalent linkages.

Several types of linkages have been proposed in LCC. For example, these include an ether linkage of the hydroxyl group (OH) at the γ-position of the lignin side chain with the 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), and ester 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).

Most LCC bonds were identified based on degradation analysis under acid hydrolysis and alkaline hydrolysis conditions. However, it is not possible to fully depolymerize or degrade LCCs into their constituent units quantitatively using common methods. Therefore, many undesirable modifications occur during their degradation reactions. Hence, a very small significant evident to this linkage has been obtained (Sipilfi and Brunow, 1991).
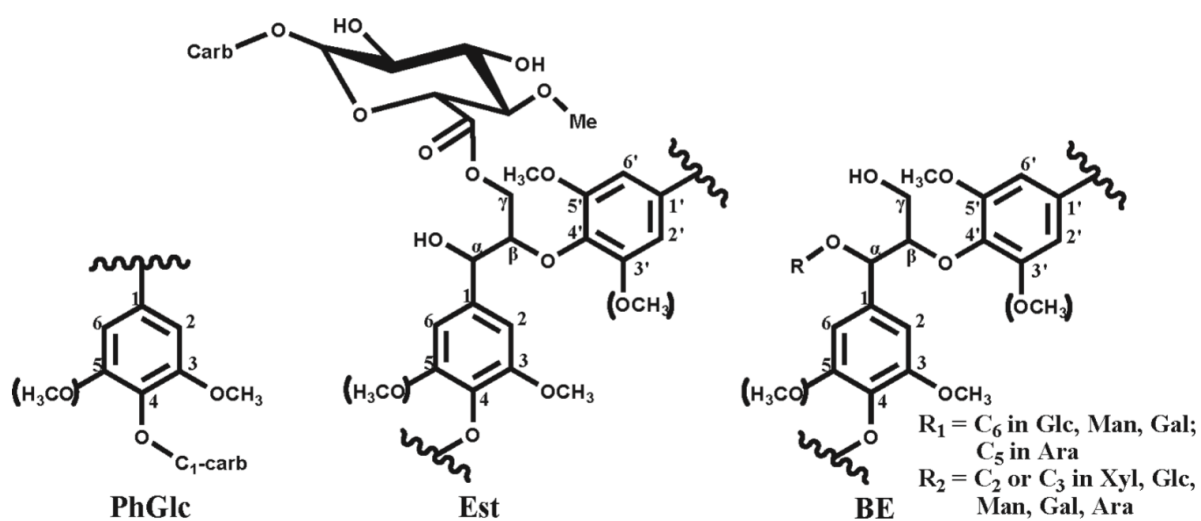
Fig. 1.6 Linkages between lignin-carbohydrate complex (LCC): PhGlc, phenyl glycoside; Est, γ-ester; and BE, benzyl ether (Balakshin *et al*., 2007; Yuan *et al*., 2011; You *et al*., 2015).

1.4.2 Condensed-type Lignin

Condensed-type 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. Condensed structures are stable under alkaline delignification conditions and are thought to be present in the residual lignin of alkaline pulp, causing the residual lignin to be unreactive. Condensation reactions occur during alkaline cooking conditions. Therefore, residual lignins should contain much higher concentrations of condensed structures. Evidence for the formation of condensed structures during alkaline pulping has also been presented (Kringstad and Morck, 1983; Gellerstedt and Robert, 1987; Gierer *et al*., 1976).

1.5 Pyrolysis-Gas Chromatography-Mass Spectrometry

Currently, conventional chemical methods for characterizing lignin are being replaced by instrumental techniques that are less troublesome and time-consuming. The most common analysis techniques used are Fourier transform infrared spectroscopy (Faix, 1992), ultraviolet spectroscopy (Bolker and Somerville, 1962), nuclear magnetic resonance spectroscopy (Leary and Newman, 1992) or pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) (Meier and Faix, 1992).

Previously, sample preparation such as the isolation process for conventional chemical methods for characterizing lignin was time-consuming. Furthermore, the natural or original chemical bonds in lignin and between lignins are partially affected or destroyed during the isolation process, altering the original lignin structure. Recently, isolation has been considered an unnecessary step in the characterization of residual lignin (Al-Dajani and Gellerstedt, 2002). One of the techniques used to study the chemical structure of lignin directly is discussed below.

14

Py-GC/MS (equipment is shown in Fig. 1.7) is an important technique for characterizing polymeric samples. It is widely utilized for the characterization of lignin (Meier and Faix, 1992; Kuroda et al., 2002a). The technique involves fragmentation of the sample in an inert gas at a high temperature. After the fragments are separated in a gas chromatograph column, they can be identified by mass spectrometry. The analysis is carried out with a flame ionization detector. Furthermore, identification can be conducted directly with a mass spectrometer without separation by gas chromatography.

The analysis requires a minimal sample amount and it can be accomplished directly without an isolation procedure (Fig. 1.8), and thus only minor modifications are made to the lignin structures. The technique allows the determination of p-hydroxyphenyl, guaiacyl, and syringyl structures (Rodrigues et al., 1999; del Río et al., 2001; Sonoda et al., 2001) as well as some other lignocellulosic materials, and the description of the structures of lignin (Meier and Faix, 1992; Ralph and Hatfield, 1991). Furthermore, this method has also been applied for in situ lignin determination of the S/G ratio in eucalyptus wood (Rodrigues et al., 1999; Yokoi et al., 1999, 2001). Information regarding the chemical structure of residual lignin is obtainable directly from pulp samples.

Del Rio et al. (2001) reported that Py-GC/MS is valuable for the characterization of residual lignin markers because of its sensitivity when individual-ion chromatographic profiles for selected lignin markers are used. Selection of lignin markers is very important for lignin characterization as described previously (Lima, et al. 2008; Nunes et al., 2010).

Fig. 1.7 Photo of equipment used for Py-GC/MS.
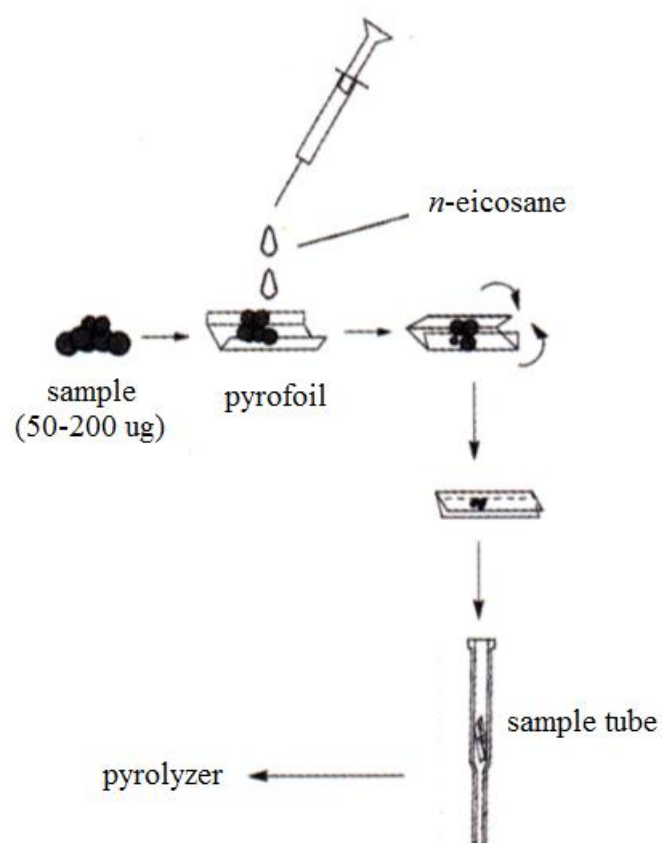
Fig. 1.8 Sample preparation for Py-GC/MS (Kuroda and Nakagawa-Izumi, 2006).

1.6   Syringyl/Guaiacyl and Syringaldehyde/Vanillin ratio

In studies of lignin model compounds, syringyl type lignin was cleaved much faster than guaiacyl type lignin by soda and soda-AQ treatment (Tsutumi et al., 1995). This high-reactivity lignin may contribute to the faster delignification rate of the materials. The delignification rate depends on the S/G ratio (Chang et al., 1973). Several studies have shown that wood with an increased S/G ratio is more easily delignified and provides higher pulp yields (Collins et al., 1990; Wallis et al. 1996., Goncalez-villa et al., 1999; Del Rio et al., 2005).

In the current study, the S/G ratio is typically obtained using the Py-GC/MS analytical method, whereas the Sa/Va ratio is obtained by NBO. Generally, higher syringaldehyde yield is attributed to a lower value of uncondensed syringyl units, and the high Sa/Va ratio results from the condensation of guaiacyl units. Furthermore, the Sa/Va ratio has been found to correlate with the proportion of the condensed type lignin (Xie, 2004). It has also been reported that lower product yields obtained from NBO occur because of the higher content of condensed lignin (Jin et al., 2012).

Comparison between the Py-GC/MS and NBO analytical methods for the determination of the S/G and Sa/Va ratio, respectively, has been conducted in several previous studies to test the efficiency of both methods (Lima et al., 2008; Nunes et al., Ohra-aho et al., 2013). The results showed that both the S/G and Sa/Va ratio are compatible and are well-correlated. However, the S/G and Sa/Va ratios were similar but not equal (Ohra-aho et.al., 2013). Lower values were obtained by Py-GC/FID (Izumi et al., 1997; Izumi et al., 1995). This may be because only β-O-4 linkages are cleaved in the NBO process, while in Py-GC/MS, other linkages of lignin are also cleaved to some extent (Ohra-aho et al., 2013). Py-GC/MS is preferred because of its advantages such as more rapid operation and lower chemical consumption.

1.7   Objectives of This Study

The first objective was to characterize the changes in lignin structure in EFB thermomechanical pulp (TMP) by using Py-GC/MS and nitrobenzene (NBO) to improve the understanding of the difference in the TMP long fiber obtained using the thermomechanical pulping process. Next, we aimed to clarify the reliability of Py-GC/MS as a method for analyzing the syringyl and guaiacyl structures in TMP.

The second objective was to develop Py-GC/MS analysis as a novel method for determining enzyme quantity, S/G ratio of lignin, and other components present in the residual lignin enzymatically isolated from EFB pulps, as well as to characterize the residual lignin present in soda-AQ and PH-soda-AQ EFB pulps.

References

Akgul, M., Tozluoglu, A., 2009. A comparison of soda and soda-AQ pulps from cotton stalks. African J. of Biotech. 22, 6127-6133.

Akiyama, T., Goto, H., Nawawi, D.S., Syafii, W., Matsumoto, Y., Meshitsuka, G., 2005. Holzforschung, 59 (3), 276-281.

Al-Dajani, W.W., Gellerstedt, G., 2002. On the isolation and structure of softwood residual lignin. Nord. Pulp Pap. Res. J. 17:2, 193-198.

Balakshin, M.Y., Capanema, E.A., Chang, H.M., 2007. MWL fraction with a high concentration of lignin-carbohydrate linkages: isolation and 2D NMR spectroscopic anlaysis. Holzforschung 61, 1-7.

Bjorkman, A., 1957. Lignin and lignin-carbohydrate complexes: extraction from wood meal with neutral solvents. Ind. Eng. Chem. 49, 1395-1398.

Blain, T. J., 1993. Anthraquinone pulping: fifteen years later. TAPPI J. 76, 137-146.

Boerjan, W., Ralph, J., Baucher, M., 2003. Lignin biosynthesis. Annu. Rev. Plant Biol. 54, 519-546.

Bolker, H.I., Somerville, N.G., 1962. Ultraviolet spectroscopic studies of lignin in the solid state. I. Isolated lignin preparations. TAPPI J. 45, 826-829.

Chang. H.M., Sarkanen, K.V., 1973. Species variation in lignin - Effect of species on the rate of kraft pulping. TAPPI 56, 132-134.

Campbell, M.M., Sederoff, R.R., 1996. Variation in lignin content and composition. Mechanism of control and implications for the genetic improvement of plants. Plant Physiol. 110, 3-13.

Casey, J.P., 1981. Pulp and Paper Chemistry and Chemical Technology, 3rd Edition, A Wiley Interscience Publication, New York, 304 – 310.

Del Rıo, J.C., Gutierrez, A., Martınez, M.J., Martınez, A.T., 2001. Py-GC/MS study of *Eucalyptus globulus* wood treated with different fungi. J. Anal. Appl. Pyrolysis 58-59, 441-452.

Del Rıo, J.C., Gutierrez, A., Hernando, M., Landın, P., Romero, J., Martınez, A.T., 2005. Determining the influence of eucalypt lignin composition in paper pulp yield using Py-GC/MS. J. Anal. Appl. Pyrolysis 74, 110–115.

Enoki, A, Koshijima, T., 1978. The mode of linkage of lignin with carbohydrates, In: Nakano J (ed) Chemistry of lignin. Uni-Koho, Tokyo, 54.

Eriksson, O., Goring, D., Lindgren, B., 1980. Structural studies on the chemical bonds between lignins and carbohydrates in spruce wood. Wood Sci. Technol. 14, 267-279.

Faix, O., 1992. Fourier Transform Infrared Spectroscopy. In: Lin, S.Y. and Dence, C.W. (eds) Method in Lignin Chemistry. Berlin, Springer-Verlag, 233-241.

Francis, R.C., Shin, S.J., Omori, S., Amidon, T.E., Blain, T.J., 2006. Soda pulping of hardwoods catalyzed by AQ and methyl substituted AQs. J. Wood Chem. Technol. 26(2), 141-152.

Freudenberg, K., 1965. Lignin: its constitution and formation from *p*-hydroxycinnamyl alcohols. Sci. 148, 595-600.

Gellerstedt, G.; Robert, D., 1987. Structural changes in lignin during kraft cooking. (7). Quantitative C (13) NMR Analysis of Kraft Lignins. Acta. Chem. Scand., B41 (7), 541-546.

Gellerstedt, G., Pranda, J., 1994. Structural and molecular properties of residual birch kraft lignins J. Wood Chem. Technol. 14, 467-482.

Ghosh, K.L., Venkatesh, V., Chin, W.J. and Gratzl, J.S., 1977. TAPPI, 1977, 60(11), 127.

Gierer, J.; Imsgard, F.; Pettersson, I., 1976. Reactions of lignin during sulfate pulping (14) Possible condensation and polymerization reactions of lignin fragments during alkaline pulping processes. J. Appl. Polym. Sci. (Appl. Polym. Symp.) 28, 1195- 1211.

Gonzalez-Vila, F.J., Almendros, G., Del Rio, J.C., Martin, F., Gutierrez, A., Romero, J., 1999. Ease of delignification assessment of wood from different *Eucalyptus* species by pyrolysis (TMAH)-GC/MS and CP/MAS ^{13}C-NMR spectrometry. J. Anal. Appl. Pyrolysis 49, 295-305.

Gorski, D., Hill, J., Engstrand, P., Johansson, L., 2010. Review: Reduction of energy consumption in TMP refining through mechanical pre-treatment of wood chips. Nord. Pulp Pap. Res. J. 25, 151-161.

Goyal, G.C., 1997. Anthraquinone Pulping: A Tappi Press Anthology of Published Papers, 1977–1996. TAPPI Press, Atlanta, GA.

Grima-Pettenati J., Goffner, D., 1999. Lignin genetic engineering revisited UMR CNRS: UPS 5546, Poˆle de Biotechnologie Veˊgeˊtale, 24 Chemin de Borde Rouge, BP 17, Auze6ille, 31 326 Castanet Tolosan, France.

Hatfield, R., Vermerris, W., 2001. Lignin formation in plants. The dilemma of linkage specificity. Plant Physiol. 126, 1351-1357.

Hayashi, A., 1961. Study on the chemical combination between lignin and carbohydrate. X. The existence of phenol-fl-glycoside bond. J Agr Chem Soc Jpn 35, 83-86.

Harsono, H., Putra, A.S., Maryana, R., Rizaluddin, A.T., H'ng, Y.Y., Nakagawa-Izumi, A., Ohi, H., 2016. Preparation of dissolving pulp from oil palm empty fruit bunch by prehydrolysis soda-anthraquinone cooking methods. J. Wood Sci. 62, 65-73.

Holton, H. H., 1977. Soda additive softwood pulping: A major new process, Pulp Paper Can. 78 (10), T218-T223 (1977).

Hortling, B., Ranua, M., Sundquist, J., 1990. Investigation of the residual lignin in chemical pulps Part 1. Enzymatic hydrolysis of the pulps and fractionation of the products. Nord. Pulp Pap. Res. J. 5 (1), 33-37.

Ibarra, D., Del Rio, J.C., Gutierrez, A., Rodriguez, I.M., Romero, J., Martinez, M.J., Martinez, A. T., 2005. Chemical characterization of residual lignin from eucalypt paper pulps. J. Anal. Appl. Pyrolysis 74, 116-122.

Ibrahim, M. M., El-Zawawy, W. K., Abdel-Fattah, Y. R., Soliman, N. A.., Agblevor, F. A., 2011. Comparison of alkaline pulping with steam explosion for glucose production from rice straw. Carbohydrate Polymers 83, 720-726.

Iiyama, K., Stone, B., 1994. Lignin and hydroxycinnamic acids in walls of brown midrib mutant of sorgham, pearl millet and maize stems. In: Proceedings of the 39th Lignin symposium, Fukuoka, Japan, 1994, 9-12.

Izumi, A., Kuroda, K., 1997. Pyrolysis-gas chromatography analysis of dehydrogenation polymers with various syringyl to guaiacyl ratio. Mokuzai Gakkaishi 43, 194-202.

Izumi, A., Kuroda, K., Ohi, H., Yamaguchi, A., 1995. Structural analysis of lignin by pyrolysis-gas chromatography (III) - comparative studies of pyrolysis-gas chromatography and nitrobenzene oxidation for the determination method of lignin composition in hardwood. Jpn. TAPPI J. 49, 1339-1346.

Jääskelä, A.S., Sun, Y., Argyropoulos, D., Tamminen, T., Hortling, B., 2001. The effect of isolation method on the chemical structure of residual lignin, in: Proc. 11th Int. Symp. on Wood and Pulping Chem., Nice, France, vol. 1, 239–243.

Jin, Z., Jin, G., Shao, S., 2012. Lignin characteristics of bast fiber and core in kenaf, bark and wood of paper mulberry and mulberry. J. Wood Sci. 58. 144-152.

Kamarudin, H., Ariffin, D., Johari, S., 1999. Pulp and paper from oil palm biomass. Oil palm and the environment: a Malaysian perspective 213-221.

Kato, A., Azuma, J., Koshijima, T., 1983. A new feruloylated trisaccharide from bagasse. Chem. Lett 1983, 137-140.

Khristova, P., Kordsachia, O., Patt, R., Khider, T., Karrar, I., 2002. Alkaline pulping with additives of kenaf from Sudan. Ind. Crops and Prod. 15, 229–235.

Kringstad, K., Morck, R., 1983. 13C-NMR spectra of kraft lignin. Holzforschung 37 (5), 237-244.

Kuroda, K., Izumi, A., Mazumder, B.B., Ohtani, Y., Sameshima, K., 2002a. Characterization of kenaf (*Hibiscus cannabinus*) lignin by pyrolysis-gas chromatography-mass spectrometry in the presence of tetramethylammonium hydroxide. J. Anal. Appl. Pyrolysis 64, 453-463.

Kuroda, K., Nakagawa-Izumi, A., 2006. Application of tetramethylammonium hydroxide (TMAH) thermochemolysis for elucidation of lignin structure, in: Silva, J.A.T. (eds.), Floriculture, Ornamental and Plant Biotech., Advances and Topical Issues, Volume IV, 64-83.

Kuroda, K., Nishimura, N., Izumi, A., Dimmel, D.R., 2002b. Pyrolysis of lignin in the presence of tetramethylammonium hydroxide: A convenient method for S/G ratio determination. J. Agric. Food Chem. 50, 1022-1027.

Labid, J., Tejado, A., Garcia, A., Jimenez, L., 2008. Simulation of tagasaste pulping using soda-anthraquinone, Biores. Technol. 99(15), 7270-7277.

Lachenal, D., Fernandes, J.C., Froment, P., 1995. Behavior of residual lignin in kraft pulp during bleaching, J. Pulp Pap. Sci. 21 (5) J173–J177.

Lai, Y. Z., Sarkanen, K.V., 1957. Isolation and structural studies. In: Sarknen KV, Ludwig CH (eds) Lignins: occurrence, formation,135 structure and reactions, Wiley-Interscience, New York, 165-230.

Lain, T.B.T., Iiyama, K., Stone, B.A., 1992. Changes in phenolic acids from internode walls of wheat and PHALA RIS during maturation. Phytochemistry 31, 2655-2658.

Leary, G.J., Newman, R.H., 1992. Cross polarization/magic angle spinning nuclear magnetic resonance (CP/MAS NMR) spectroscopy, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany, 146-161.

Li, J., Henriksson, G., Gellerstedt, G., 2005. Carbohydrate reactions during high-temperature steam treatment of aspen wood. Appl. Biochem. Biotech. 125, 175–188.

Li, X., Weng, J.K., Chapple, C., 2008. Improvement of biomass through lignin modification. Plant J. 54, 569-581.

Lima, C.F., Barbosa, L.C.A., Marcelo, C.R., Silverio, F.O., Colodette, J.L., 2008. Comparison between analytical pyrolysis and nitrobenzene oxidation for determination of syringyl/guaiacyl ratio in *Eucalyptus* spp. lignin. BioResource 3, 707-712.

Lundquist, K., 1992. Acidolysis, in: Lin, S.Y., Dence, C.W., eds, Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany, 289-300.

Meier, D., Faix, O., 1992. Pyrolysis-gas chromatography-mass spectrometry, in Lin, S.Y., Dence, C.W., eds., Methods in Lignin Chemistry, Springer-Verlag, Berlin, Germany, 177-199.

Minja, R.J.A., Karlsen, T., Kleppe, P.J., 1997. Modified polysulphide (AQ)-pulping of softwood. In: TAPPI Pulping Conference Proceedings, 721.

Mulyantara, L.T., Harsono, H., Maryana, R., Ohi, H., 2016. Properties of thermomechanical pulps from sugarcane bagasse and oil palm empty fruit bunch as non-wood materials. Proceedings of 83rd Pulp Pap. Res. Conf. Jpn TAPPI, 22-23 June, Tokyo, 119-122.

Nomura, Y., Nakamura, M., 1978. Studies on quinone additive cooking. Part 1 Effect of quinone addition on alkaline cooking. Jpn. TAPPI J. 32, 713-721.

Nunes, C.A., Lima, C.F., Barbosa, L.C.A., Colodette, J.L., Gouveia, A.F.G., Silcwrio, F.O., 2010. Determination of *Eucalyptus* spp. lignin S/G ratio: a comparison between methods. Bioresour. Technol. 101, 4056-4061.

Ohi, H., 1994. Function of anthraquinone as pulping additive and its possibilities. Jpn. TAPPI J. 48, 531-544.

Ralph, J., Hatfield, R.D., 1991. Pyrolysis-GC-MS characterization of forage materials. J. Agric. Food Chem. 39, 1426-1437.

Rodrigues, J., Meier, D., Faix, O., Pereira, H., 1999. Determination of tree to tree variation in syringyl/guaiacyl ratio of *Eucalyptus globulus* wood lignin by analytical pyrolysis, J. Anal. Appl. Pyrolysis, 48:2, 121-128.

Rowell, R.M., 2005. Handbook of Wood Chemistry and Wood Composites, CRC Press, Boca Raton, Florida.

Sipilfi, J., Brunow, G., 1991. On the mechanism of formation of noncyclic benzyl ethers during lignin biosynthesis. Holzforschung 45(suppl), 3-7.

Sixta, H., 2006. Handbook of pulp. 2006, 1st edn." WILEY-VCH, Weinheim

Smook, G.A., 1992. Handbook for Pulp and Paper Technologist, 2nd Edition, Angus Wilde Publications, Vancouver.

Schultz, T.P., Templeton, M.C., 1986. Proposed mechanism for nitrobenzene oxidation of lignin. Holzforschung 40, 93-97.

Smelstorius, J.A., 1974. Chemical composition of wood of Australian-grown *Pinus radiata*. III. Lignin-polysaccharide complexes. Holzforschung 28(3), 99-101.

Sonoda, T., Ona, T., Yokoi, H., Ishida, Y., Ohtani, H., Tsuge, S., 2001. Quantitative analysis of detailed lignin monomer composition by pyrolysis-gas chromatography combined with preliminary acetylation of the samples, Anal. Chem., 73:22, 5429-5435.

Studer, M, H., DeMartini, J.D., Bavis, M.F., Sykes, R.W., Davison, B., Keller, M., Tsukan, G.A., Wyman, C.E., 2011. Lignin content in natural *Populus* variants affects sugar release. Proc. Natl. Acad. Sci. U.S.A. 108, 6300-6305.

Takayama, M., Johjima, T., Yamanaka, T., Wariishi, H., Tanaka, H., 1997. Fourier transform Raman assignment of guaiacyl and syringyl marker bands for lignin determination. Spectrochimica Acta Part A 53, 1621-1628.

Tanahashi, M., 1990. Characterization and degradation mechanism of wood components by steam explosion and utilization of exploded wood. Wood Res. 77, 49–117.

Tsutumi, Y., Kondp, R., Imamura, H., 1995. The difference of reactivity between syringyl lignin and guaiacyl lignin in alkaline systems. Holzforschung 49, 423-428.

Vanholme, R., Demedts, B., Morreel, K., Ralph, J., Boerjan, W., 2010.Lignin biosynthesis and structure. Plant Physiol. 153, 895-905.

Wan Rosli, W. D., Law, K.N., Valade, J.L., 1998. Chemical pulping of oil palm empty fruit bunches. Cellulose Chem. Technol. 32, 133-143.

Wan Rosli, W. D., Leh, C.P., Zainudin, Z., Tanaka, R., 2003. Optimisation of soda pulping variables for preparation of dissolving pulps from oil palm fibre. Holzforschung 57, 106-114.

Wan Rosli, W.D., Leh, C.P., Zainuddin, Z., Tanaka, R., 2004. Effects of prehydrolysis on the production of dissolving pulp from empty fruit bunches. J. of Tropical Forest Sci 16 (3), 343-349.

Xie, Y., 2004. Difference of condensed lignin structures in *Eucalyptus* species. Nord. Pulp Pap. Res. J. 19, 18-21.

Yaku, F., Yamada, Y., Koshijima, T., 1976. Lignin carbohydrate complex. II. Enzymic degragation of acidic polysaccharides in Björkman LCC. Holzforschung 30, 148-156.

Yamasaki, T., Chen, C.L., Gratzl, J.S., Chang, H.M., 1981. Characterization of residual lignin in kraft pulp. In: Proceedings of the 1st International Symp. on Wood and Pulping Chem. 2, Stockholm, Sweden: 34-42.

Yokoi, H., Ishida, Y., Ohtani, H., Tsuge, S., Sonoda, T., Ona, T., 1999. Characterization of within-tree variation of lignin components in *Eucalyptus camaldulensis* by pyrolysis-gas chromatography. Analyst 124, 669–674.

Yokoi, H., Nakase, T., Ishida, Y., Ohtani, H., Tsuge, S., Sonoda, T., Ona, T., 2001. Discriminative analysis of *Eucalyptus camaldulensis* grown from seeds of various origins based on lignin components measured by pyrolysis-gas chromatography. J. Anal. Appl. Pyrolysis 57, 145–152.

You, T., Zhang, L., Zhou, S., Xu, F., 2015. Structural elucidation of lignin-carbohydrate complex (LCC) preparations and lignin from *Arundo donax* Linn. Ind. Crops Prod. 71, 65-74.

Yuan, T., Sun, S., Xu, F., Sun, R., 2011. Characterization of lignin structures and lignin-carbohydrate complex (LCC) linkages by quantitative ^{13}C and 2D HSQC NMR spectroscopy. J. Agri Food Chem 59, 10604-10614.

Yunos, N.S.H.M., Baharuddin, A.S., Yunos, K.F.M., Hafid, H.S., Busu, Z., Mokhtar, M.N., Sulaiman, A., Som, A.M., 2015. The physicochemical characteristics of residual oil and fibers from oil palm empty fruit bunches. BioResources 10, 14-29.

Zier, N., Schiene, R., Fischer, K., 1997. Structural characterization of an organosolv lignin by analytical pyrolysis and wet chemical degradation methods. J. Anal. Appl. Pyrolysis 40-41, 525-551.

Chapter 2 Characterization of Syringyl and Guaiacyl Lignins in Thermomechanical Pulp from Oil Palm Empty Fruit Bunch by Pyrolysis-Gas Chromatography-Mass Spectrometry Using Ion Intensity Calibration

2.1 Introduction

Oil palm (*Elaeis guineensis*) is cultivated in West and Central Africa, where it originated, and is one of the largest commercial crops in Malaysia, Indonesia, and Thailand. However, the empty fruit bunch (EFB) of oil palm also has many negative impacts on the biodiversity and the environment (Obidzinski *et al*., 2012). Moreover, global production of paper and paperboard has increased year by year because of significant growth of the global economy, resulting in a shortage of wood. EFB fibers are abundantly available and could potentially replace wood in a variety of industries. EFB fibers could be useful for preparing paperboard, dissolving pulp, and have other applications in treating agricultural waste (Harsono *et al*., 2015, 2016).

Development of thermomechanical pulp (TMP) was established in the 1970s. Today, thermomechanical pulping is the most dominant among all mechanical pulping methods (Gorski *et al*., 2010). In thermomechanical pulping, pressurized steam is introduced to the refining process to soften the lignin of the materials. The main purpose of refining is to apply a mechanical force to the materials in order to separate and fibrillate the fibers, which is achieved by removing the middle lamella from the cell walls (Li *et al*., 2011).

In addition to paperboard production, dried fibers of EFB TMP are useful in medium-density fiberboard (MDF) as an alternative to wood. In previous studies, TMP was successfully obtained from raw EFB material by dry- and wet-blowing methods, and the paper properties of the pulp were clarified (Mulyantara *et al*., 2016a, 2016b). However, the characteristics of

lignin chemical feature which should affect bleaching ability and paper properties for EFB TMP have not yet been clarified.

Nitrobenzene oxidation (NBO) has been widely used to study the characteristics of lignin structures (Chen, 1992; Nishimura *et al*., 2002; Claudio *et al*., 2008). Syringaldehyde (S_a) and vanillin (V_a), the main aromatic aldehydes, are obtained from lignin by NBO. S_a and V_a represent the non-condensed structures of lignin, whereas the condensed structures of carbon–carbon linkages between the side chains and the aromatics of lignin do not form S_a and V_a. Cupric oxide oxidation and NBO with organic solvent extraction and gas chromatography (GC) are very time consuming and chemically consuming for lignin characterization. Thus, pyrolysis-gas chromatography (Py-GC) has been recommended as an alternative analytical method because it is faster, safer, and more economical than other methods (Kuroda *et al*., 2005a; Claudio *et al*., 2008).

Analytical pyrolysis has been widely applied to the characterization of lignocellulosic materials (Galletti and Bocchini, 1995; Moldoveanu 1998; Yokoi *et al*., 1999; Kuroda and Nakagawa-Izumi, 2005b; Alves *et al*., 2006). This method incorporates Py-GC with a flame ion detector (FID) and Py-GC/mass spectrometry (MS) (Faix *et al*., 1988; Kleen *et al*., 1993; Sjoberg *et al*., 2002; Rencoret *et al*., 2009).

Py-GC/MS analysis of various hardwood materials gives a good relationship in syringyl/guaiacyl (S/G) and syringaldehyde/vanillin (S_a/V_a) ratios between Py-GC/FID and GC analysis of the NBO products (Izumi *et al*., 1995). However, the reliability of Py-GC/MS using total ion (TI) intensity to obtain a similar relationship between the Py-GC/MS and the NBO method has not been clarified yet. S/G and S_a/V_a molar ratios are important indicators of the reactivity of lignin (Ibarra *et al*., 2005; Rovia *et al*., 2011), and they have been roughly calculated from the TI response of selected S- and G-type products (Lima *et al*., 2008; Nunes *et al*., 2010).

In this study, lignin in EFB TMP was characterized by Py-GC/MS and NBO to better understand the structural changes of TMP long fibers and fines in the process. First, fractions of TMP long fibers and fines were compared, and then the reliability of Py-GC/MS as a method of analyzing the syringyl and guaiacyl structures was clarified.

2.2 Experimental

2.2.1 Materials

Oil palm EFB was obtained from PT. Madukismo in Yogyakarta, Indonesia, and PT. Perkebunan Nusantara VIII in Bogor, West Java, Indonesia. The materials, which had a 10% moisture content, were cut into lengths of 0.4–0.5 cm by a laboratory disk mill for preparing thermomechanical pulping. Japanese hardwoods materials (*Pupulus sieboldii, Quercus myrsinaeforia, Quercus crispula, Castanopsis cuspidate*, and *Aesculus turbinate*) as shown in Fig. 2.1 were provided by the Forestry and Forest Products Research Institute, Tsukuba, Japan. The materials were milled into particles (60–80 mesh size) for acetone extraction and chemical analysis.

2.2.2 TMP Fabrication and Dry Fractionation with Screens

EFB TMP was made 160 °C using the dry-blowing method according to a procedure previously reported (Mulyantara *et al*., 2016b). The dried TMP fibers and fines were separated into four fractions using screens with 850, 355, and 180 μm openings (20, 45, and 80 mesh, respectively). Approximately 10 g of dried TMP was placed into the upper screen (850 μm opening) with 30 stainless steel balls (12.5 mm diameter) to disperse the fibers so that they dropped smoothly in subsequent screens during shaking for 2 min. These four fractions were denoted as "On 850 μm opening," "850-355 μm opening," "355-180 μm opening," and "Pass 180 μm opening" as shown in Fig. 2.2

2.2.3 Analysis of Chemical Composition for EFB Materials and TMP

The contents of acid-insoluble lignin, acid-soluble lignin, extractives, and ash were determined using TAPPI Test Methods T 222 om-15, T 204 cm-07, and T 211 om-02. The amount of glucose, xylose, and others sugars in the acid hydrolysate of materials and TMP were determined using ion chromatography (Harsono et al. 2016).

2.2.4 Pyrolysis-gas Chromatography-mass Spectrometry

2.2.4.1 Making Calibration Lines of Lignin Pyrolysis Products Using Main Ion Intensity

The conditions of Py-GC/MS were as follows: Pyrolyzer, JHP-5 (Japan Analytical Industry Co. Ltd. Japan); pyrolysis conditions, 500 °C for 4 s; GC/MS system, QP-5050A (Shimazdu, Japan); column, HP 1-MS (30 m × 0.25 mm; film thickness: 1.0 µm). Temperature profile for GC analysis: 1 min at 50 °C, 50–280 °C (at a rate of 5 °C/min), and 13 min at 280 °C. Approximately 150–180 µg of each sample prepared with n-eicosane (0.1 µg) as an internal standard (IS) was placed on a ferromagnetic pyrofoil and tightly wrapped (Geib et al. 2008). Then, the sample-loaded pyrfoil was inserted into a quartz sample tube. The sample holder with the sample tube was centered in the pyrolyzer, and then Py-GC/MS started. The main ion intensity (MI) conversion factors (weight-to-MI area on the chromatogram) for the lignin pyrolysis products were obtained by making calibration lines between the weight and area ratios of commercial monomeric lignin model compounds and the internal standard. Approximately 15 mg of each compound was dissolved in 50 mL of ethanol. Calibration lines were created by injections of various amounts (approximately 150, 300, 600, 900, and 1200 µg) of the compound with 0.1 µg of IS. Three replicas were performed for each sample.

2.2.4.2 Determination of Lignin Pyrolysis Products Yields and Syringyl/ Guaiacyl Molar Ratios

The amount of each lignin pyrolysis product was determined using the MI conversion factor of the product. The yields, which were based on the weight of the prepared sample (or its acid-insoluble lignin content) and the S/G molar ratio, were then evaluated.

2.2.5 Nitrobenzene Oxidation

According to a modified NBO method (Chen, 1992), approximately 200 mg (oven-dried weight) of the prepared sample, 7.0 mL of 2 N NaOH, and 0.4 mL of nitrobenzene (chemical analysis pure grade) were placed together in a 10 mL steel reactor and oxidized at 170 °C for 2 h. Then, ethyl vanillin (2 mg) as an internal standard was added to the reaction mixture. The mixture was extracted three times using 20 mL of ether. The lower (water) layers of the mixture were gathered and acidified to pH 3–4 using 2 N HCl. The mixture was again extracted three times using 25 mL of ether. The upper (ether) layers were gathered and washed with 5 mL of water to remove the remaining acid. Then, approximately 20 g of anhydrous Na_2SO_4 was added to the collected ether layers, and the ether fraction was stored for 24 h. After filtration of the mixture, the ether was evaporated. S_a and V_a were concentrated and ready for GC. Above procedures of each sample were done for three replicas.

The conditions for GC equipped with FID were as follows: GC system, GC-17A (Shimadzu, Japan); column, DB-1 (30 m × 0.25 mm; film thickness: 0.25 μm) with helium gas as a carrier. Temperature profile for analysis: 15 min at 110 °C, 110–160 °C (at a rate of 5 °C/min), 160–280 °C (at a rate of 20 °C/min), and 7 min at 280 °C. The FID response factors for estimating the amount of S_a and V_a were obtained by making calibration lines between the weight ratios and area ratios of S_a and V_a to the internal standard. Calibration lines were created by injection with various concentrations of the compound (approximately 2, 4, and 2 mg/mL)

with the internal standard (2, 1, and 0.2 mg/mL). The amounts of S_a and V_a and the S_a/V_a molar

ratio were determined using the FID response factors of S_a and V_a.
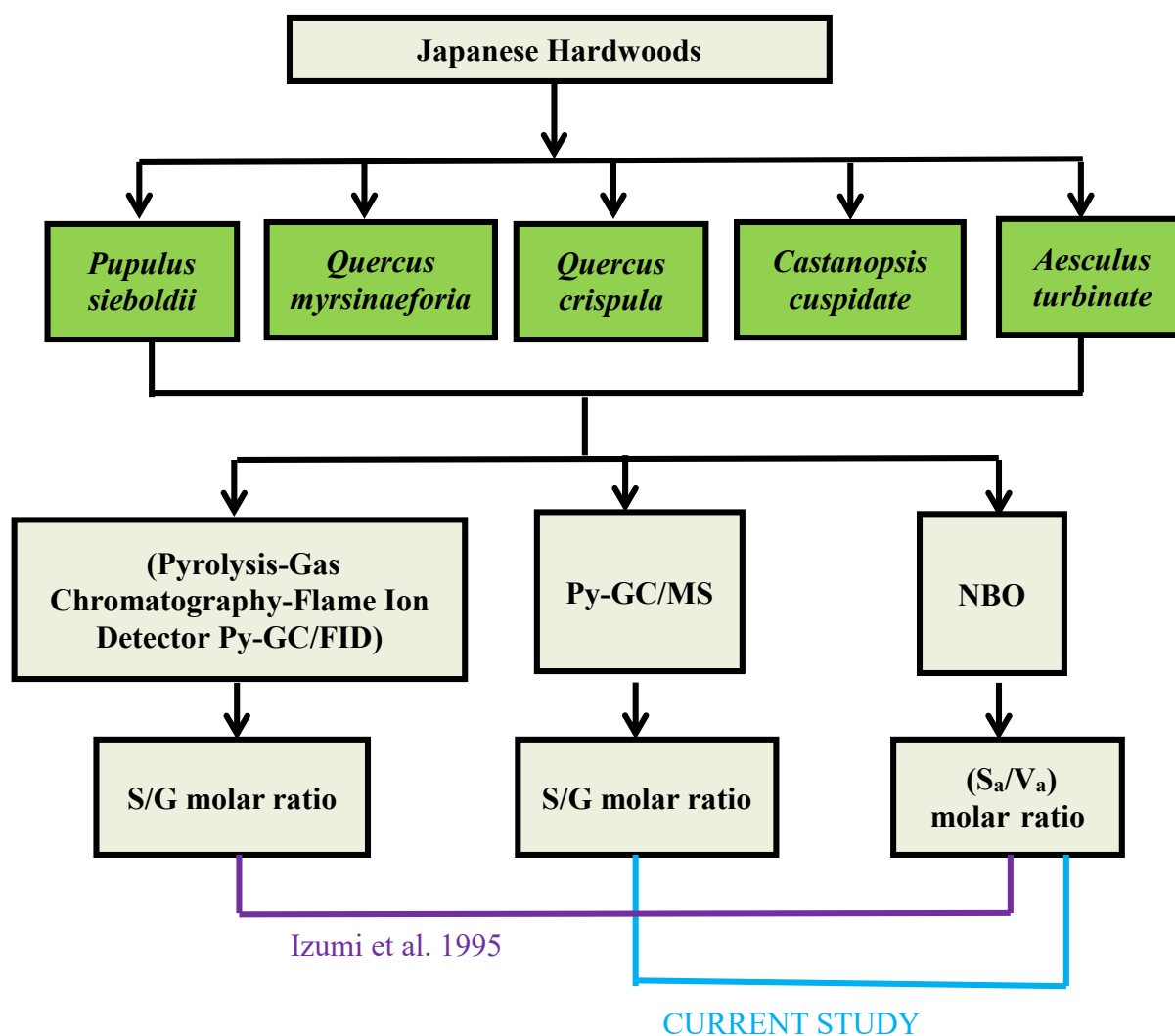
Fig. 2.1 Scheme of analytical methods for Japanese hardwoods.
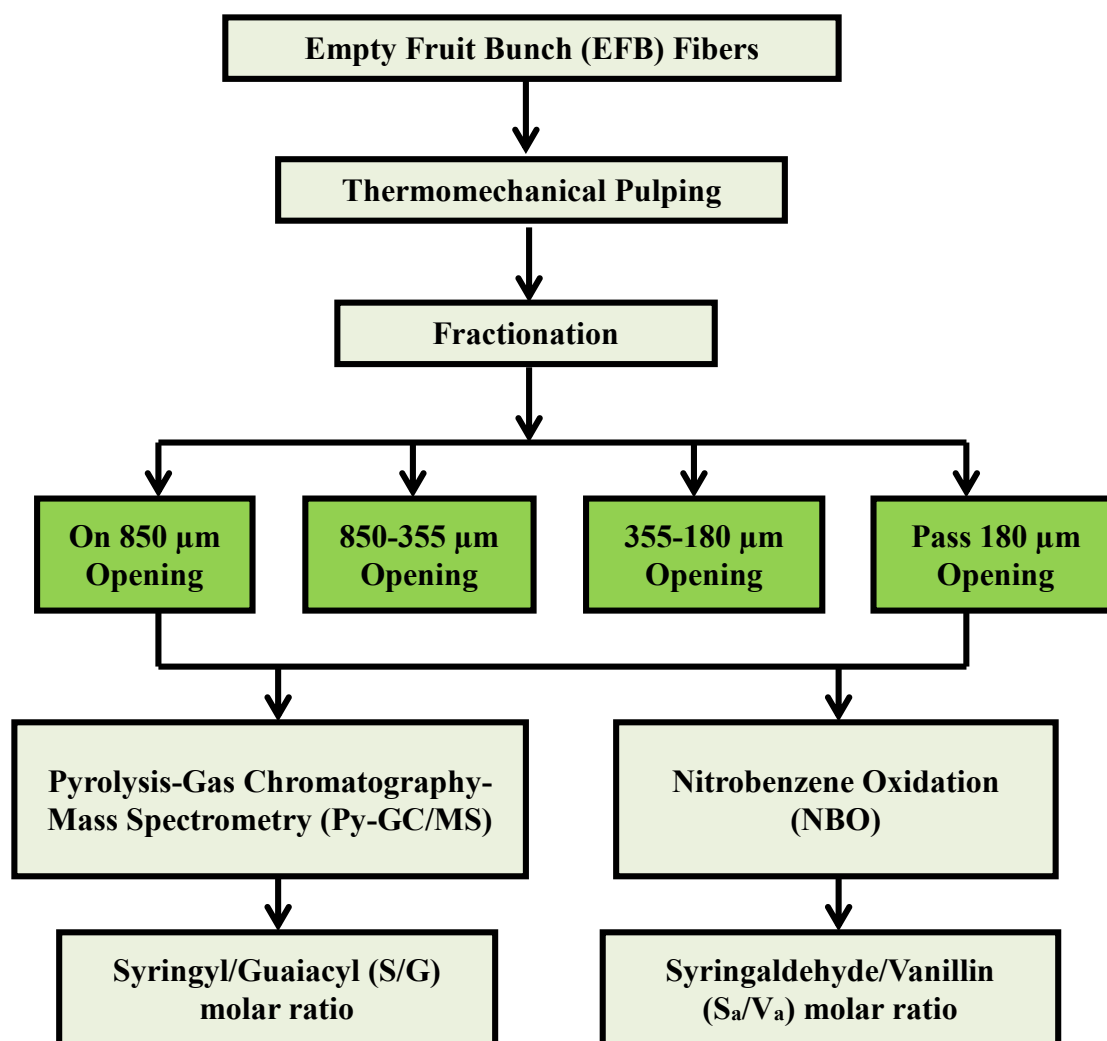
Fig. 2.2 Scheme of sample preparation and analytical methods for fractionated EFB TMPs.

2.3 Results and discussion

2.3.1 Chemical Compositions of Empty Fruit Bunch Raw Materials and Thermomechanical Pulps

As shown in Table 2.1, the raw EFB material and EFB TMP mainly consisted of lignin, glucan (cellulose), xylan, extractives, and ash. The EFB TMP showed relatively large amounts of other organics, such as those formed from hemicellulose, extractives, and components of ash during the TMP process. The total lignin contents of the raw EFB materials and EFB TMP were 29.6% and 28.4%, respectively. The slight decrease of lignin might have been the result of the small loss of lignin-rich fines peeled from the middle lamella of the EFB fibers according to a previous study on TMP from wood (Li *et al*., 2011).

Table 2.1 Chemical compositions of EFB raw materials and EFB TMP.

	Acid-insoluble lignin (%)	Acid-soluble lignin (%)	Glucan [a] (%)	Xylan (%)	Other sugars [b] (%)	Extractives [c] (%)	Ash (%)	Other organics [d] (%)
Materials	26.7 ± 0.2	2.9 ± 0.1	35.1 ± 0.2	19.8 ± 0.2	1.9 ± 0.6	5.4 ± 0.2	5.9 ± 0.2	2.3 ± 0.3
TMP	25.6 ± 0.3	2.8 ± 0.1	35.1 ± 0.2	17.9 ± 0.2	0.9 ± 0.6	3.7 ± 0.4	3.4 ± 0.5	10.6 ± 1.3

[a] As polymer of glucose

[b] Arabinose, galactose and mannose

[c] Acetone extractives

[d] Organic acids and other extractives

2.3.2 Pyrolysis-gas Chromatography-Mass Spectrometry of Empty Fruit Bunch Raw Materials and Thermomechanical Pulps

Py-GC/MS analysis of the raw EFB materials clearly showed eight pyrolysis products from lignin and one from palmitic acid (Harsono *et al*., 2016). Each compound was identified using the clear information of mass spectrum with a retention time of main ion chromatography. The Py-GC/MS analysis of the EFB TMP also clearly showed the same peaks (G1–G4, S1–S4, and P) in the total ion chromatogram (TIC) shown in Fig. 2.3, and structure of G1–G4 and S1–S4 are shown in Fig. 2.4. The intensities were similar to those of the raw EFB materials and were free of overlapping signals such as those from carbohydrates.
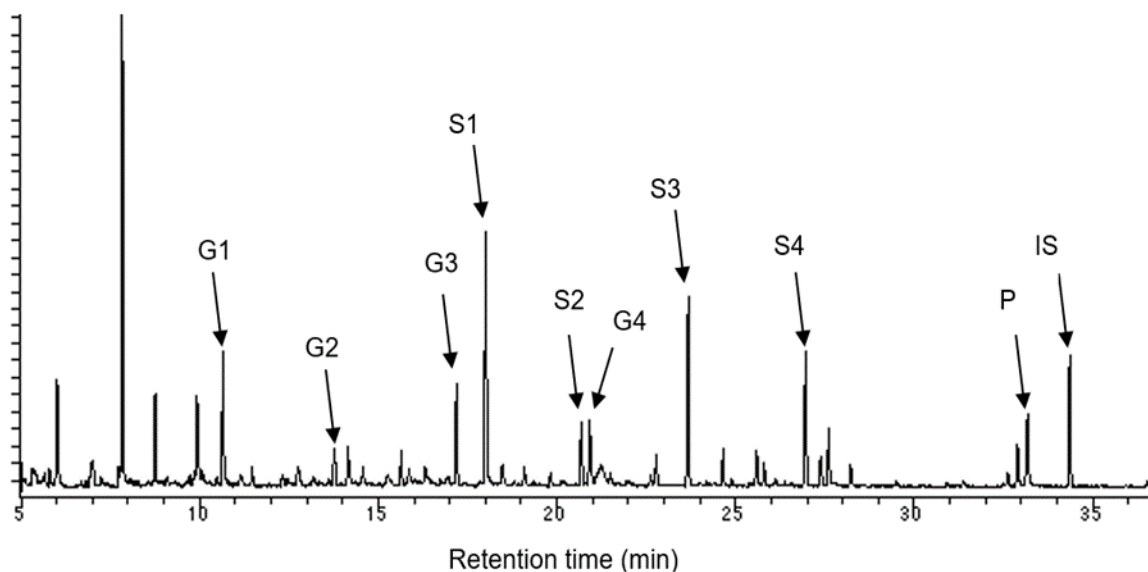
Fig. 2.3 Total ion chromatogram of pyrolysis products of EFB TMP by Py-GC/MS.

Note:

Peaks	Pyrolysis products	Main ions	Retention time (min)
G1	Guaiacol	124, 109	10.8
G2	4-Methylguaiacol	138, 123	14.9
G3	4-Vinylguaiacol	150, 135	17.2
G4	*trans*-Isoeugenol	164,149	21.1
S1	Syringol	154, 139	18.1
S2	4-Methysyringol	168, 153	20.8
S3	4-Vinylsyringol	180, 165	23.8
S4	4-*trans*-Prophenylsyringol	194,179	27.0
P	Palmitic acid	256	33.2
IS	*n*-Eicosane	282	34.3

G1: Guaiacol	G2: 4-Methylguaiacol	G3: 4-Vinylguaiacol	G4: *trans*-Isoeugenol
S1: Syringol	S2: 4-Methylsyringol	S3: 4-Vinylsyringol	S4: 4-*trans*-Prophenylsyringol
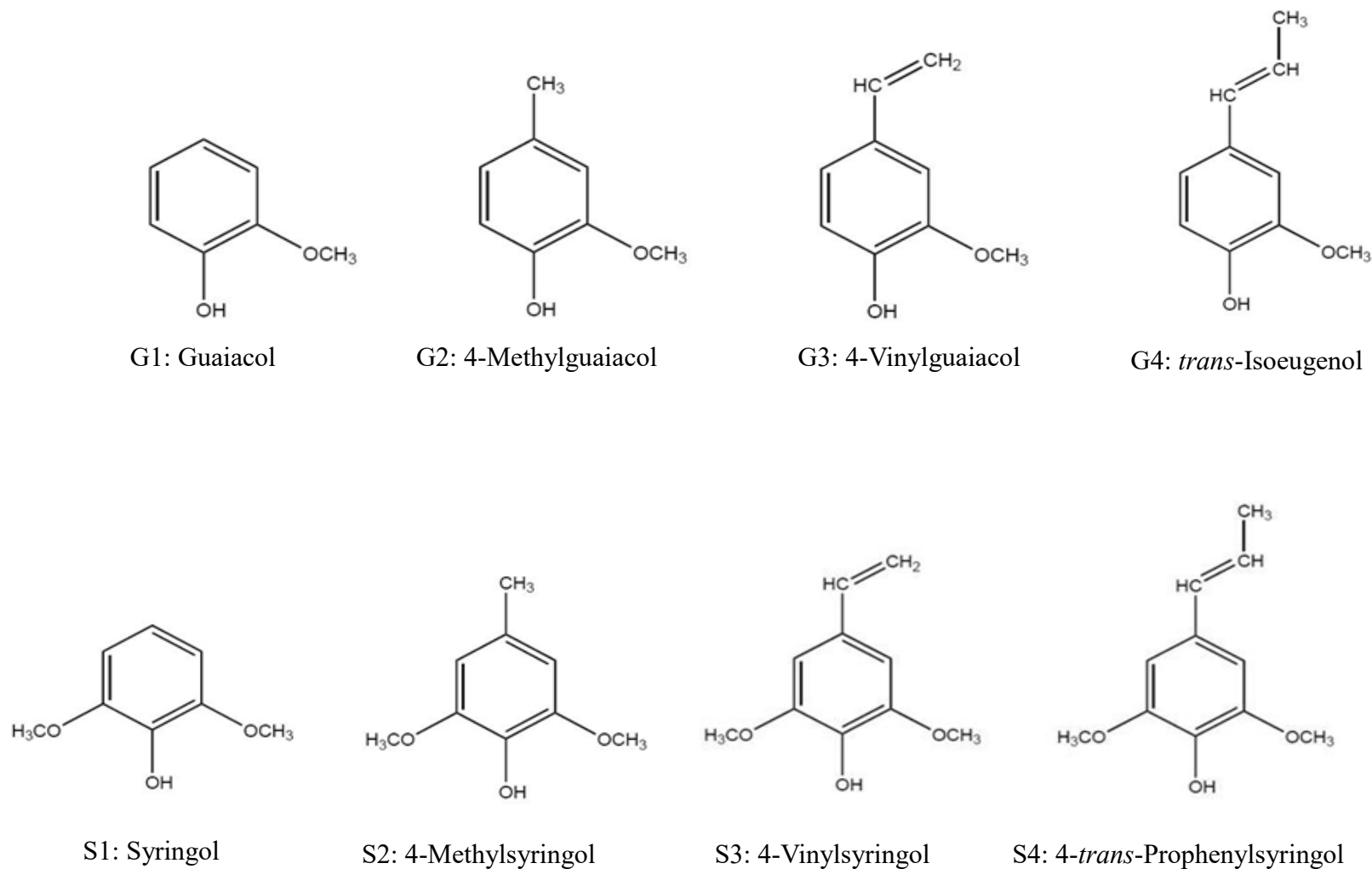
Fig. 2.4 Structures of main lignin pyrolysis products obtained by Py-GC/MS.

2.3.3 Determination of Main Ion Intensity Conversion Factors from Calibration Lines by Pyrolysis-Gas Chromatography-Mass Spectrometry

By using Py-GC/MS, two calibration lines for guaiacol (G1) and 4-*trans*-prophenylsyringol (S4) were obtained, which are shown in Fig. 2.5 and Fig. 2.6 as examples. The slope (11.47) of the calibration line is the conversion factor of the MI area ratio into the weight ratio using G1 and *n*-eicosane (IS). Conversion factors for G1–G4, S1, and S4 are listed in Table 2.2. The conversion factors for S2 and S3 were assumed to be the same as those for G2 and G3, respectively, because similar MI mass spectra intensities were obtained from the authentic compounds. For comparison, FID response factors obtained by Py-GC/FID (Izumi *et al*., 1995) are shown in Table 2.2, even though the MI response of each compound is theoretically different from its FID response. Py-GC/MS can generally provide high sensitivity and reliability as a precise analytical method for the characterization of lignin structures as compared to Py-GC/FID. Therefore, these MI conversion factors are very useful for determining the lignin in wood and non-wood materials and pulps.
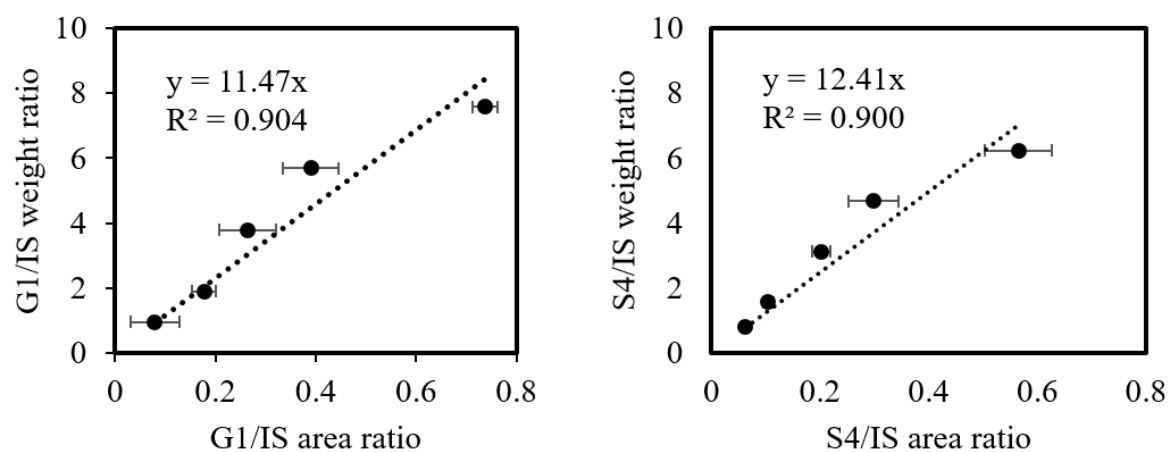
Fig. 2.5 Guaiacol (G1) and 4-*trans*-prophenylsyringol (S4) calibration lines by Py-GC/MS.
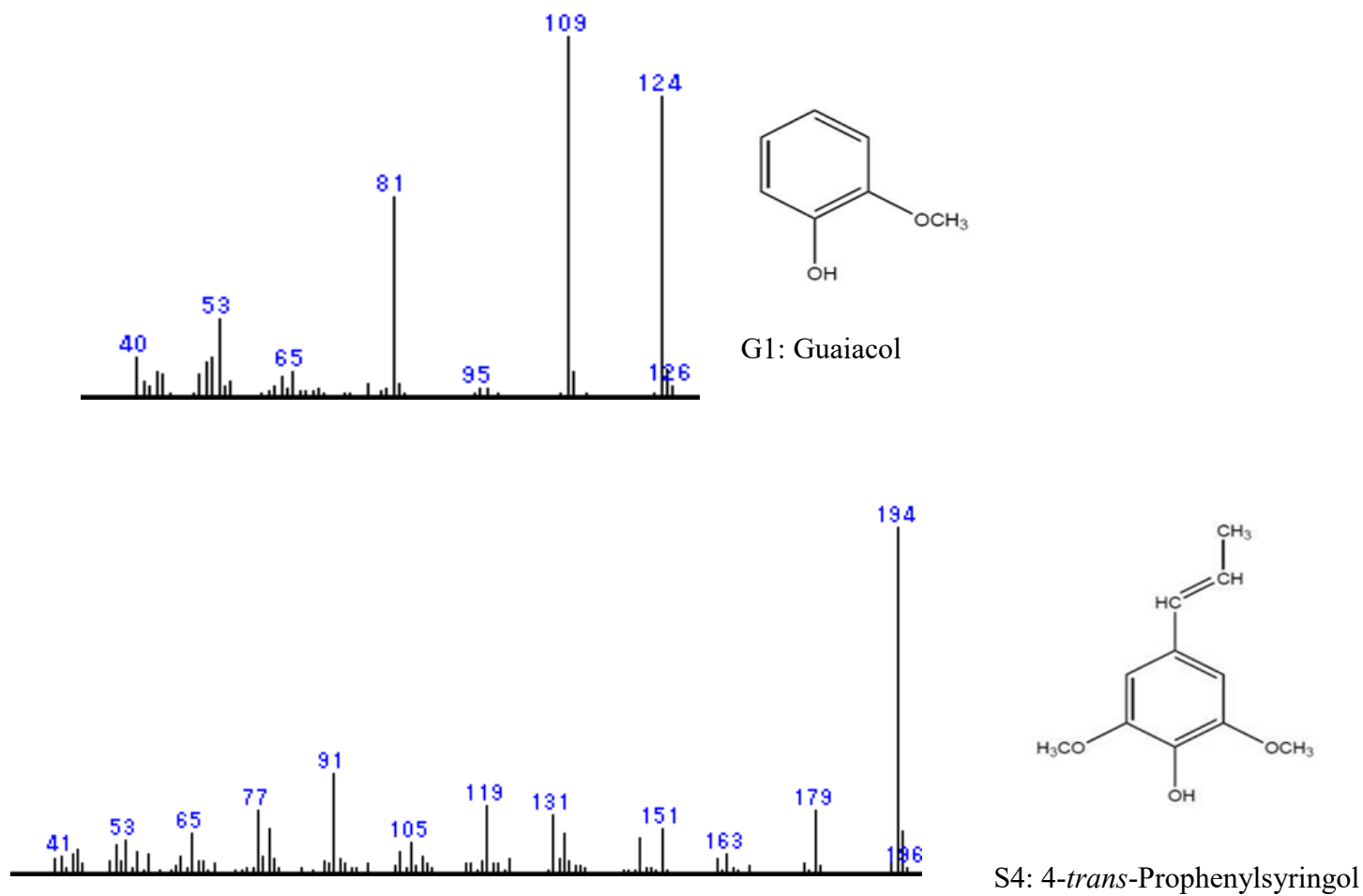
Fig. 2.6 Main ion (MI) intensity of G1 and S4.

Table 2.2 Conversion factors of pyrolysis products from lignin and palmitic acid by Py-GC/MS.

Peaks	Pyrolysis products	MI conversion factors by Py-GC/MS	FID response factors by Py-GC/FID [a]
G1	Guaiacol	11.47 ± 0.31	1.247
G2	4-Methylguaiacol	9.16 ± 0.52	1.162
G3	4-Vinylguaiacol	12.93 ± 0.41	1.673
G4	*trans*-Isoeugenol	10.86 ± 0.64	1.817
S1	Syringol	10.17 ± 0.71	1.601
S2	4-Methysyringol	$9.16^{b} \pm 0.52$	1.549
S3	4-Vinylsyringol	$12.93^{c} \pm 0.41$	1.904
S4	4-*trans*-Prophenylsyringol	12.41 ± 0.84	1.820
P	Palmitic acid	1.513 ± 0.08	-

[a] Izumi *et al.*, (1995); compound weight (µg) ratio to peak area (µV·s) $\times 10^{6}$ (no internal standard).

[b] Assumed to be the same as G2.

[c] Assumed to be the same as G3.

2.3.4 Determination of Lignin Pyrolysis Products Yields by Pyrolysis-Gas Chromatography-Mass Spectrometry

The yields of the pyrolysis products were determined by comparing the MI area ratios of the peaks (G1–G4 and S1–S4) to the IS peak according to the following equations as examples:

$$\text{G1 content (\%)} = 100 \times \frac{[11.47 \times (\text{Peak G1 area ratio})] \times \text{Weight of IS}}{\text{Weight of EFB TMP}}$$

$$\text{S4 content (\%)} = 100 \times \frac{[12.41 \times (\text{Peak S4 area ratio})] \times \text{Weight of IS}}{\text{Weight of EFB TMP}}$$

In these equations, approximately 150–180 µg of EFB TMP and 0.1 µg of the IS was subjected to Py-GC/MS. As shown in Table 2.3, the total yield of EFB materials was 3.46% and those of the EFB TMP fractions were in the range of 2.63–2.73%. These decrement of total yields indicated that lignin was slightly condensed under thermomechanical pulping conditions and thus slightly changed the lignin structure. Although the S1 (syringol), S3 (4-vinylsyringol), G1 (guaiacol), and G3 (4-vinylguaiacol) yields from raw EFB material were decreased by the TMP process, but the yields among the fractions of TMP long fibers and fines did not show any significant difference.

Table 2.3 Determination for eight pyrolysis products from lignin in EFB materials and TMP fractions by Py-GC/MS.

	Yield of pyrolysis products (% based on weight of sample preparation)								
	S1	S2	S3	S4	G1	G2	G3	G4	Total
EFB materials	0.64 ± 0.05	0.15 ± 0.03	0.68 ± 0.05	0.19 ± 0.05	0.66 ± 0.03	0.13 ± 0.05	0.70 ± 0.05	0.30 ± 0.05	3.46 [a] ± 0.36
EFB TMP fractions									
On 850 µm opening	0.42 ± 0.05	0.16 ± 0.03	0.47 ± 0.05	0.16 ± 0.05	0.47 ± 0.05	0.11 ± 0.01	0.53 ± 0.04	0.29 ± 0.01	2.63 ± 0.29
850-355 µm opening	0.41 ± 0.03	0.17 ± 0.02	0.49 ± 0.05	0.17 ± 0.01	0.47 ± 0.03	0.11 ± 0.02	0.56 ± 0.05	0.28 ± 0.02	2.65 ± 0.23
355-180 µm opening	0.42 ± 0.05	0.16 ± 0.02	0.48 ± 0.05	0.16 ± 0.03	0.48 ± 0.05	0.15 ± 0.02	0.57 ± 0.05	0.31 ± 0.02	2.73 ± 0.29
Pass 180 µm opening	0.40 ± 0.05	0.16 ± 0.03	0.49 ± 0.03	0.16 ± 0.01	0.44 ± 0.03	0.15 ± 0.02	0.54 ± 0.04	0.29 ± 0.01	2.63 ± 0.22

[a] 13.0% based on acid-insoluble lignin content (26.7%).

2.3.5 Correlation between Syringaldehyde/Vanillin Ratio by Nitrobenzene Oxidation and Syringyl/Guaiacyl Ratio by Pyrolysis-Gas Chromatography-Flame Ionization Detector and Pyrolysis-Gas Chromatography-Mass Spectrometry

Izumi *et al.* (1995) reported that there was a good relationship between S_a/V_a yield ratios by the NBO method and S/G yield ratios by Py-GC/FID. In this study, five hardwoods with various S_a/V_a ratios were selected to test this theory, and good correlation was found between the S_a/V_a molar ratios and the S/G molar ratios (Fig. 2.7). It is well known that hardwoods species and non-wood fibers show various S_a/V_a molar ratios which are, for example, in the range of 0.5 and 4.0 (Izumi et al. 1995; Chen, et al. 1992). By using the MI response of Py-GC/MS, the S/G molar ratios by Py-GC/MS exhibited good correlation with syringaldehyde/vanillin (S_a/V_a) molar ratios by NBO for raw EFB material and five hardwoods (Fig. 2.8). This good correlation is supported by a previous study (Ohra-aho *et al.*, 2013) which has clarified that the S/G ratios are similar to the S_a/V_a ratios but usually a little lower than S_a/V_a ratios. Py-GC/MS was therefore found to be a reliable analytical method for characterization of lignin in TMPs fabricated from either wood or non-wood fibers.
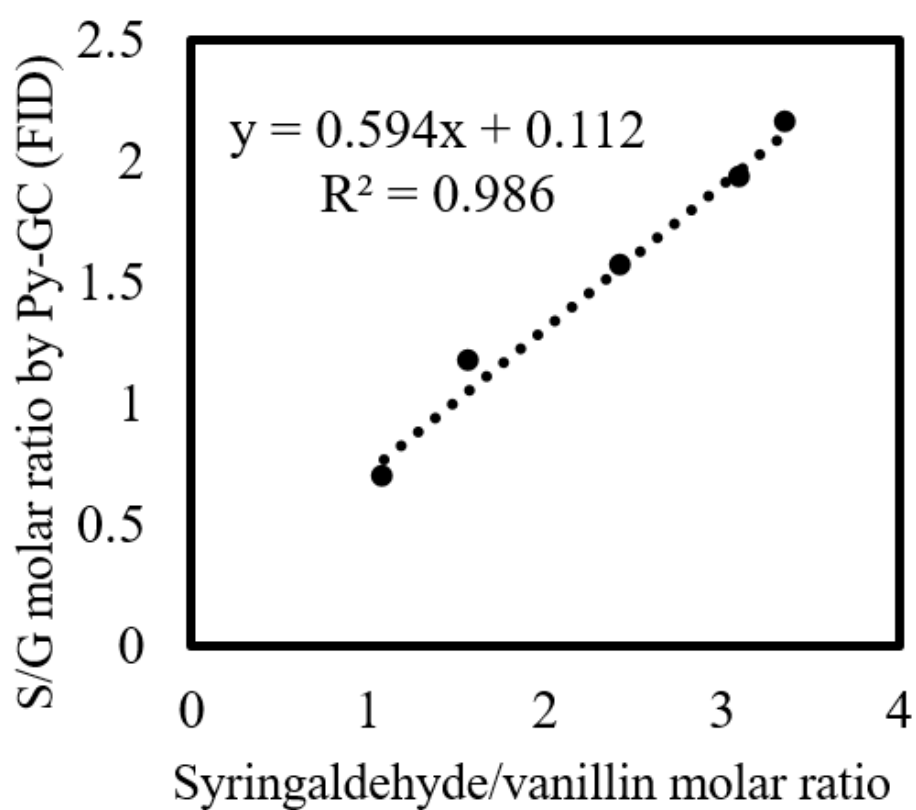
Fig. 2.7 Correlation of S_a/V_a molar ratios by NBO and S/G molar ratios by Py-GC/FID.
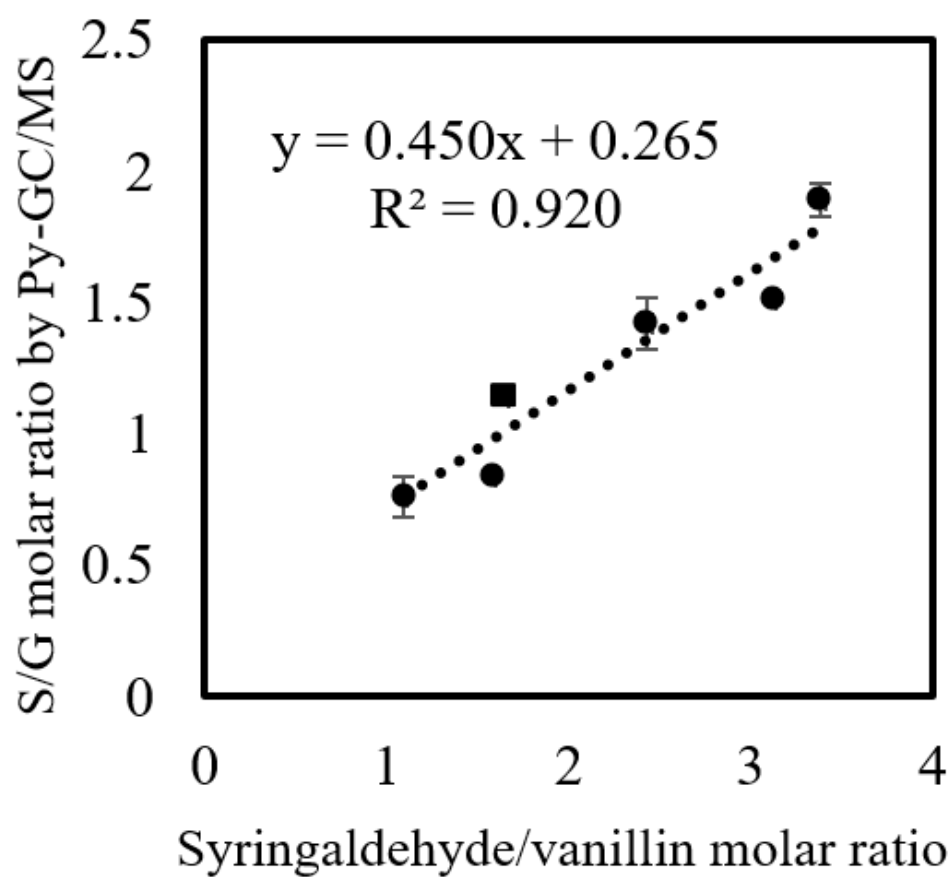
Fig. 2.8 Correlation of S_a/V_a molar ratios by NBO and S/G molar ratios by Py-GC/MS (MI response).

Legend: ■: EFB, ●: hardwoods.

2.3.6 Chemical Features of Lignin in Long Fibers and Fine Fractions of Thermomechanical Pulps

The low yield of V_a represents for a high amount of condensed type lignin (Chen, 1992; Lapierre, 2010), and the lower yields of S_a and V_a indicates that the higher contents of condensed structure of lignin (Wayman *et al*., 1979; Jin *et al*., 2012). Furthermore, a lower S_a/V_a ratio resulted in a high degree of lignin condensation (Ohra-aho *et al*., 2013; Xie, 2004) and a lower amount of β-O-4 linkages (Silva *et al*. 2012).

The yields of S_a and V_a based on the weights of the raw EFB materials and the TMP fractions were in the range of 5.85–6.08%, and the S_a/V_a molar ratios were approximately 1.61–1.66 (Table 2.4). There was no significant difference between the raw EFB materials and the TMP fractions. On the other hand, the S/G molar ratios of the EFB TMP fractions (0.96–1.03) were slightly lower than that of raw EFB materials (1.14). There was no significant difference in the chemical features of the long fibers or fine fractions.

Table 2.4 S_a/V_a molar ratios by NBO and S/G molar ratios by Py-GC/MS of EFB materials and TMP fractions.

	NBO			Py-GC/MS
	S_a (%) [a]	V_a (%) [a]	S_a/V_a molar ratio	S/G molar ratio
EFB materials	4.04 ± 0.64	2.04 ± 0.24	1.65 ± 0.02	1.14 ± 0.03
EFB TMP fractions				
On 850 µm opening	3.89 ± 0.18	1.96 ± 0.15	1.66 ± 0.01	0.97 ± 0.03
850-355 µm opening	3.92 ± 0.15	2.03 ± 0.35	1.61 ± 0.06	0.96 ± 0.02
355-180 µm opening	3.99 ± 0.19	2.03 ± 0.14	1.64 ± 0.05	1.03 ± 0.01
Pass 180 µm opening	3.95 ± 0.22	1.99 ± 0.32	1.66 ± 0.05	0.97 ± 0.02

[a] based on sample preparation weight

2.4 Conclusions

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) identified the analogous chemical features of lignin in EFB TMP fractions by determining eight pyrolysis products from its syringyl (S) and guaiacyl (G) types. The amount of each pyrolysis product was successfully determined through a calibration line obtained by a conversion factor of main ion intensity to the authentic compound weight. Moreover, the S/G molar ratios obtained by Py-GC/MS showed good correlation with syringaldehyde/vanillin (S_a/V_a) molar ratios obtained by nitrobenzene oxidation for raw EFB material and some hardwoods. The yields of lignin pyrolysis products for the EFB TMP fractions were somewhat lower than that for the raw EFB material. The thermomechanical pulping process slightly changed the structure of lignin, whereas there were no significant chemical differences between the long fibers and fines fractions. Py-GC/MS is therefore thought to be a reliable analytical method for characterization of lignin in TMPs fabricated from either wood or non-wood fibers.

References

Alves, A., Schwanninger, M., Pereira, H., Rodrigues, J., 2006. Analytical pyrolysis as a direct method to determine the lignin content in wood. Part 1: Comparison of pyrolysis lignin with Klason lignin. J. Anal. Appl. Pyrolysis. 76, 209–213.

Chen, C.L., 1992. Nitrobenzene and cupric oxide oxidations, in: Lin, S.Y., Dence, C.W. (Eds.), Methods in Lignin Chemistry. Springer-Verlag, Berlin, pp. 301–306.

Claudio, F.L., Luiz, C.A.B., Cristiana, R.M., Flaviano, O.S., Jorge, L.C., 2008. Comparison between analytical pyrolysis and nitrobenzene oxidation for determination of syringly/guaiacyl ratio in *Eucalyptus* spp. Lignin. BioResources. 3, 701–712.

Faix, O., Meier, D., Fortmann, I., 1988. Pyrolysis-gas chromatography-mass spectrometry of 2 trimeric lignin model compounds with alkyl-aryl ether structure. J. Anal. Appl. Pyrolysis. 14, 135–148.

Galletti, G.C., Bocchini, P., 1995. Pyrolysis/gas chromatography/mass spectrometry of lignocellulose. Rapid Commun. Mass Spectrom. 9, 815–826.

Geib, S.M., Filley, K., Hatcher, P.G., Hoover, K., Carlson, J.E., Maria del Mar Jimenez-Gasco, M. del M., Nakagawa-Izumi, A., Sleighter, R.L, Tien, M., 2008. Lignin degradation in wood-feeding insects. PNAS 105, 12932-12937.

Gonzalez, I., Alcala, M., Arbat, G., Vilaseca, F., Mutje, P., 2013. Suitability of rapeseed chemithermomechanical pulp as raw material in papermaking. BioResoureces 8, 1697–1708.

Gorski, D., Hill, J., Engstrand, P., Johansson, L., 2010. Review: Reduction of energy consumption in TMP refining through mechanical pre-treatment of wood chips. Nord Pulp Paper Res. J. 25, 156–161.

Harsono, Mulyantara, L.T., Rizaluddin, A.T., Nakagawa-izumi, A., Ohi, H., Nakamata, K., 2015. Properties of fibers prepared from oil palm empty fruit bunch for use as corrugating medium and fiberboard. Jpn TAPPI J. 69, 1349–1359.

Harsono, H., Putra, A.S., Maryana, R., Rizaluddin, A.T., H'ng, Y.Y., Nakagawa-Izumi, A., Ohi, H., 2016. Preparation of dissolving pulp from oil palm empty fruit bunch by prehydrolysis soda-anthraquinone cooking method. J. Wood Sci. 62, 65–73.

Ibarra, D., Del Rio, J.C., Gutierrez, A., Rodriguez, I.M., Romero, J., Martinez, M.J., Martinez, A.T., 2005. Chemical characterization of residual lignin from eucalypt paper pulps. J. Anal. Appl. Pyrolysis. 74, 116–122.

Izumi, A., Kuroda, K., Ohi, H., Yamaguchi, A., 1995. Comparative studies of pyrolysis-gas chromatography and nitrobenzene oxidation for the determination method of lignin composition in hardwood. Structural analysis of lignin by pyrolysis-gas chromatography (III). Jpn TAPPI J. 49, 1339–1346.

Jin, Z., Jin, G., Shao, S., 2012. Lignin characteristic of bast fiber and core in kenaf, bark and wood of paper mulberry and mulberry. J. Wood Sci. 58, 144–152.

Kleen, M., Lindblad, G., Backa, S., 1993. Quantification of lignin and carbohydrates in kraft pulps using analytical pyrolysis and multivariate data-analysis. J. Anal. Appl. Pyrolysis 25, 209–227.

Kuroda, K., Nakagawa-Izumi, A., Mazumder, B.B., Ohtani, Y., Sameshima, K., 2005a. Evaluation of chemical composition of the core and bast lignins of variety Chinpi-3 kenaf (*Hibiscus cannabinus* L.) by pyrolysis-gas chromatography/mass spectrometry and cupric oxide oxidation. Ind. Crops Prod. 22, 223–232.

Kuroda, K., Nakagawa-Izumi, A., 2005b. Tetramethylammonium hydroxide of guaiacyl-syringyl and guaiacyl dehydrogenation polymers. Org. Geochem. 36, 53–61.

Lapierre, C., 2010. Determinating lignin structure by chemical degradations, in: Heitner, C., Dimmel, D.R., Schmidt, J.A. (Eds.), Lignin and Lignans Advances in Chemistry. CRC Press, 11–18.

Li, B., Li H., Zha, Q., Bandekar, R., Alsaggaf, A., Ni, Y., 2011. Review: Effects of wood quality and refining process on TMP pulp and paper quality. Bioresources 6, 3569–3584.

Lima, C. F., Barbosa, L.C. A., Marcelo, C.R., Silverio, F.O., Colodette, J.L., 2008. Comparison between analytical pyrolysis and nitrobenzene oxidation for determination of syringyl/guaiacyl ratio in *Eucalyptus* spp. lignin. BioResources 3, 701-712.

Moldoveanu, S.C., 1998. Analytical pyrolysis of natural organic polymers. Techniques and Instrumentation in Anal. Chemistry. 20, 510.

Mulyantara, L.T., Harsono, H., Maryana, R., Ohi, H., 2016a. Properties of thermomechanical pulps from sugarcane bagasse and oil palm empty fruit bunch as non-wood materials. Proceedings of 83rd Pulp Pap. Res. Conf. Jpn TAPPI, 22–23 June, Tokyo, 119–122.

Mulyantara, L.T., Maryana, R., Do, V.T., Das, A.K., Ohi, H., Nakamata, K., 2016b. Modified operation of a laboratory refiner for obtaining dried thermomechanical pulp from sugarcane bagasse and oil palm empty fruit bunch as non-wood fibers. Jpn TAPPI J. J-STAGE Advance Published: August 31, 2016.

Nishimura, N., Izumi, A., Kuroda, K., 2002. Structural characterization of kenaf lignin: Differences among kenaf varieties. Ind. Crops Prod. 15, 115–122.

Nunes, C.A., Lima, C.F., Barbosa, L.C.A., Colodette, J.L., Gou-veia, A.F.G., Silverio, F.O., 2010. Determination of *Eucalyptus* spp lignin S/G ratio: A comparison between methods. Bioresour. Technol. 101, 4056–4061.

Obidzinski, K., Andriani, R., Komarudin, H., and Andrianto, A., 2012. Environmental and social impacts of oil palm plantations and their implications for biofuel production in Indonesia. Ecol. Soc 17, 25.

Ohra-aho, T., Gomes, F.J.B., Colodette, J.L., Tamminen, T., 2013. S/G ratio and lignin structure among *Eucalyptus* hybrids determined by Py-GC/MS and nitrobenzene oxidation. J. Anal. Appl. Pyrolysis 101, 166–171.

Rencoret, J., Marques, G., Gutierrez, A., Nieto, L., Jimenez-Barbero, J., Martinez, A.T., Del Rio, J.C., 2009. Isolation and structural characterization of the milled-wood lignin from *Paulownia fortune* wood. Ind. Crops Prod. 30, 137–143.

Rovia, S., Kuitunen, S., Ohra-Aho, T., Alakurtti, S., Kalliola, A., Tamminen, T., 2011. Lignin oxidation mechanisms under oxygen delignification conditions. Part 2: Advanced methods for the detailed characterization of lignin oxidation mechanisms. Holzforschung. 65, 575–585.

Silva, T.C.F., Gomide, J.L., Santos, R.B., 2012. Evaluation of chemical composition and lignin structural features of Simarouba versicolor wood on its pulping performance. BioResources 7, 3910-3920.

Sjoberg, J., Kleen, M., Dahlamn, O., Agnemo, R., Fabriker, D., Sundvall, H., 2002. Analyses of carbohydrates and lignin in the surface and inner layers of softwood pulp fibers obtained employing various alkaline cooking processes. Nordic Pulp Paper Res. J. 17, 295–301.

TAPPI Test Methods T 222 om-15, 2015. Acid-insoluble lignin in wood and pulp. TAPPI Atlanta GA, USA.

TAPPI Test Methods T 204 cm-07, 2007. Solvent extractives of wood and pulp. TAPPI Atlanta GA, USA.

TAPPI Test Methods T 211 om-02, 2002. Ash in wood, pulp, paper and paperboard. TAPPI Atlanta GA, USA.

Wayman, M., Chua, M. G. S., 1979. Characterization of autohydrolysis aspen (*P. tremuloides*) lignins. Part 2. Alkaline nitrobenzene oxidation studies of extracted authohydrolysis lignin. Can. J. Chem 57, 2599-2602.

Xie. Y., 2004. Difference of condensed lignin structure in Eucalyptus species. Nordic Pulp Paper Res. J. 19, 18-21.

Yokoi, H., Ishida, Y., Ohtani, H., Tsuge, S., 1999. Characterization of within-tree variation of lignin components in *Eucalyptus camaldulensis* by pyrolysis-gas chromatography. Analyst 124, 669–674.

Chapter 3 Characterization of Residual Lignin Obtained by the Enzymatic Hydrolysis of Oil Palm Empty Fruit Bunch Pulps

3.1 Introduction

Non-wood lignocellulosic biomass is considered a highly promising alternative to wood. In particular, oil palm (Elaeis guineensis) empty fruit bunch (EFB) is abundantly available as a by-product of the palm oil industry. Indeed, the chemical and physical properties of oil palm EFB have been well examined (Law *et al.* 2007; Wan Rosli *et al.* 2011), and it has been utilized as a raw material for producing a range of fiber-based products, including pulp and paper. Wan Rosli *et al.* (1998) demonstrated that superior quality pulp with low shives content was obtained from EFB fibers through alkaline cooking using only sodium hydroxide, compared to that obtained by other chemical cooking methods. Biorefinery processes have also been used to produce cellulose derivatives, cellulose nanofibers, and other chemicals from lignocellulosic materials. Prehydrolysis (PH) soda cooking has been demonstrated to significantly improve delignification compared to that afforded by soda cooking (Wan Rosli *et al.* 2004). Hence, PH-soda-anthraquinone (AQ) cooking has been developed as an environmentally friendly biorefinery process, because it involves prehydrolysis that aids in removing fragments of hemicellulose and in modifying lignin prior to cooking (Harsono *et al.* 2016).

Although many types of unbleached EFB pulps have been produced, the characteristics of the residual lignin in the EFB pulps have not yet been fully understood. Such an understanding could help in enhancing delignification in the bleaching process. Previous studies have mainly concentrated on the structure of residual lignin in wood chemical pulps (Jaaskelainen *et al.* 2003), and as such, similar studies are also required for non-wood residual lignin for future applications. In the 1980s, the enzymatic hydrolysis of pulp was introduced to

study the characteristics of residual lignin (Yamasaki *et al*. 1981). It was found that no structural changes occurred in the isolated residual lignin, although carbohydrates were dissolved by the enzyme, leaving behind solid residues referred to as residual lignin. While this enzymatic method provides structurally intact lignin, the purities tend to be low, and traces of the mixed enzyme remain in the residue, although this has yet to be quantified.

The efficiency of enzymes in the enzymatic hydrolysis process for lignocellulosic materials depends on the characteristics of lignin remaining in the materials following pretreatment for the saccharification. This is an important consideration for designing enhanced bioethanol production processes for non-wood materials such as wheat straw and softwood (Yang *et al*. 2016; Morales *et al*. 2014). It is therefore important to enhance our understanding on the adsorption of enzymes on residual lignin when EFB is used as a raw material for bioethanol production. In a previous study (Tanifuji *et al*. 2011), the enzyme activity in terms of filter paper units (FPU) adsorbed onto residual lignin has been estimated by pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS).

Lignin, which is often regarded as an undesirable component in the conversion of wood into bleached pulp, is a polymerized compound consisting of phenylpropane units known as p-hydroxyphenyl, syringyl (S), and guaiacyl (G) units. The most common method used to determine the S/G ratio is nitrobenzene oxidation that involves a number of steps and affords syringaldehyde and vanillin. However, this procedure is time consuming. Thus, an alternative analytical technique such as Py-GC/MS is recommended, as it is a faster, safer, and economical method for the determination of lignin (Kuroda *et al*. 2005). Py-GC/MS is based on the thermal degradation of lignin to produce pyrolysis compounds (G and S types), which can be detected by GC/MS. Previously, Py-GC/MS and nitrobenzene oxidation have been considered only in the context of characterizing lignin in wood and non-wood materials (Lima et al. 2008), but not for analyzing the residual lignin in non-wood chemical pulps.

Thus, we herein report the development of Py-GC/MS as a novel method to determine the enzyme quantity, the S/G ratio of lignin, and other components present in the residual lignin enzymatically isolated from EFB pulps. Furthermore, the residual lignin present in soda-AQ and PH-soda-AQ EFB pulps is also characterized.

3.2 Experimental

3.2.1 Preparation of Empty Fruit Bunch Fibers, Prehydrolysis Fibers, and Chemical Pulps

EFB fibers were provided by the United Oil Palm Industries Sdn. Bhd, Nibong Tebal, Pulau Pinang, Malaysia. The EFB fibers were washed with filtered tap water to remove any contaminants such as dust and sand. The washed fibers were then air-dried until the solid content reached over 90%. As shown in Fig. 3.1, to obtain the PH-EFB fibers, the washed EFB fibers were treated with water (water/fiber ratio of 7/1) in a 4 L stationary stainless steel vessel at 165 °C for 60 min and air-dried until the solid content was over 90%.

Soda-AQ cooking was carried out at 160 °C for 120 min in a 4 L stationary stainless steel vessel with an AQ dosage of 0.1%, active alkali: (NaOH as Na_2O) (AA) dosages of 17–19%, and a water/fiber ratio of 7/1. The pulp slurry was subsequently screened on a TAPPI standard flat screen (slit width: 0.8 mm).

3.2.2 Preparation of the Residual Lignin and Black Liquor Lignin

Enzymatic hydrolysis was carried out according to previous literature procedures (Chang 1992) using a mixture of cellulolytic enzymes (GC220, Genencore Kyowa Co. Ltd., Japan) as shown in Fig. 3.1. The pulp was continuously shaken for 24 h in an acetate buffer at pH 4.5 and temperature 45 °C using 30 filter paper units per 1 g pulp (FPU/g) of GC220 and then for further 24 h with an additional 15 FPU/g. Subsequently, the residue was washed with

distilled water and separated by centrifugation as the residual lignin. The residual lignins were repeatedly prepared twice from a pulp. These are referred to as soda-AQ lignin 1, PH-soda-AQ lignin 1, soda-AQ lignin 2, and PH-soda-AQ lignin 2. Further, the enzymatically isolated residue (80 mg) was treated with cellobiose (24 mg) in distilled water (50 mL) either once, twice, or thrice. The residual lignins were then freeze-dried.

Meanwhile, lignin was isolated from spent black liquor of soda-AQ and PH-soda-AQ cooking according to a previous literature procedure (Lin, 1992). The black liquor was acidified to pH 2 using 2 M hydrochloric acid in one step. Subsequently, the mixture was centrifuged, and the precipitate was washed thrice with distilled water and then freeze-dried. These are referred to as soda-AQ black liquor lignin and PH-soda-AQ black liquor lignin (Fig. 3.2).

3.2.3 Analytical Methods

3.2.3.1 Elemental Analysis of the Residual Lignin

Elemental (CHN) analysis of carbon, hydrogen, and nitrogen in the enzymatically isolated residual lignin was carried out at the Chemical Analysis Division, Research Facility Center for Science and Technology, University of Tsukuba, Japan, using a Perkin-Elmer 2400 CHN Elemental Analyzer.

3.2.3.2 Estimation of Lignin Content by Pyrolysis-Gas Chromatography-Mass Spectrometry and Acetyl Bromide Method

The Py-GC/MS conditions employed herein were as follows: Pyrolyzer, JHP-5 (Japan Analytical Industry Co. Ltd., Japan); pyrolysis conditions, 500 °C for 4 s; GC/MS system, QP-5050A (Shimadzu, Japan); column, HP 1-MS (30 m × 0.25 mm; film thickness: 1.0 µm). First, 100 µg of the enzymatically isolated residual lignin was subjected to Py-GC/MS with 0.1 µg of n-eicosane as an internal standard. Yields of lignin pyrolysis products were obtained using

the parameters reported in the previous study (Nakagawa-Izumi *et al.* 2016), where the calibration lines authentic compounds with were made by Py-GC/MS. Black liquor lignins were also analyzed, and the lignin contents in the enzymatically isolated residual lignin were estimated using a conversion factor derived from the pyrolysis products yield and the lignin content in black liquor lignin. In addition, the acetyl bromide method (Dence, 1992) was employed to estimate the lignin content in the enzymatically isolated residual lignin.

3.2.3.3 Determination of Enzyme and Fatty Acid in the Residual Lignin by Pyrolysis-Gas Chromatography-Mass Spectrometry

The Py-GC/MS conditions were identical to those described previously. The parameter for estimating the enzyme content in the residual lignin was obtained from the calibration lines between the authentic compounds (indole and methylindole) and the freeze-dried GC220. The GC220 solution (1 mL) contained 426 mg of the freeze-dried solid, and exhibited an activity of 63.2 FPU. The nitrogen content of the solid was 5.6%, and 1 mL of the enzyme solution contained 149 mg of protein.

The parameter for estimating the fatty acid content in the isolated residue was obtained from the calibration line of an authentic palmitic acid sample.

3.2.3.4 Determination of Acid-insoluble Lignin and Carbohydrate Composition

The contents of the acid-insoluble lignin (Klason lignin), acid-soluble lignin, and ash were determined using TAPPI Test Method T 222 om-15 and T 211 om-02, and the amounts of glucose, xylose, and other sugars in the acid hydrolysate were determined using ion chromatography according to previously published procedures (Harsono *et al.* 2016).
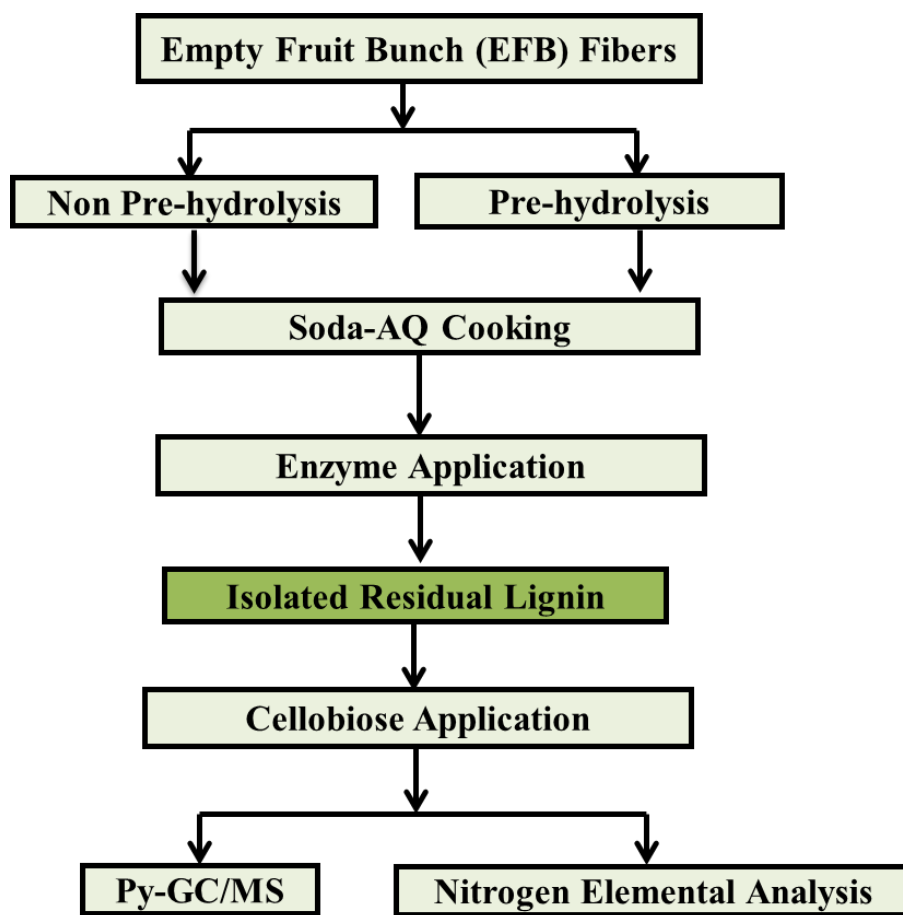
Fig. 3.1 Scheme of EFB pulps preparation and the analytical methods for enzymatically

isolated residual lignin.
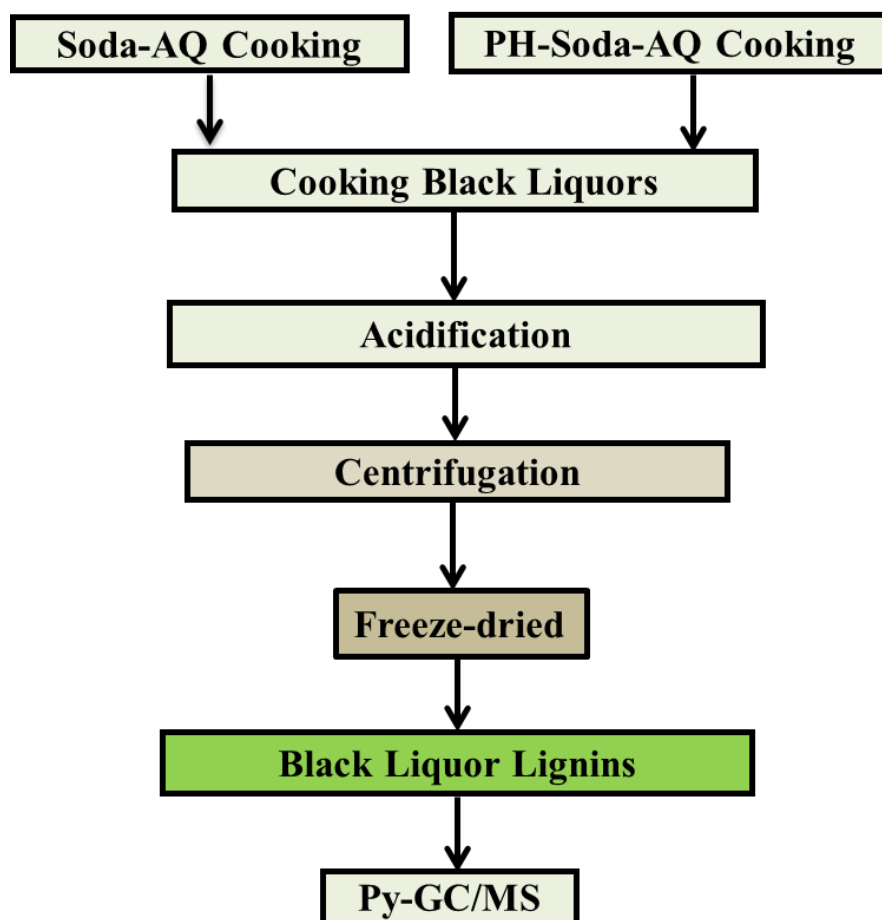
Fig. 3.2 Scheme of sample preparation and the analytical methods for EFB black liquor

isolated lignin.

3.3 Results and Discussion

3.3.1 Fiber and Pulp Preparation

EFB fibers and pulps contained glucan, xylan, lignin, extractive, and ash (Table 3.1). The prehydrolysis process can increase the dissolution of lignin during alkaline cooking and thus reduce the ash content substantially (Wan Rosli *et al*. 2004).

Prehydrolyzed pulp exhibited a much lower kappa number (5.8) and rejects yield (4.2%) than those of the non-prehydrolyzed pulp (12.3 and 5.7%, respectively). In addition, the prehydrolyzed pulp gave a significantly lower screened yield than the non-prehydrolyzed pulp (Table 3.2).

Table 3.1 Chemical composition of EFB fibers and pulps.

	Acid-insoluble lignin (%)	Acid-soluble lignin (%)	Glucan (%)	Xylan (%)	Ash (%)	Other organics (%)
EFB material[a]	19.1	3.7	35.6	19.6	3.1	18.9[a]
PH-EFB material	18.2	2.0	35.2	9.2	0.6	5.8[b]
Soda-AQ pulp	1.3	0.6	31.0	14.1	0.8	7.1
PH-soda-AQ pulp	0.2	0.4	29.6	3.3	0.2	7.2

[a,b] Acetone extractives contents: [a] 2.1% and [b] 2.8%, respectively.

Table 3.2 Kappa number and pulp yield of EFB chemical pulps.

	Active alkali (%)	Kappa number	Rejects yield (%)	Screened yield (%)
Soda-AQ pulp	18	12.3	5.7	45.3
PH-soda-AQ pulp	18	5.8	4.2	33.9

3.3.2 Solubility of the Enzymatically Isolated Lignin

The yields of enzymatically isolated lignins from soda-AQ and PH-soda-AQ pulp were 1.6 and 0.8% based on pulp weights, respectively. Lignin isolated using the enzymatic method was insoluble in most standard solvents such as tetrahydrofuran, dimethyl sulfoxide, acetone, and chloroform (Chang, 1992). This insolubility is mainly due to the inability of the enzymatic treatment to break the covalent bonds between carbohydrates and lignin; thus, the solubility of the resulting lignin-carbohydrate structure is low in these solvents. Indeed, previous studies have suggested protocols to alleviate these solubility problems of the enzymatically isolated lignin (Hortling *et al*. 1990; Fukagawa *et al*. 1992; Duarte *et al*. 2001). Furthermore, the enzymatically isolated lignin contained impurities originating from the enzyme, cellulose, and hemicellulose, which cannot be removed by prolonged and repetitive enzymatic treatments or by any purification procedure (Yamasaki *et al*. 1981). Therefore, a reliable method is required for determining the enzyme content in the isolated lignin.

3.3.3 Elemental Carbon, Hydrogen and Nitrogen Analysis of the Residual Lignin

The enzymatically isolated lignin contained significant quantities of nitrogen (5.3 to 9.2%) because of the presence of enzyme protein, despite thorough washing and purification following enzymatic hydrolysis (Table 3.3). Typical nitrogen contents of enzymatically isolated lignin from unbleached pulps have been reported in the range 2.5 to 7.3% (Jiang *et al*. 1987). The enzyme amounts calculated from the values in this study using a nitrogen protein conversion factor of 6.25 (Hortling *et al*. 1990) were 33 to 58%, suggesting that more than one-third of weight for the isolated lignin is indeed protein.

Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (NMR) have been used for the characterization of residual lignin and lignocellulosic materials (Fukagawa *et al*. 1992; Del Rio *et al*. 2007). In a supplemental

experiment using FTIR, the enzymatically isolated lignin exhibited only one signal at 1650 cm−1, which was reported to be corresponding to protein impurities (Hortling *et al*. 1990), whereas signals corresponding to an OH stretch (3400 cm⁻1), palmitic acid (2920 cm⁻1), CH2 deformation (1460 cm⁻1), S unit (1330 cm⁻1), and G unit (1270 cm⁻1) were observed in the EFB materials. Therefore, FTIR spectroscopy was unfortunately not useful for the characterization of the residual lignin. Because of poor solubility of the enzymatically isolated lignin in testing solvents, only minimal information could be obtained by NMR. Hence, the use of Py-GC/MS was examined.

Table 3.3 CHN composition of enzymatically isolated residual lignin.

	Carbon (%)	Hydrogen (%)	Others (%)	Nitrogen (%)	Nitrogen×6.25 (%)
Soda-AQ lignin 1	49.7	7.1	37.9	5.3	33
PH-soda-AQ lignin 1	50.4	7.3	35.7	6.6	41
Soda-AQ lignin 2	44.2	6.1	43.5	6.2	39
PH-soda-AQ lignin 2	47.0	6.4	37.4	9.2	58

3.3.4 Pyrolysis-Gas Chromatography-Mass Spectrometry of the Residual Lignin

In the total ion chromatograms (TIC), eight compounds (G1–G4, S1–S4) corresponding to lignin pyrolysis products and two compounds (indole: E1, methylindole: E2) corresponding to the cellulolytic enzyme in GC220 were identified (Fig. 3.3). The result clearly indicates that the majority of the lignin pyrolysis products signals did not overlap with signals corresponding to cellulose and hemicellulose, and hence, determination of the chemical features of lignin was relatively straightforward by this method. In addition, although a number of cellulose and hemicellulose pyrolysis products could often be observed on TIC of the isolated lignin, their contents were very low compared to those of the EFB material.

For the lignin isolated from EFB pulps, an additional signal (P) was observed corresponding to a long-chain fatty acid. This long-chain fatty acid has been identified as palmitic acid (Harsono *et al*. 2016). However, as the EFB material does not actually have oil palm seed and long-chain fatty acids, it is suspected that this long-chain fatty acid originates from the crude palm oil processing process. On the other hand, the signal P was not observed for black liquor lignin. Palmitic acid was expected to be bound to the residual lignin of EFB pulps.

3.3.5 Determination of Enzyme and Palmitic Acid Contents in the Residual Lignin

Tanifuji *et al*. (2011) used Py-GC/MS to measure the enzyme activity in terms of FPU adsorbed to residual lignin. However, the amount of enzyme present in the form of protein was not estimated. We therefore expected that the enzyme protein and palmitic acid contents can be determined using Py-GC/MS based on the parameters that were obtained from the calibration lines (Fig. 3.4). The palmitic acid and enzyme contents were calculated using the ratio of total ion (TI) response area of peak P to that of an internal standard (IS, *n*-eicosane), or of peaks E1 and E2 to that of IS (Fig. 3.5).

According to the following equations, palmitic acid and enzyme contents in the isolated residual lignins were calculated as 0.11 to 0.28% and 12 to 44%, respectively (Table 3.4). The cellobiose treatments resulted in a decrease in the enzyme contents (%) in the residual lignin.

$$\text{Palmitic acid content (\%)} = 100 \times \frac{[1.41 \times (\text{Peak P area ratio})] \times \text{Weight of IS}}{\text{Weight of the isolated residual lignin}}$$

$$\text{Enzyme content (\%)} = 100 \times \frac{[138 \times (\text{Peak E1 and E2 area ratio}) + 108] \times \text{Weight of IS}}{\text{Weight of the isolated residual lignin}}$$

3.3.6 Determination of Eight Lignin Pyrolysis Products

The lignin pyrolysis products yield based on material weight were determined using main ion (MI) intensity parameters (response factors of MI area to weight) according to the previous literature (Nakagawa-Izumi *et al*. 2016). The S/G molar ratio was also calculated using molar yields of S1–S4 and G1–G4 (Table 3.4). The yields of soda-AQ and PH-soda-AQ black liquor lignins were 7.4% and 7.8%, respectively. Meanwhile, the yields of enzymatically isolated residual lignin were only 1.7 to 4.2%.

Furthermore, lignin in the PH-EFB material has a higher S/G ratio (1.70) than that in the EFB material (1.27). In general, lignocellulosic materials containing higher S/G ratio lignin undergo easier delignification under alkaline cooking (Wallis *et al*. 1996; Del Rio *et al*. 2005). This is because the *β*-aryl ether-containing S units are easier to cleave during alkaline cooking than G units (Shimizu *et al*. 2012). Interestingly, the S/G ratios of PH-soda-AQ lignin (0.86 to 0.98) were also higher than those of soda-AQ lignin (0.76 to 0.97), and hence, it is the preferred material for the subsequent oxygen bleaching stage and the production of bleached dissolving pulp. PH-soda-AQ black liquor lignin has a higher S/G ratio (1.70) than the soda-AQ black liquor lignin (1.44).

For native lignin in EFB material, the lignin pyrolysis products yield based on acid-insoluble lignin content (19.1% in Table 1) were 12.0% (2.3÷0.191). These are similar values to the yields for hardwoods, which are shown in the previous chapter. The yields for soda-AQ and PH-soda-AQ black liquor lignins were 9.0% and 8.9%, respectively, which were lower than 12.0% for native lignin in EFB materials.

The S1–S4 and G1–G4 yields based on acid-insoluble lignin contents are shown in Table 3.5. Interestingly, S2, S4, G2, and G4 were increased by the prehydrolysis while S1, S3, G1, and G3 were decreased. These changes should be probably due to the cleavage of lignin-carbohydrate bonds at Cα or Cγ positions by prehydrolysis. In addition to these data, the S1–S4 and G1–G4 yields based on materials for enzymatically isolated lignins are shown in Table 3.5.
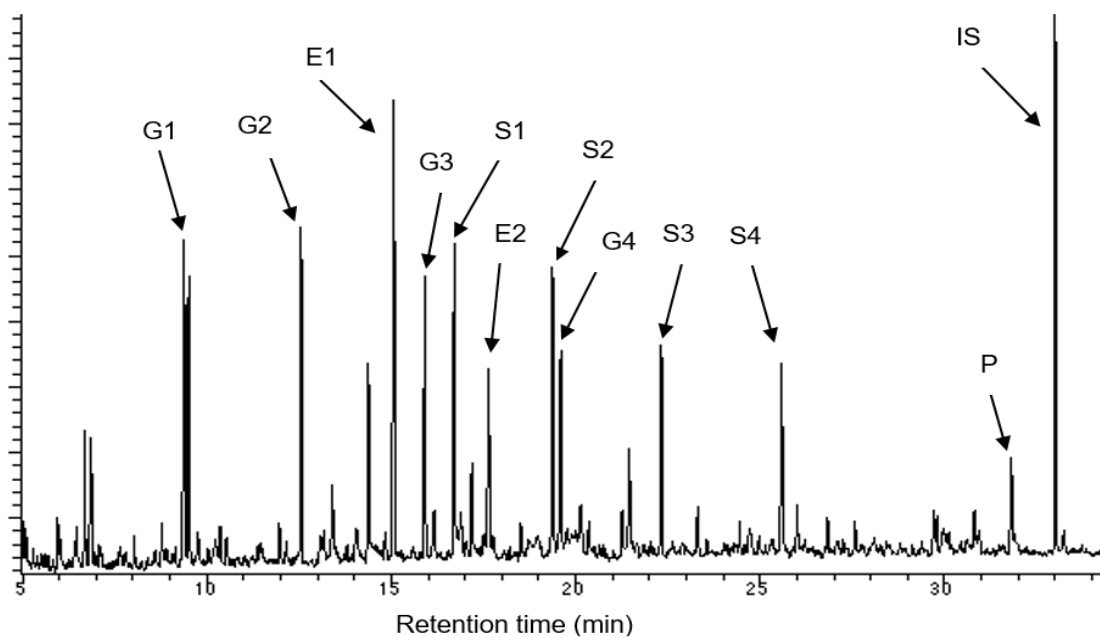
Fig. 3.3 Total ion chromatogram of enzymatically isolated residual lignin by Py-GC/MS.

Note:

| Peaks	Pyrolysis products	Main ions	Retention time (min)
G1	Guaiacol	124, 109	9.5
G2	4-Methylguaiacol	138, 123	12.5
E1	Indole	117	15.0
G3	4-Vinylguaiacol	150, 135	15.9
S1	Syringol	154, 139	16.7
E2	Methylindole	130	17.5
S2	4-Methysyringol	168, 153	19.6
G4	*trans*-Isoeugenol	164, 149	19.8
S3	4-Vinylsyringol	180, 165	22.3
S4	4-*trans*-Prophenylsyringol	194, 179	25.5
P	Palmitic acid	256	31.8
IS	*n*-Eicosane	282	33.0
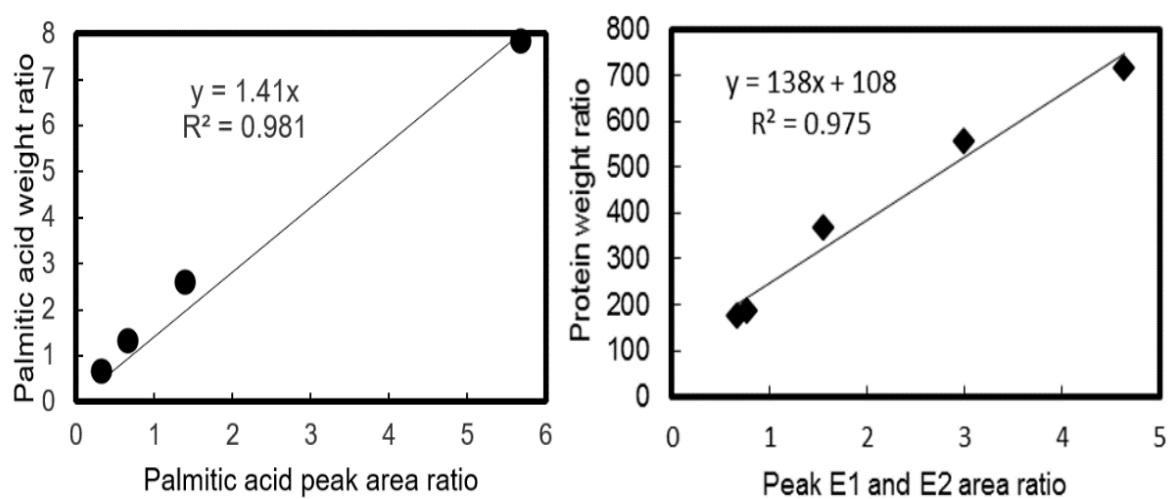
Fig. 3.4 Calibration line for palmitic acid and enzyme obtained by Py-GC/MS.
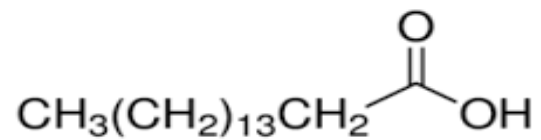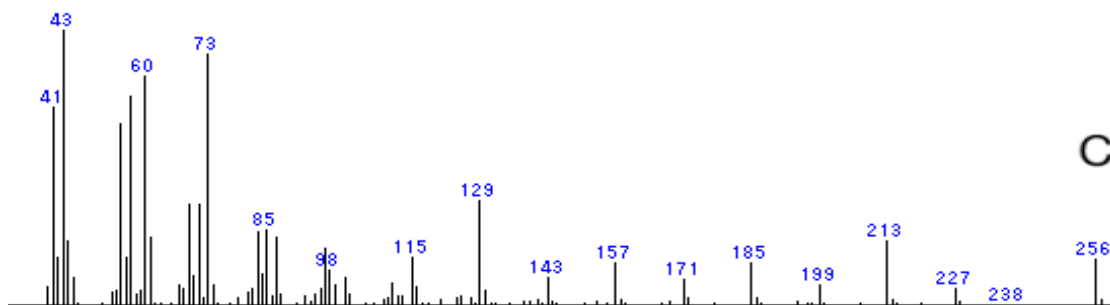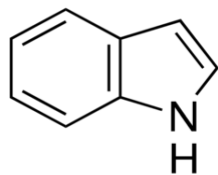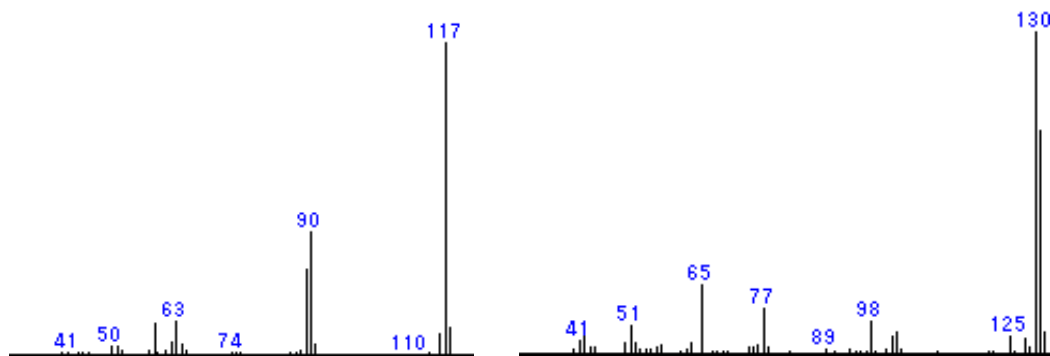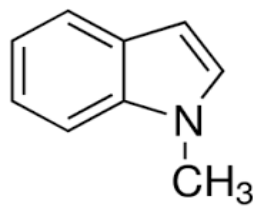
Fig. 3.5 Main ion (MI) intensity of palmitic acid (P), indole (E1) and methyindole (E2).

Table 3.4 Determination of the palmitic acid, eight lignin pyrolysis product, and enzyme by Py-GC/MS analysis.

	Lignin pyrolysis products yield (%)	S/G ratio	Palmitic acid yield (%)	Enzyme yield (%)
EFB material	2.3	1.27	0.31	-
PH-EFB material	2.3	1.70	0.25	-
Soda-AQ lignin 1	4.0	0.91	0.28	22
Soda-AQ lignin 1-3[a]	4.2	0.97	0.10	12
PH-soda-AQ lignin 1	3.5	0.94	0.20	27
PH-soda-AQ lignin 1-3[a]	4.1	0.98	0.17	24
Soda-AQ lignin 2	3.1	0.76	0.11	34
PH-soda-AQ lignin 2	1.7	0.86	0.11	44
Soda-AQ black liquor lignin[b]	7.4	1.44	-	-
PH-soda-AQ black liquor lignin[c]	7.8	1.70	-	-

[a] Cellobiose treatment repeated in triplicate.

[b,c] Acid-insoluble lignin contents (%): [b] 82.4% and [c] 87.0%, respectively.

Table 3.5 Determination of eight pyrolysis products from EFB materials, black liquor

lignins, and enzymatically isolated lignin.

	Yield of pyrolysis products (% based on acid-insoluble lignin)								
	S1	S2	S3	S4	G1	G2	G3	G4	Total
EFB materials	2.36	0.74	2.34	1.27	1.98	0.62	2.08	0.60	12.0
PH-EFB material	1.98	1.46	2.21	2.31	1.34	0.90	1.69	0.70	12.6
Soda-AQ black liquor lignin	2.46	0.92	1.42	0.53	1.55	0.56	1.29	0.29	9.0
PH-soda-AQ black liquor lignin	1.92	1.79	1.26	0.62	1.08	0.95	0.92	0.34	8.9
	Yield of pyrolysis products (% based on materials)								
	S1	S2	S3	S4	G1	G2	G3	G4	Total
Soda-AQ lignin 2	0.41	0.29	0.38	0.27	0.55	0.39	0.52	0.32	3.1
PH-soda-AQ lignin 2	0.27	0.18	0.21	0.14	0.26	0.24	0.31	0.12	1.7

3.3.7 Estimation of Lignin Contents in Enzymatically Isolated Lignin

The contents of lignin as acid-insoluble lignin in the enzymatically isolated residual lignin can be estimated when we assume that a conversion factor from the lignin pyrolysis products yield based on lignin (as acid-insoluble lignin) weight is 9.0% for the isolated residual lignin. As shown in Table 3.6, the lignin contents in the isolated residual lignins were 19 to 44%. Meanwhile, the acetyl bromide method indicated that the lignin contents in the isolated residual lignins were 25 to 49%.

Analysis of the carbohydrate composition indicated that the isolated residual lignins contained arabinose, galactose, and mannose in addition to glucose and xylose. The contents of glucan, xylan, arabinan, galactan and mannan in freeze-dried GC220 were 22.8, 1.0, 1.2, 1.2 and 44.8%, respectively. EFB material contains little amounts of arabinan, galactan and mannan. Relative increments of xylan, arabinan, and galactan in the residual lignins strongly suggest that the hemicelluloses should chemically combined with lignin.

Table 3.6 Chemical composition of enzymatically isolated residual lignin.

	Lignin	Relative composition (%)				
	(%)	Glucan	Xylan	Arabinan	Galactan	Mannan
Soda-AQ lignin 1	44[a] (49[b])	13	11	4.6	2.9	2.1
PH-soda-AQ lignin 1	39[a] (48[b])	16	10	5.1	1.7	1.4
Soda-AQ lignin 2	34[a] (32[b])	14	11	3.1	2.0	1.8
PH-soda-AQ lignin 2	19[a] (25[b])	16	11	5.2	2.9	2.3

[a] Yield of lignin pyrolysis products (%) $\div 0.09$

[b] Acetyl bromide method.

3.4 Conclusions

Py-GC/MS was employed to identify lignin pyrolysis products, enzyme pyrolysis products, and palmitic acid, and to determine their respective contents of the enzymatically isolated residual lignin in oil palm EFB alkali pulps. Residual lignin in PH-soda-AQ pulp exhibited a higher S/G ratio than that in soda-AQ pulp. Besides that, palmitic acid was found to be bound to the residual lignin of EFB pulps. Furthermore, the enzyme content adsorbed on the residual lignin was estimated, and it was reduced by cellobiose treatment. On the other hand, the enzymatically isolated residual lignin still contained significant quantities of both carbohydrates and enzymes. Black liquor lignin exhibited a lower total yield of lignin pyrolysis products than the native lignin in EFB materials. Py-GC/MS was a useful method for estimating contents of lignin and enzyme present in enzymatically isolated lignin.

References

Chang, H. M., 1992. Isolation of lignin from pulp, in: Lin, S. Y., and Dence, C. W. (eds.), Methods in Lignin Chemistry, Springer-Verlag, Berlin, 71-74.

Del Rio, J. C., Gutiérrez, A., Hernando, M., Landin, P., Romero, J., Martinez, A. T., 2005. Determining the influence of eucalypt lignin composition in paper pulp yield using Py-GC/MS. J. Anal. Appl. Pyrolysis. 74, 110-115.

Del Rio, J. C., Gutiérrez, A., Rodríguez, I. M., Ibarra, D., Martinez, A. T., 2007. Composition of non-woody plant lignins and cinnamic acids by Py-GC/MS, Py/TMAH and FT-IR. J. Anal. Appl. Pyrolysis 79, 39-46.

Dence, C. W., 1992. The determination of lignin, in: S. Y. Lin and C. W. Dence (eds.), Methods in Lignin Chemistry, Springer-Verlag, Berlin, 44-46.

Duarte, A. P., Robert, D., Lachenal, D., 2001. *Eucalyptus globulus* kraft pulp residual lignins. Part 1. Effect of extraction methods upon lignin structure. Holzforschung 54, 365-372.

Fukagawa, N., Meshitsuka, G., Ishizu, A., 1992. 2D NMR study of the residual lignin in beech kraft pulp combined with selective cleavage with pivaloyl iodine. J. Wood Chem. Technol. 12, 425-445.

Harsono, H., Putra, A. S., Maryana, R., Rizaluddin, A. T., H'ng, Y. Y., Nakagawa-Izumi, A., Ohi, H., 2016. Preparation of dissolving pulp from oil palm empty fruit bunch by prehydrolysis soda-anthraquinone cooking method. J. Wood Sci. 62, 65-73.

Hortling, B., Ranua, M., Sundquist, J., 1990. Investigation of the residual lignin in chemical pulps part 1. Enzymatic hydrolysis of the pulps and fractionation of the products. Nordic Pulp Pap. Res. J. 5, 33-37.

Jääskeläinen, A. S., Sun, Y., Argyropoulos, D. S., Tamminen, T., Hortling, B., 2003. The effect of isolation method on the chemical structure of residual lignin. Wood Sci. Technol. 37, 91-102.

Jiang, J. E., Chang, H. M., Bhattacharjee, S. S., Kwoh, D. L. W., 1987. Characterization of residual lignin isolated from unbleached and semi-bleached softwood kraft pulps. J. Wood Chem. Technol. 7, 81-96.

Kuroda, K., Nakagawa-Izumi, A., Mazumder, B. B., Ohtani, T., Sameshima, K., 2005. Evaluation of chemical composition of the core and bast lignins of variety Chinpi-3 kenaf (*Hibiscus cannabinus* L.) by pyrolysis-gas chromatography/mass spectrometry and cupric oxide oxidation. Ind. Crops Prod. 22, 223-232.

Law, K. N., Wan Rosli, W. D., Arniza, G., 2007. Morphological and chemical nature of fiber strands of oil palm empty fruit bunch (OPEFB). BioResources 2, 351-362.

Lima, C. F., Barbosa, L. C. A., Marcelo, C. R., Silvério, F. O., Colodette, J. L., 2008. Comparison between analytical pyrolysis and nitrobenzene oxidation for determination of syringyl/guaiacyl ratio in *Eucalyptus* spp. lignin. BioResources 3, 701-712.

Lin, S. Y., 1992. Commercial spent pulping liquors, in: S. Y. Lin and C. W. Dence (eds.), Methods in Lignin Chemistry, Springer-Verlag, Berlin, 75-80.

Morales, L., Iakovlev, M., Martin-Sampedro, R., Rahikainen, J., Laine, J., Heiningen, A., Rojas, O., 2014. Effects of residual lignin and heteropolysaccharides on the bioconversion of softwood lignocellulose nanofibrils obtained by SO_2-ethanol-water fractionation. Bioresour. Technol. 161, 55-62.

Shimizu, S., Yokoyama, T., Akiyama, T., Matsumoto, Y., 2012. Reactivity of lignin with different composition of aromatic syringyl/guaiacyl structures and *erythro*/*threo* side chain structures in β-O-4 type during alkaline delignification: As a basis for the different degradability of hardwood and softwood lignin. J. Agric. Food Chem. 60, 6471–6476.

Tanifuji, K., Takahashi, S., Kajiyama, M., Ohi, H., 2011. Advantage of acid sulfite cooking as processes of bioethanol production. Jpn. TAPPI J. 65, 494-497.

Wallis, A. F. A., Wearne, R. H., Wright, P. J., 1996. Analytical characteristics of plantation eucalypt woods relating to kraft pulps yields. Appita J. 49, 427-432.

Wan Rosli, W. D., Law, K. N., 2011. Oil palm fiber as papermaking material: Potentials and challenges. BioResources 6, 901-917.

Wan Rosli, W. D., Law, K. N., Jacques, L. V., 1998. Chemical pulping of oil palm empty fruit bunches. Cellul. Chem. Technol. 32, 133-143.

Wan Rosli, W. D., Leh, C. P., Tanaka, R., 2004. Effects of prehydrolysis on the production of dissolving pulp from empty fruit bunches. J. Trop. For. Sci. 16, 343-349.

Yamasaki, T., Chen, C. L., Gratzl, J. S., Chang, H. M., 1981. Characterization of residual lignin in kraft pulp. Proceed. Int. Symp. Wood Pulping Chem., 1st, 2, Stockholm, Sweden, 34-42.

Yang, H., Xie, Y., Zheng, X., Pu, Y., Huang, F., Meng, X., Wu, W., 2016. Comparative study of lignin characteristics from wheat straw obtained by soda-AQ and kraft pretreatment and effect on the following enzymatic hydrolysis process. Bioresour. Technol. 207, 361-369.

Chapter 4   General Conclusion

This overall study is about the characterization of lignin in oil palm empty fruit bunch (EFB) mechanical chemical pulps by Pyrolysis-gas chromatography mass-spectrometry (Py-GC/MS).

Thermomechanical pulp (TMP) was fabricated from EFB of oil palm (*Elaeis guineensis*) by using a laboratory pressurized refiner, and separated with screens (850, 355 and 180 μm opening) into four fractions. Py-GC/MS indicated analogous chemical feature of lignin in the TMP fractions by determining eight pyrolysis products (Guaiacol, 4-Methylguaiacol, 4-Vinylguaiacol, Syringol, 4-Methysyringol, *trans*-Isoeugenol, 4-Vinylsyringol, 4-*trans*-Prophenylsyringol) from its syringyl (S) and guaiacyl (G) types. The amount of each pyrolysis product was successfully determined through a calibration line obtained by a conversion factor of total ion intensity to an authentic compound weight. Moreover, S/G molar ratios by Py-GC/MS provided a good correlation (R^2 value: 0.920) with syringaldehyde/vanillin (S_a/V_a) molar ratios by nitrobenzene oxidation for EFB and some hardwoods.

The total yields of lignin pyrolysis products for the EFB TMP fractions (2.66%) were showed lower than that for the raw EFB material (3.46%). Therefore, it can be concluded that the TMP fabrication process slightly changed the lignin structure. However, there were no significant chemical differences between the EFB TMP long fibers and fines fractions.

Black liquor lignin exhibited a lower total yield of lignin pyrolysis products (9%) than the native lignin in EFB materials (12%).

Residual lignin present in alkali pulps prepared from EFB was isolated using an enzymatic method and characterized successfully by Py-GC/MS. These pulps were prepared by soda-anthraquinone (AQ) and prehydrolysis (PH) soda-AQ cooking methods; the pulp yields were 45.3% and 33.9%, respectively. Py-GC/MS of the residual lignin showed that two pyrolysis products which were indole and methylindole originating from the enzyme; its

85

contents were in the range of 12% to 44%. In addition to the eight pyrolysis products originated from syringyl (S) and guaiacyl (G) structure of lignin. Furthermore, palmitic acid was also detected in the residual lignin; its contents were in the range of 0.11% to 0.28%. The residual lignin was subjected to further purification by a cellobiose treatment method, and the quantity of enzyme present in the residual lignin was found to decrease after the treatment. Residual lignin in prehydorolysis-soda-AQ pulp exhibited a higher S/G ratio (0.86 to 0.98) than that in soda-AQ pulp (0.76 to 0.97). The lignin contents in enzymatically isolated lignin were estimated to be in the range of 19% to 44%.

Finally, as a conclusion, this study shows that the contents of lignin and enzyme present in enzymatically isolated lignin can be estimated by the Py-GC/MS method. Py-GC/MS was found to be a reliable analytical method for the characterization of lignin in EFB mechanical and chemical pulps.
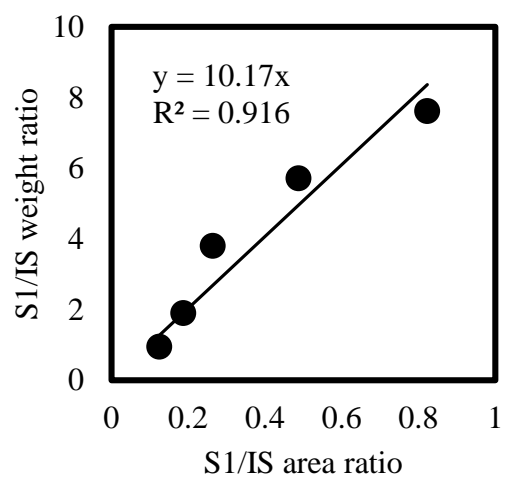
APPENDIXES

A1

Calibration Lines of G-type Monomeric Lignin Model Compounds by Py-GC/MS.
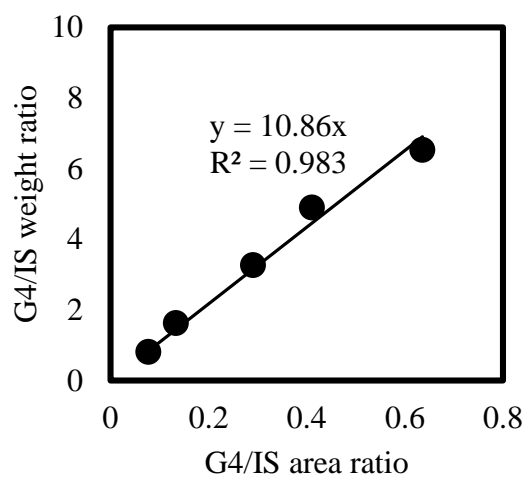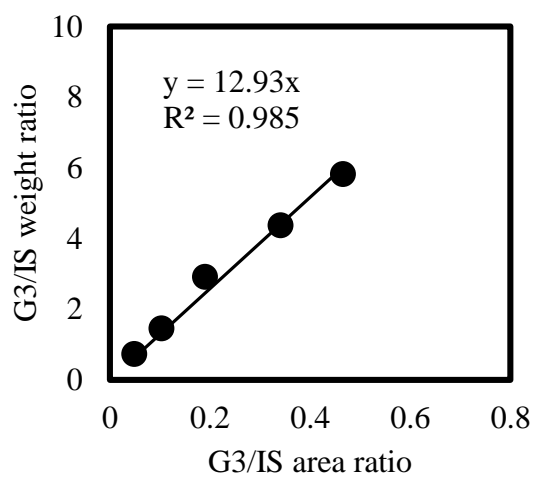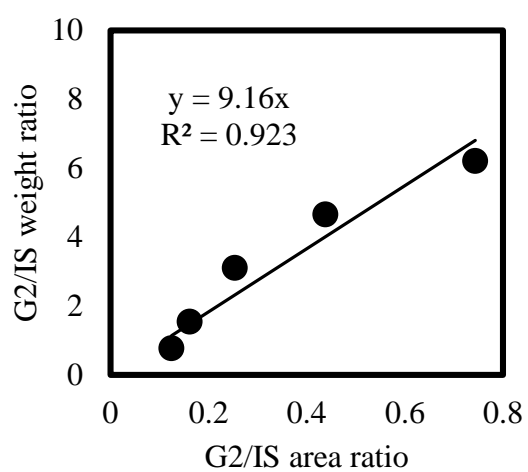
A2

Calibration Line of S-type Monomeric Lignin Model Compound by Py-GC/MS.

$y = 10.17x$
$R^2 = 0.916$

(x-axis: S1/IS area ratio; y-axis: S1/IS weight ratio)

A3

Yields of syringaldehyde (S_a) and vanillin (V_a) by nitrobenzene oxidation method and syringyl (S1-S4) and guaiacyl (G1-G4) pyrolysis compounds by Py-GC/FID for hardwood materials [a].

|	Nitrobenzene oxidation			Py-GC/FID		
	(mmol/ g of Klason lignin)		S_a/V_a molar ratio	(mmol/ g of Klason lignin)		S/G molar ratio
	S_a	V_a		S1-S4	G1-G4	
Pupulus sieboldii	1.89	0.56	3.38	0.285	0.133	2.15
Quercus myrsinaefolia	1.68	0.54	3.12	0.309	0.160	1.92
Quercus crispula	1.68	0.69	2.43	0.192	0.123	1.56
Castanopsis cuspidata	1.52	0.96	1.58	0.182	0.156	1.16
Aesculus turbinata	1.02	0.94	1.09	0.217	0.312	0.69

[a] Izumi at al., (1995).

A4

Yields of syringaldehyde (S$_a$) and vanillin (V$_a$) by nitrobenzene oxidation method and syringyl (S1-S4) and guaiacyl (G1-G4) pyrolysis compounds by Py-GC/MS for EFB raw material and hardwood materials.

	Nitrobenzene oxidation			Py-GC/MS		
	(mmol/ g of Klason lignin)		S$_a$/V$_a$ molar ratio	(mmol/ g of Klason lignin)		S/G molar ratio
	S$_a$	V$_a$		S1-S4	G1-G4	
Japanese hardwoods						
Pupulus sieboldii	1.89	0.56	3.38	0.405	0.214	1.89
Quercus myrsinaefolia	1.68	0.54	3.12	0.475	0.316	1.51
Quercus crispula	1.68	0.69	2.43	0.402	0.284	1.42
Castanopsis cuspidata	1.52	0.96	1.58	0.328	0.390	0.84
Aesculus turbinata	1.02	0.94	1.09	0.299	0.394	0.76
EFB materials	0.82	0.49	1.66	0.45	0.40	1.14

A5

Total ion chromatography of cellobiose-treated enzymatically isolated lignin by Py-GC/MS.
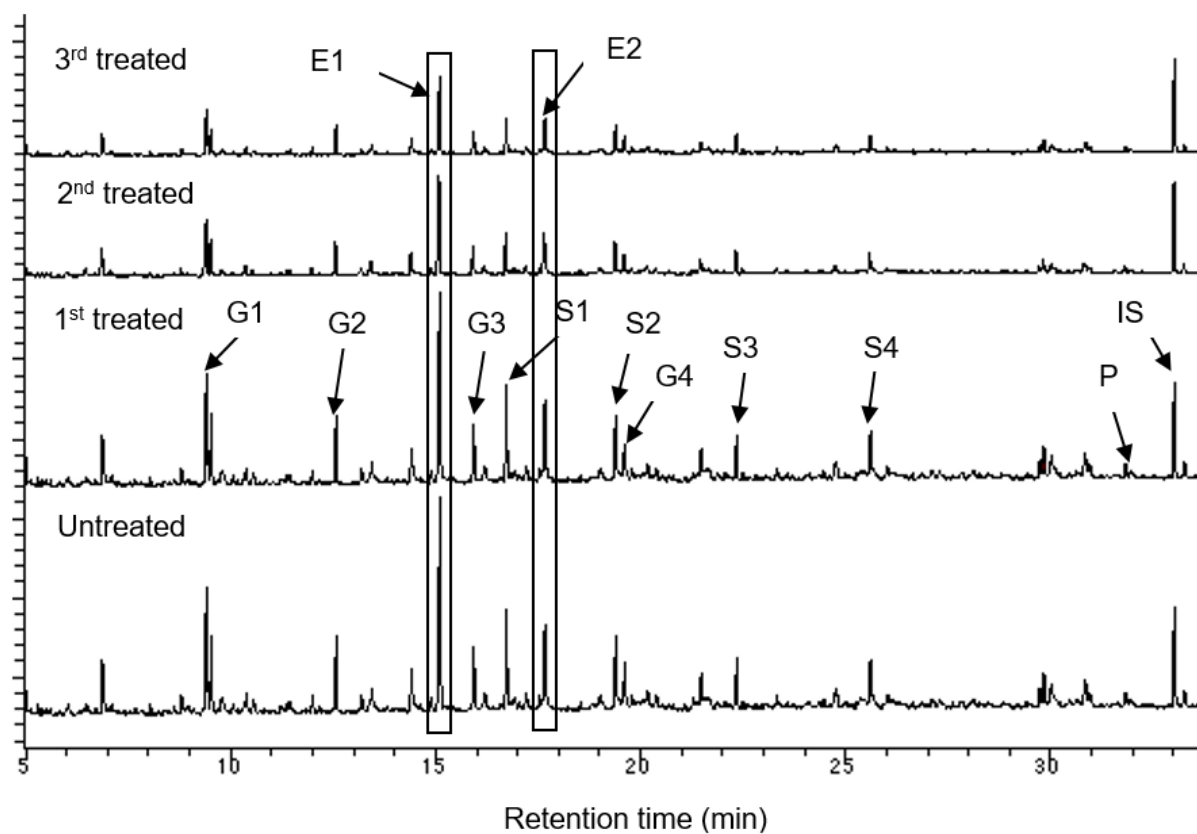
Acknowledgement

Firstly, I would like to express my deepest appreciation to my supervisor, Prof Hiroshi Ohi from Laboratory of Chemistry of Bio-Materials, Graduate School of Life and Environmental Sciences, University of Tsukuba for his helpful advice and constructive guidance throughout the study. I am thankful to my co-supervisor, Associate Professor Akiko Nakagawa-Izumi for her knowledge that I acquired from her comments and suggestions.

Besides, I would like to thank the committee members from Doctoral program in Appropriate Technology and Sciences for Sustainable Development, University of Tsukuba. They are Professor Tosiharu Enomae and Associate Professor Mikio Kajiyama who giving their advices, suggestions, and discussion to me for completing this study.

Furthermore, I would like to thank my previous supervisor Associate Professor Leh Cheu Peng from Bio-resource, Paper, and Coatings Devision, School of Industrial Technology USM who is being so helpful and supportive throughout the project. I also would like to thank my fellow lab mates, Mr. Roni Maryana, Mr. Agusta Samodra Putra, Mr. Lilik Tri Mulyantara, Ms. Maya Ismayati, Miss Nadia Nuraniya Kamaluddin, Ms. Umi Hamidah, Mr. Ayoub Salaghi, Mr. Vu Thang Do, and Mr. Atanu Kumar Das for their co-operation to take turn to use the facilities and always help each other during the study. Tolerance and advices from them are much appreciated.

Apart from that, not forgotten is RU Grant 1001/PTEKIND/811088 of USM and Scholarship Donations of Hokuetsu Kishu Paper Co., Ltd. (ECF Bleached Pulp and Dewaterability of Pulp for Paperboard) 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.

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.

<div align="right">

Yin Ying H'NG

2016 November

</div>