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A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

**Peracetic acid treatment of lignins for selective
production of valuable chemicals**

리그닌 유래 유용화합물의 선택적 생산을 위한
과초산 처리

By Se-Yeong Park

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DEPARTMENT OF FOREST SCIENCES
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Abstract

Peracetic acid treatment of lignins for selective production of valuable chemicals

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This study was conducted to investigate the degradation characteristics of lignin depending on the peracetic acid (PAA) and hydrogen peroxide (HP) concentrations, and to produce the low-molecular weight lignins by controlling the reaction conditions. In addition, reaction conditions for producing the definite products from lignin were investigated. Fractionation of the PAA-treated lignin depending on their molecular weight range was carried out by using open-column with selecting several solvents.

Acetic acid (AA) and HP were mixed at a ratio of 4:1, 1.5:1, 1:1, 1:1.5, and 1:5 (v/v) for preparing the PAA solution. After PAA treatment, the highest solid degradation rates were obtained as 29.0% and 65.3% at 1:1.5 (v/v) for alkali and kraft lignin, respectively. However, degradation characteristics of two lignins were different; the degradation rates were more rapid in kraft lignin (KL), which was made from hardwood species. This is due to the difference in reactivity depending on the lignin species. Meanwhile,

the lignin-derived compounds detected in the liquid fraction showed decrease in molecular weight and polydispersity than the untreated lignins.

Based on these results, PAA reaction was carried out to induce complete dissolution and lower molecular weight of the lignin. AA and HP were mixed at 4:1, 1:1, and 1:4 (v/v) ratios and employed as reaction media. After PAA treatment at 80°C, lignin was completely dissolved. When the high HP concentration in PAA solution was used, portion of the solvent-extracted fraction (ethyl acetate) gradually decreased. Both PAA and HP changed the lignin solubility at elevated temperature during the oxidation. Meanwhile, when the PAA reaction was carried out with high HP concentration (1:4, v/v), the molecular weight of lignin-derived compounds was the lowest. However, the lignin aromatic skeleton was not retained in that condition, and it was confirmed that lignin was converted to a specific structure containing a large amount of the carboxylic groups. On the other hand, when reacted with high AA concentration (4:1, v/v), low-molecular weight fragments retaining lignin aromatic structure were more produced. Hence, selective production of target compounds from lignin is possible by controlling PAA and HP concentrations.

This study aimed at the selective fractionation of lignin that have a uniform structures and lower molecular weight than untreated lignin. Before the column fractionation, lignin liquefaction was performed using a certain PAA solution (4:1, (v/v)) that is capable of maintaining the lignin structures. PAA-treated lignin was extracted by ethyl acetate (EA) to divide into organic and water-soluble fractions. And, EA fraction was fractionated by open-column using three solvents (chloroform-ethyl acetate, methanol, water), sequentially. Thus, when the least polar solvent was used in fractionation, the lignin-derived compounds in that fraction showed the lowest molecular weight. Meanwhile, some fractions did not show aromatic lignin structures and were identified as carboxylic acid containing polymers. From these results, it was concluded that separation of lignin-derived polymers by both molecular

weight and structural characteristic could be possible through open-column fractionation.

In this study, PAA solution was made directly for complete liquefaction of technical lignins and it was shown that selective production of the final lignin-derived products was possible depending on the PAA and HP concentrations. In addition, a simple separation method of the column fractionation can be applied to provide the method capable of selectively separating compounds with different molecular weight and structural properties.

Keywords: lignin depolymerization, peracetic acid treatment, technical lignin, open-column fractionation, selective production of lignin-derived compounds,

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List of Abbreviations

AA	acetic acid
AL	alkali lignin
Ar	Aromatics
DCA	dicarboxylic acid
EA	ethyl acetate
FT-IR	fourier-transform infrared spectroscopy
GC/MS	gas chromatography-mass spectrometry
GPC	gel permeation chromatography
HP	hydrogen peroxide
KL	kraft lignin
NBO	nitrobenzene oxidation
NMR	nuclear magnetic resonance
PAA	peracetic acid

Chapter 1

Introduction

1. Background

1.1. Potential of lignin as a bio-based source

Global energy demand is growing rapidly, and about 88% of this demand is currently provided by fossil fuels. Petroleum-based energy production is continually emitting carbon dioxide (CO₂) gas and heat playing the most important role in global warming and climate changes (Biller and Ross, 2014; Bondesson et al., 2013). For this reason, biomass including lignocellulosics as renewable and sustainable resources are attracting great attention to substitute the petroleum-based products. Lignocellulosic biomass has been projected as an abundant carbon-neutral source, which can decrease CO₂ emissions and atmospheric pollution (Mäki-Arvela et al., 2010; Sun et al., 2011).

For changing the physical and chemical properties of the lignocellulosic matrix, pretreatment of lignocellulosic biomass, which is an expensive procedure with respect to cost and energy, is required (Bildirici and Ersin, 2015; Martín and Grossmann, 2013). Besides, frequent fluctuation in oil prices and the shale gas boom result in decrease of overall price competition of the pretreatment process. That makes production of fuels and chemicals from biomass difficult to use of lignocellulosic biomass. Nevertheless, the world leading global corporations including Abengoa and Dupont and research institutes are persistently making efforts to resolve the economic limit.

In cellulosic ethanol plant, pulp and paper industries using the lignocellulosic biomass, lignin-rich residues are produced as a byproduct. It is usually burned to generate heat and electricity and is underestimated until the last few decades (Guo et al., 2013; Mansouri and Salvadó, 2006; Yang and

Wyman, 2008). Lignin is second abundant natural polymer synthesized by precursor of *p*-coumaryl, coniferyl, and sinapyl alcohol. It is composed of a diversely linked network of aromatic compounds with mainly β -O-4, β -5, and biphenyl linkages, and hence it can provide a potential source for sustainable and renewable production of fuels and aromatic chemicals, which are generally obtained from petroleum-based feedstock (Hatakeyama and Hatakeyama, 2009; Isikgor and Becer, 2015). To increase the value of this byproduct and the profitability of lignocellulosic-based production, many research efforts have been made to convert the lignin into value-added products such as chemicals, fuels and biomaterials (Azadi et al., 2013; Laurichesse and Avérous, 2014).

Global lignin market size is currently predicted to exceed 17.4 million tons and the industry is likely to be valued at more than USD 6 billion by 2022. Furthermore, it has been reported that U.S market size was estimated at over 3,000 kilo tons in 2015 (Figure 1-1). Currently, main consumption of lignin is focused on liginosulfonates, kraft lignin, and aromatics. Particularly, rising in demand for the lignin-based materials will be among key factor for global lignin market growth (Global Market Insights, 2016).

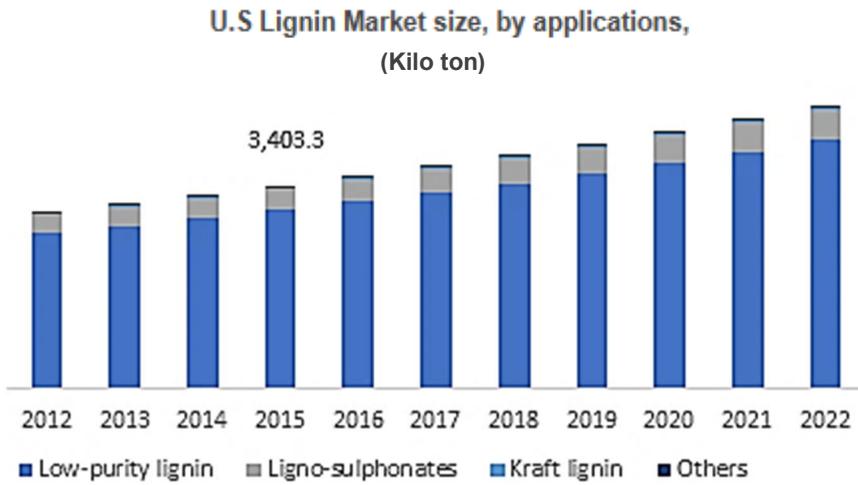
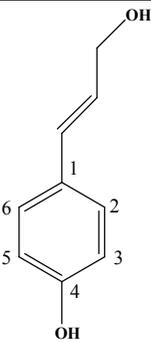
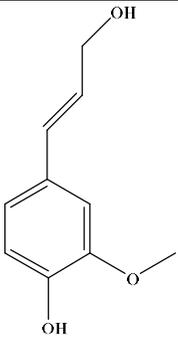
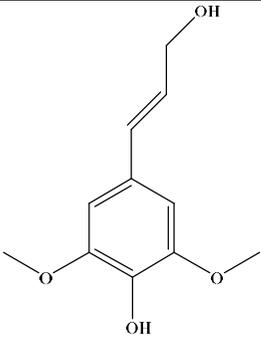


Figure 1-1. U.S lignin market size, by applications (Global Market insights, 2016).

1.2. Lignin structures & technical lignins

Lignin varies in structure according to their plant sources based on the ratio of different type of monomer: *p*-coumaryl, coniferyl, and sinapyl alcohol (Table 1-1). Grasses typically contain all three monomers, while softwood lignins contain mainly coniferyl alcohol and hardwood lignins contain both coniferyl and sinapyl alcohol (Boerjan et al., 2003). With differing monomer content, bonds formed during polymerization via an enzyme-mediated dehydrogenation also differ, and they are comprised of carbon-oxygen (C-O) and carbon-carbon (C-C) bonds. Repeating units of the monolignols linked by the two bonds give heterogeneous and complex properties to the final lignin polymer molecules (Norgren and Edlund, 2014; Sakakibara, 1980).

Table 1-1. Chemical structures of lignin monomeric precursors

Lignin monomer type		
<i>p</i> -Coumaryl alcohol	Coniferyl alcohol	Sinapyl alcohol
		

With the lignin biosynthetic formation systems, isolation method of the lignin from raw biomass is a major factor giving differences between lignins. Lignocellulosic biomass is comprised of cellulose, hemicellulose and lignin by complex linkages. Initial biomass structures depending on plant sources and composition of these constituents influence on controlling the lignin (Carere et al., 2008; Chandra et al., 2007). Therefore, isolation method of lignin from lignocellulosic biomass can be an important factor in consideration of the end-use of lignin. Generally, lignin isolation processes can be divided according to their operation conditions involved in additive chemicals, solvents, reaction temperature, which lead to different lignin properties (Figure 1-2). By the method that is usually being conducted, two principal lignins can be categorized, which are sulfur-containing and sulfur-free lignins (Cotana et al., 2014; Zhou and Lu, 2014). The widely used lignins are as the sulfur-containing, which are being commercialized for various applications. Kraft lignin, and sulfite lignin are included representatively. On the other hands, sulfur-free lignins resemble more closely to the structure of native lignin, and hence it is able to provide the high-purity of lignin. Sulfur-free lignin can be obtained through the processes such as organic solvent pulping, soda pulping, and biomass conversion technologies (Brudin and Schoenmakers, 2010; Li et al., 2009; Mansouri and Salvadó, 2006; Ragauskas et al., 2014).

Various technical lignins have been considered as a potentially interesting raw material, since they are produced in processes dealing with the treatment of lignocellulosic biomass and readily available in large amounts (Laurichesse and Avérous, 2014). However, utilization of the technical lignins in high value-added applications is limited because there are several factors restricting the use of lignin. Different interunit linkages, molecular weight distribution, chemical reactivity originated from the separation methods as well as plant sources of lignin result in challenges to be solved (Vishtal and

Kraslawski, 2011).

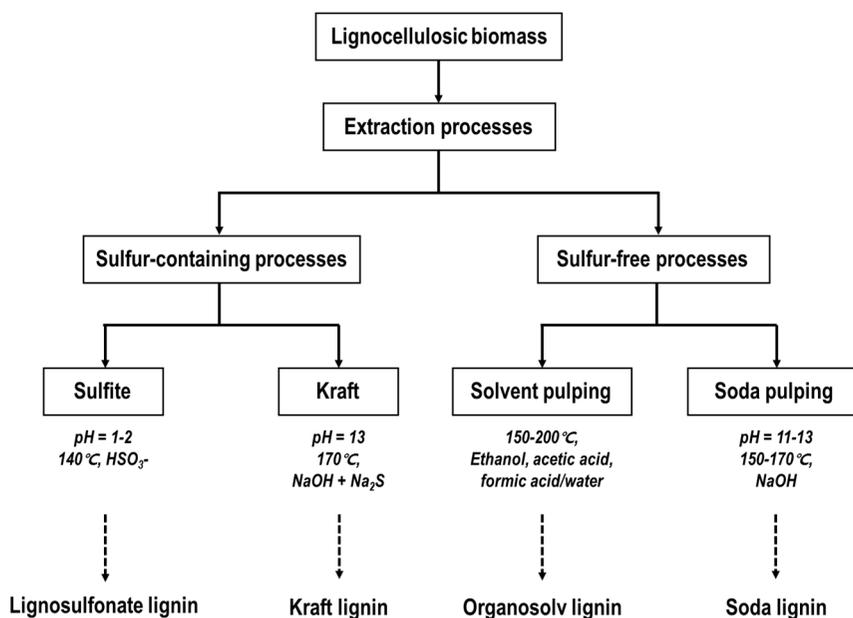


Figure 1-2. Different extraction processes to isolate lignin from lignocellulosic biomass and produced technical lignins (Laurichesse and Avérous, 2014).

1.3. Complete valorization of lignin

Lignin valorization is an essential task based on techno-economic analyses of lignocellulosic biorefineries. However, current plant designs dispose lignin to produce heat and power by combustion process. To overcome the profitable limitation of existing lignocellulosic-based production, stream of complete valorization of lignin should have been established for value-added utilization.

Among various processes using lignin, combustion is a well-established commercial technology producing power and electricity, which are the best cost-effective process in the paper and biomass-related industries. In addition to this process, gasification has been attracted for many years since it can produce a syngas from lignin under high temperature and pressure conditions. The final gaseous products by gasification are mainly consisted of carbon monoxide (CO), hydrogen (H₂) and methane (CH₄). These syngas can be obtained easier and more versatile to use compared to other processes. For example, the syngas can be directly applied in gas engines and gas turbines. It can also be converted into liquid fuels through a Fischer-Tropsch synthesis producing green diesel (Liu et al., 2013; Yan et al., 2013). Meanwhile, pyrolysis is a leading method that it can convert dry lignin to a liquid product in the absence of oxygen known as pyrolysis oil or bio-oil. This pyrolytic lignin has potential as transportation fuels since they have a plenty of aromatic and hydrocarbon structures.

All current commercial uses of lignin, except combustion, take advantage of polyelectrolyte and polymeric properties. One of the commercial use of lignin as macromolecules is widely applied to a dispersant using sulfonated lignin generated by sulfite pulping, which can be acted as a surfactant based on their polyelectrolytic properties. Use of the lignosulfonate accounts for the greater part of commercial lignin applications and it is expected that consumption of lignosulfonate will continually increase. In addition, polyurethanes have attracted interests since lignin structures is consisted of a plenty of hydroxyl groups and hence its properties can be advantageous for polymer applications. Another interesting approach for the direct use of macromolecular lignin consists in the exploitation of its high carbon content in the range of 55-65% to prepare precursors for carbon-based materials. For example, carbon fibers and activated carbons can be the final products using the carbon-rich lignin resources.

Lignin is the only renewable source of an important and high-volume class of compounds as aromatics. Direct and efficient conversion of lignin to discrete molecules or classes of low-molecular weight aromatics is an attractive long-term opportunity. In the early studies for aromatics, production of benzene-toluene-xylene (BTX) from lignin as an alternative bio-based chemical were considered as a target for chemical production (Bozell, 2008; Zakzeski et al., 2010). Since then, production of vanillin, phenols and other lignin monomers can be also possible by various processes, especially depolymerization technology. In addition to aromatics, small fragments are able to be produced from lignin by breaking the aromatic structures and they are considered as new building blocks. Dicarboxylic acids (DCAs) such as succinic acid, muconic acid are the main representative small fragments as value chemicals, and they can be produced by thermal reaction as well as biological systems of organisms (Isikgor and Becer, 2015; Ma et al., 2014).

Although extensive research is currently being undertaken all over the

world, system buildup for total utilization of lignin based on the concept of biorefinery has not been established completely yet. Nevertheless, recent trends are on the path of establishing a worldwide bio-based economy, and hence lignin may have a great contribution in this context (Figure 1-3).

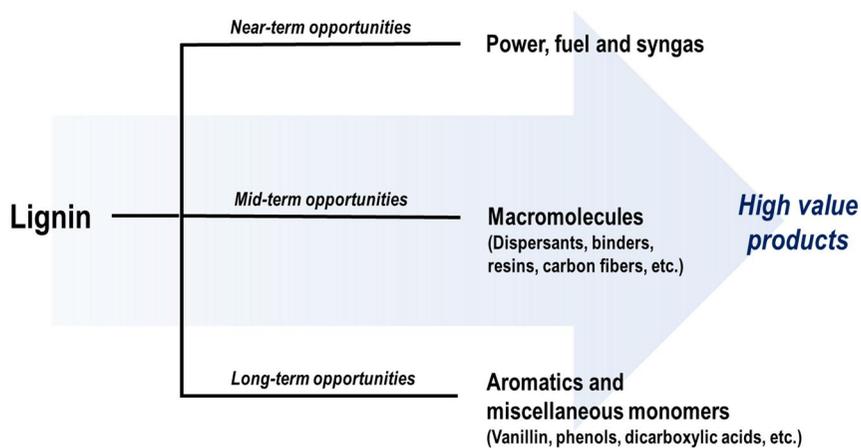


Figure 1-3. Products potentially derived from lignin conversion.

1.4. Promising conversion processes for valued-products

Even when lignin is isolated, it often needs to be depolymerized into smaller molecules for more valued-uses of lignin. There are several thermal processes, which can transform macromolecule lignin into low-molecule chemicals under high temperature condition. Pyrolysis with or without catalyst, and supercritical depolymerization are represented as the main techniques. Through optimization of the reaction conditions, targeted compounds can be selectively obtained and its yield also can be improved (Brebou and Vasile, 2010; Gosselink et al., 2012). Especially, lignin depolymerization in supercritical fluid takes center stage as a promising thermal decomposition method, where solvents in that state behave like non polar enhancing the solvolysis of many types nonpolar substances lignin (Ding et al., 2011).

In nature, lignin is utilized by specialized microorganisms encoding metabolic pathway that can break down components of lignin. Microorganisms such as fungal and bacterial species breaking down lignin are able to use it as a carbon and energy source for metabolite production (Leonowicz et al., 1999). Many scientists have studied on lignin degrading enzyme for several decades. The ligninolytic enzyme including ligninolytic peroxidase (LiP), manganese dependent peroxidase (MnP), laccases can cleavage heterogeneous C-C and C-O bonds, and hence it is considered as potential bio-catalyst to produce small fragments from lignin (Abdel-Hamid et al., 2013; Sigoillot et al., 2012).

Properties of technical lignin and its derived compounds can be controlled by pre-process for targeted final products. But high structural diversity of the lignin and its broad molecular distribution makes its commercial use a difficult task. The size of the lignin molecules can vary between 1000 Da and 100,000 Da within a same sample, therefore, fractionation technique has become one of the best ways to obtain specific lignins (Saito et al., 2014; Toledano et al., 2010). Fractionation can provide desirable properties related to molecular weight, and functional group content.

Precipitation is traditional method to obtain lignin powders, and it consists in extracting lignin from liquor decreasing gradually the pH of the solution. Simple addition of a strong mineral acid such as sulfuric acid is enough to reach the objective (Sun and Tomkinson, 2001). In other words, how to precipitate the lignin is an important factor, which provide different properties of lignin. Another promising method consists in using several organic solvents for the lignin extraction. Solvent fractionations are the processes of isolating components of any mixture based on their different solubilities in a solvent or mixture of solvents. Especially, successive solvent extraction could provide various information according to the characteristics of fractionated lignin and help to facilitate the desired application (Li et al., 2012; Shuerch, 1952; Sun et al., 2000a).

The huge potential of lignin gives several opportunities to take advantage of its versatility for multiple applications. The main uses of lignin have been divided into two different groups: (i) Without chemical modification, where lignin is directly incorporated into matrix to give new or improved properties, and (ii) with chemical modification to prepare a large range of chemicals, building blocks and polymers. Of them, chemical modification of lignin can be classified into following categories: (i) modification by creating new chemical active sites, and (ii) chemical modification of hydroxyl groups.

Chemically, lignin has a variety of functional groups, namely hydroxyl, methoxyl, carbonyl and carboxyl groups. Higher end uses of lignin have not previously been achieved because of its structure complexity. To overcome this limitation, lignin can be modified to increase the range of their applications. Different types of modification have been proposed to increase its chemical reactivity, reduce the brittleness of lignin-derived polymers, increase its solubility in organic solvents, and improve the ease of processing the lignin. On the basis of the previously described lignin structure, its reactivity is based by its particular structure with both specific functional groups (mainly hydroxyl groups) and specific position (mainly ortho position) of the aromatic ring. So far, different chemical modifications pathways have been investigated to introduce new chemical active sites in the lignin chemical structure. Several modifications such as nitration, amination, alkylation/dealkylation, carboxylation, and halogenation have been investigated, though less extensively than reactions involving hydroxyl groups (Chen et al., 2011; Lange, 1980; Laurichesse and Avérous, 2014).

Lignins have phenolic hydroxyl groups and aliphatic hydroxyl groups at C- α and C- γ positions on the side chain. Chemical modification of the hydroxyl groups results in the formation of lignin polyol derivatives by etherification, which in turn improves the solubility of the lignin (Ahvazi et al., 2011). After modification, the majority of phenolic hydroxyl groups are converted into aliphatic hydroxyl units. Thus, more reactive hydroxyl groups become readily available. Esterification is another functionalization methods giving the reactive sites by adding ester group, which able to be used for preparation of polyester, epoxy resin and elastomeric materials (Gordobil et al., 2016; Thielemans and Woo, 2005).

In summary, a variety of techniques are involves in lignin conversion process to achieve their end-uses as shown in Figure 1-4. Through the processes, macromolecular lignin can be separated depending on their

molecular weight and also modified for changing their chemical structures by additional further process.

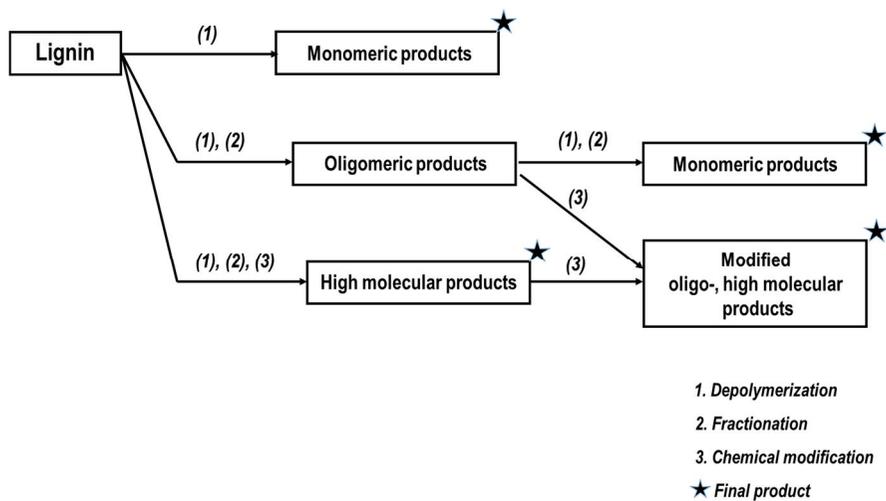


Figure 1-4. Proposed routes for the conversion of lignin for total utilization (Optional processes for target products; 1) depolymerization, 2) fractionation, and 3) chemical modification).

1.5. Peroxy acid as an emerging oxidant for lignin valorization

Peracetic acid (PAA), which is formed by synthesis of hydrogen peroxide (HP) and acetic acid (AA) with mineral acid catalysts, has been widely used as a disinfectant and sanitizer in various industries such as food processing, pharmaceutical, medical application, as well as pulp & paper (Tero and Simo, 2017). PAA has gained great attention as an alternative agent for chlorine-based chemicals because it has positive features such as antimicrobial activity, inexpensive capital cost, and non-toxicity (Mehmet, 2004; Laura et al., 2018).

Since PAA is comprised of peroxy group (HOO-) acting as a powerful oxidizer, it has newly applied in the field of biomass for selective removal of lignin from lignocellulosic materials. It can reduce the enzyme loading, hydrolysis time during the bioethanol production process by improving the enzyme accessibility (Wi et al., 2015, Lee et al., 2017). Particularly, PAA can not only remove the lignin from complex structure of biomass but also degrade or convert the macromolecular lignin into value-added chemicals as alternatives of petroleum-based chemicals (Ma et al., 2014). Thus, PAA has been gained much attention toward bio-refinery lignin.

As shown in Figure 1-5, hydroxonium (HO⁺) generated by heterolytic cleavage of the peroxy bonds attacks electron-rich sites in lignin, resulting in lignin depolymerization. In addition, PAA can lead to oxidative cleavage of the β -aryl ether linkage in lignin and degrade the aromatic ring to produce dicarboxylic acids by several reaction such as hydroxylation, demethoxylation, and displacement of phenolic moieties (Barros et al., 2010; Tan et al., 2010). However, many studies on PAA chemistry in biomass fields are generally conducted using commercial PAA solutions with different physical and

chemical properties. Although PAA has a great potential to produce commercial bio-based products, understanding of reaction mechanism of PAA with lignin is needed.

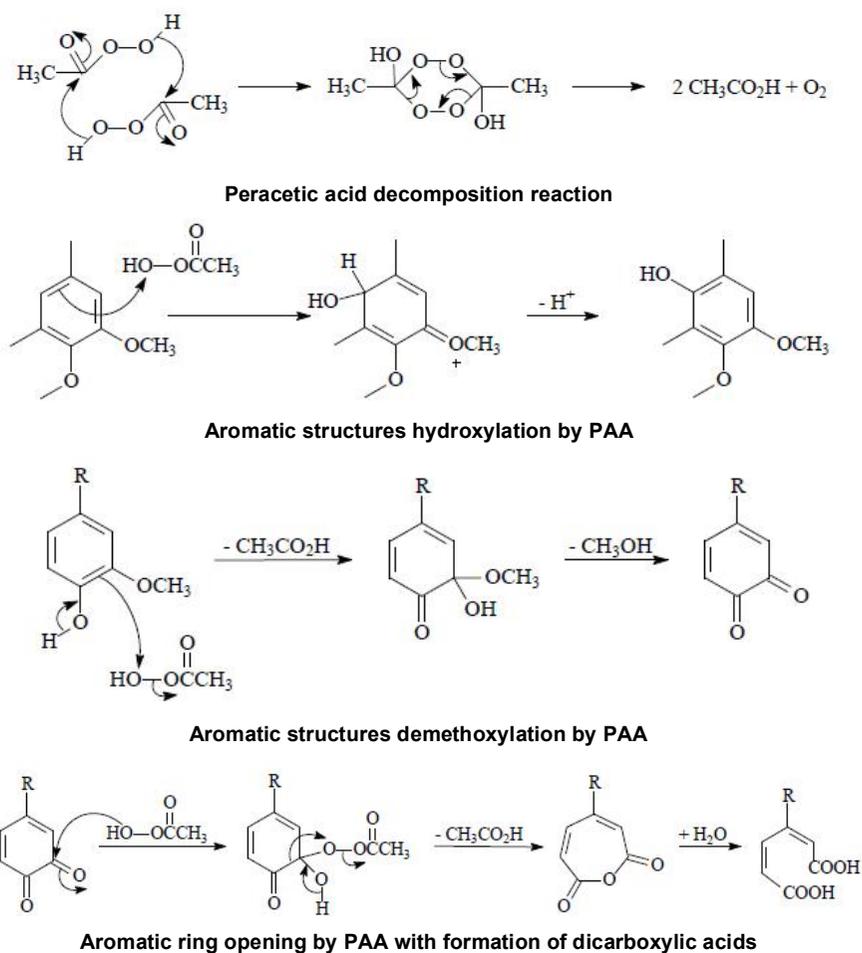


Figure 1-5 Peroxyacid oxidation mechanism of lignin (Barros et al., 2010).

2. Objectives

As mentioned before, complete utilization of lignin is needed to overcome the low profitability of lignocellulosic-based industries. While increasing the concern, depolymerization method has been great attention for producing value-added chemicals from lignin such as vanillin, succinic acid as well as high molecule compounds used for polymer applications. However, current lignin depolymerization studies have focused on increase of productivity and selectivity of targeted monomeric compounds. There are a few limitation on existing their approaches. 1) It is not easy to isolate the certain targeted compound from mixtures produced by depolymerization because so many types of products are generated simultaneously. And, 2) there is an unexpected result leading to production of undesirable side compounds during depolymerization reaction, which have high molecular and broad molecular weight distribution. For these reasons, total application of lignin-derived products including not only monomers but also high molecules, and efficient separation methods of them should be considered.

In this study, to change the lignin structure to more uniform and reactive, liquefaction process by peracetic acid (PAA) treatment was adopted using technical lignins. PAA is a promising solvent, which can solubilize solid lignin under lower temperature below 100°C compared to traditional technologies for depolymerization of lignin. Therefore, depolymerization of lignin using PAA was performed to convert heterogeneous macromolecule lignin into lower fragments. Furthermore, lignin-derived compounds produced by PAA treatment were separated by applying open-column fractionation

methods for targeted end-use.

Therefore, the objectives of this study aimed

- (1) To investigate the effects of PAA and hydrogen peroxide concentrations in lignin degradation by controlling mix ratio of acetic acid and hydrogen peroxide
- (2) To understand the degradation characteristics of different technical lignins by PAA treatment
- (3) To investigate the chemical properties of lignin-derived compounds after PAA treatment
- (4) To fractionate the PAA-treated lignin using open-column for producing lignin-derived compounds, selectively

3. Literature review

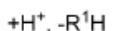
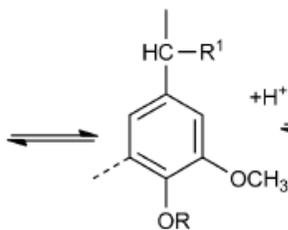
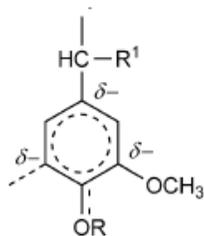
3.1. Lignin behavior in acidic and alkaline medium

Before discussing specific reaction for producing lignin-derived products, whether high or low-molecular weight, general lignin reactivity in acidic and alkaline media should be understood. The hydroxyl or methoxy group on the aromatic rings carry lone pairs of electrons on the oxygen atom, which overlap with the p -electron system of the aromatic ring, leading to relatively higher electron density centers (δ^-) in the ortho and para positions. These sites are susceptible to attack from electrophiles (Fleming, 1976). In lignin structures with an aliphatic double bond conjugated to the aromatic ring (Figure 1-6), partial negative centers (δ^-) are also extended to the β -carbon atom for structures with an aliphatic double bond and an α -carbonyl group. The equivalent resonant structures with partial negative centers (δ^-) are only observed in the O–C $_{\alpha}$ –C $_{\beta}$ region. Formation of the partial negative centers can promote oxidation by a number of cations, such as Cl $^+$ and HO $^+$, which are formed during oxidation. Conversely, the elimination of an α -substituent (γ -substituent in ring-conjugated structures) could result in the formation of enone intermediates of the quinone methide type, and the formation of centers with relatively low electron density (δ^+) at the carbonyl carbon and at carbon atoms in vinylogous position to the carbonyl carbon (Patai and Rappoport, 1974). These δ^+ sites can be actively attacked by nucleophiles. In acidic media, both phenolic and non-phenolic units can react with electrophilic and nucleophilic agents (Nimz, 1966; Dence, 1971). In alkaline media, however, a similar

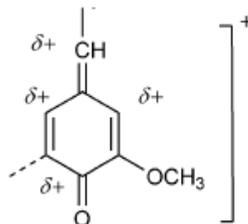
distribution of electron sites for electrophilic and nucleophilic reactions has

Acidic Condition

for electrophilic attack

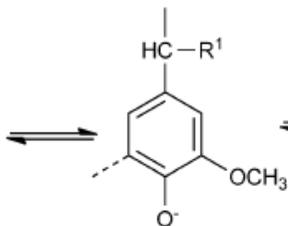
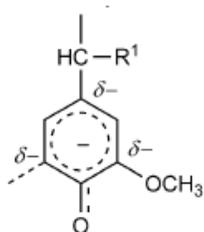


for nucleophilic attack

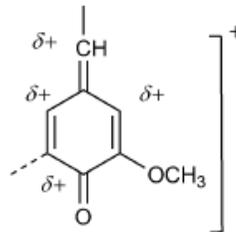


Alkaline Condition

for electrophilic attack



for nucleophilic attack



R=H, lignin
R¹=OH, OAr

only been encountered in phenolic units (Gierer, 1985). Several oxidative delignification chemistries have been significantly developed and reviewed in many researches (Ma et al., 2015).

Figure 1-6. Lignin activation in acidic and alkaline condition
(Ma et al., 2015).

3.2. Lignin oxidizing agents

Among the oxidants used for lignin oxidation, hydrogen peroxide and oxygen are widely used and their specific mechanisms are also well-established. Additionally, peroxy acid has attracted a great attention recently on conversion technique of lignin since it has been considered as a promising oxidant which able to dissolve lignin under mild reaction conditions.

Oxidation with oxygen (O₂) proceeds predominantly through a radical reaction mechanism. Since oxygen is a weak oxidizing agent in its normal state, the reaction requires basic conditions to ionize free phenolic hydroxyl groups in the lignin units. Conjugated side chain oxidation, aromatic ring cleavage, oxidative condensation are dominant pathways in overall. Several recent reports have demonstrated the application of different catalytic processes to produce phenolic aldehydes in considerable yields (e.g., vanillin, syringaldehyde, and benzaldehyde) (Deng et al., 2008; Sales et al., 2006). Due to the radical reaction mechanism, however, condensation of lignin fragments appears to be inevitable in oxygen-based lignin conversions.

Hydrogen peroxide is recognized as an environmentally mild chemicals, which makes it a common oxidant for pulp bleaching. Most hydrogen peroxide processes are targeted at destroying chromophores and aromatic ring cleavage in lignin (Gierer, 1986; Fang et al., 1999; Sun et al., 2000). For example, the unsaturated side chain of 3-(4-hydroxy-3-methoxyphenyl)-2-acrolein is oxidized to produce *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic

acid, and *p*-hydroxylguaiacol. It is also worthy to note that these fragments can be converted to quinones by hydrogen peroxide through Dakin-like oxidation of lignin phenolic units, which in turn generate new chromophore structures. *p*-Hydroquinone, *p*-quinone, and *o*-quinone have been detected in the effluent from peroxide bleaching of mechanical pulp TMP (Kadla, 1997; Crestini et al., 2010; Pan et al., 1999; Nonni et al., 2007). In addition, hydrogen peroxide is also an effective oxidant for aromatic ring cleavage reaction. The *o*- and *p*-quinone rings are comprised of dual enone elements, which offer multiple reaction sites that can be attacked by hydroperoxide anions, leading to ring opening products. The formation of dicarboxylic acids (DCAs) has been detected in the effluent of the hydrogen peroxide bleaching of chemical pulp (Sun and Argyropoulos, 1996; Holmbom et al., 1991).

A peroxy acid is an acid that contains a perhydroxy group (-OOH) in the place of the hydroxyl group of its parent acid. Peroxy acid can be derived from either mineral acids (e.g., sulfuric acid) or carboxylic acid (e.g., acetic acid, formic acid). The hydroxonium ion (HO⁺) produced from heterolytic cleavage of the peroxy bond is a strong electrophilic species that can readily react with a number of electron-rich sites in lignin, including both aromatic ring and olefinic side chain structures. The application of peroxy acids for wood pulping and bleaching has attracted a considerable amount of interest in the past and at present (Barros et al., 2010). Despite significant interest in applying peroxy acid for lignin removal from lignocellulosic biomass, there has been little effort expended towards applying peroxy acid chemistry to converting biomass lignin to value-added products. Studies of monolignol and β -aryl ether model compounds indicate the direct production of phenolic monomeric fragments (benzaldehyde and benzoic acid) by dehydration reaction and double bond cleavage. Moreover, ether bonds are hydrolyzed in the acidic media of peroxy acid solution. In addition, the hydroxylated products formed can also undergo demethoxylation and oxidation by peroxy

acid to form quinone structure and subsequently ring cleavage products (Ma et al., 2016; Sun et al., 2000b). While muconic acid and its derivatives are among the expected products, further degradation compounds such as maleic/fumaric acid, acetic acid, and formic acid were also observed in PAA medium (Johnson and Frrand, 1971; Ma et al., 2014). Meanwhile, PAA belong to the peroxy acid has different solution properties, resulting in different reaction mechanism of lignin depolymerizaiton. Although PAA is generally prepared by mixing acetic acid and hydrogen peroxide, the mixd ratio is the key factor in respect of lignin oxidation process. In Wi and coworkers' research, they used a solvent by mixing hydrogen peroxide and acetic acid (HP-AA) with different ratio (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1, v/v) to remove the lignin from oak wood and pine wood. As a result, it was concluded that delignification rate of by HP-AA pretreatment were influenced by mix ratio of HP and AA. Especially, mix ratio of 5:5 showed the highest delignification rate about 97% (Wi et al., 2015). Another study also described that the concentration of hydrogen peroxide and PAA in PAA medium with polyoxomethalates resulted in different oxidation rate of lignin model compounds such as ferulic acid, vanillin, sinapic acid (Popova et al., 2014).

3.3. Production of chemicals in lignin

Lignin has been found to be an appropriate raw material for producing low molar mass compounds like vanillin, simple and hydroxylated aromatics, quinines, aldehydes, aliphatic acids and many others chemical compounds (Carlos et al., 2001; Erdocia et al., 2012). The recognition that lignin can be degraded into phenolic materials has stimulated almost 60 years of research. It is commonly known that thermal chemical, biochemical and chemical conversions have also been developed to produce high value compounds. Various types of lignins (alkali, sulfite and kraft) from both softwood and hardwood have been subjected to these processes (Arun and Eyini, 2011; Pandey and Kim, 2011).

3.3.1. Aromatic chemicals

Various lignins have been oxidized studying the most favorable conditions (temperature, reaction time, air pressure) to obtain fractions with high value-added compounds. Among all of the products that can be produced by lignin oxidation, vanillin (4-hydroxy-3-methoxybenzaldehyde) constitute the most well-known and valuable product. Vanillin is mainly used as a flavoring agent and as a chemical feedstock in the pharmaceutical industry (Silva et al., 2009). The first hints that it might be possible to produce vanillin

from lignin-containing wastes published in 1875 based on a vanillin-type smell in spent acid sulfite pulping liquor (Hocking, 1997). This opportunity was developed, and for a long time vanillin was exclusively produced by oxidation of lignosulfonates. Recently, an interesting work has been developed by Tarabanko et al. to separate efficiently vanillin and syringaldehyde, two structurally and very similar compounds that are produced from lignin oxidation. They demonstrated the possibility to separate both compounds by a one-stage crystallization with a high yield (90%) and purity over 98% (Tarabanko et al., 2013). Besides, vanillin production has accelerated continuously through biological metabolite systems. Several microorganisms have been reported to transform ferulic acid to vanillin. The highest vanillin production from ferulic acid (more than 10 g/L with a molar yield of about 75%) was obtained with actinomycetes, such as *Amycolatopsis* sp. HR167 and *Streptomyces setonii* ATCC 39116 (Rabenhorst, 1997; Gallage and Moller, 2015).

Except for vanillin and its derivatives, previous studies related to production of alkylated aromatics and aldehydes also has been reported. In supercritical fluids, syringol, guaiacol, 4-methysyringol, benzaldehyde and other lignin-derived chemicals are produced and yield of certain chemicals can be improved by using catalysts. It was reported that supercritical treatment with formic acid improved the yield of total monomeric compounds and the selectivity of valued-products (Kim et al., 2013; Rahimi et al., 2014). Another studies described that presence of catalyst such as Pd/C, Zn, NiAu showed high conversion yields, and hence it resulted in yield for aromatic chemicals (Parsell et al., 2013; Zhang et al., 2014).

3.3.2. Dicarboxylic acid chemicals

Dicarboxylic acids (DCAs) such as succinic acid, adipic acid, maleic

acids are highly valuable industrial chemicals, and intermediates used in the fields of biopolymer, pharmaceutical and food additives (Lee et al., 2004; Sato et al., 1998). Discovering a selective route to convert lignin to DCA will pave a new avenue towards lignin valorization. Developing a green route to produce DCA from renewable biomass lignin will be of a prime interest to both chemical and biomass conversion industry. Oxidative aromatic ring cleavage to yield muconic acid and its derivatives is a well-recognized reaction (Lin et al., 2001; Ran et al., 2008). Previous study reported that DCA and their derivatives have been detected in the reaction mixture from oxidative delignification of wood pulp (Gierer, 1986).

Oxidation of lignin can be performed with or without catalytic reaction. The DCAs production technology developed by Zhang reported that the mineral chalcopyrite and hydrogen peroxide to produce high yields of DCAs including malonic acid, succinic acid, malic acid and maleic acid. Total DCAs represented approximately 94% of the total soluble products after the reaction (Ma et al., 2014).

Biological methods for producing DCA from lignin are also studied for a few decades ago. Powerful oxidative enzymes secreted by rot fungi and bacterial can degrade lignin macromolecules into aromatic monomers such as catechol and protocatechuic acid. These intermediates are involved in the β -ketoacid pathway, where muconic acid are generated by ring opening (Vardon et al., 2015). The muconic acid can be converted into innumerable downstream products including adipic acid, which is the most commercially important DCA and it has a big market volume with an annual demand growth about 3-3.5% globally. Figure 1-7 shows the detailed lignin to muconic acid pathway using *Pseudomonas putida* KT2440 as an organism presented in Polen's research paper and similar fragmentation mechanism in thermal reaction can be observed through the pathway (Polen et al., 2013).

3.3.3. Oligomeric and partially depolymerized chemicals

Oligomeric lignin or partially depolymerized chemicals readily obtained as the end product of the several fractionation and they can be used as the starting material for the preparation of a variety of value-added specialty compounds. (Ragauskas et al., 2014). Particularly, various material application fields have big interests in a lignin-based polyol for preparing a polyurethane form (PU). Depolymerization of lignin results in depolymerized lignin products with lower molecular weights and high aliphatic/total hydroxyl numbers, making them suitable for the preparation of PUs at higher replacement ratios (Cinelli et al., 2013) . For these reason, Cinelli and coworkers made the liquefied Indulin AT lignin under microwave treatment in which contained partially depolymerized lignin and they were used for preparing flexible polyurethanes (Cinelli et al., 2013). Mahmood et al., also demonstrated that hydrolytic depolymerization of kraft lignin and hydrolysis lignin could be a viable route for the preparation of depolymerized products with low-molecular weights and acceptably high hydroxyl numbers, and these depolymerized products can be incorporated in the preparation of rigid PU foams at higher replacement ratios ($\geq 50\%$) without much compromising the properties of the final foams. Another study emphasized that liquid polyol from kraft pine lignin showed good performance on density property as rigid PU forms (Li and Ragauskas, 2012).

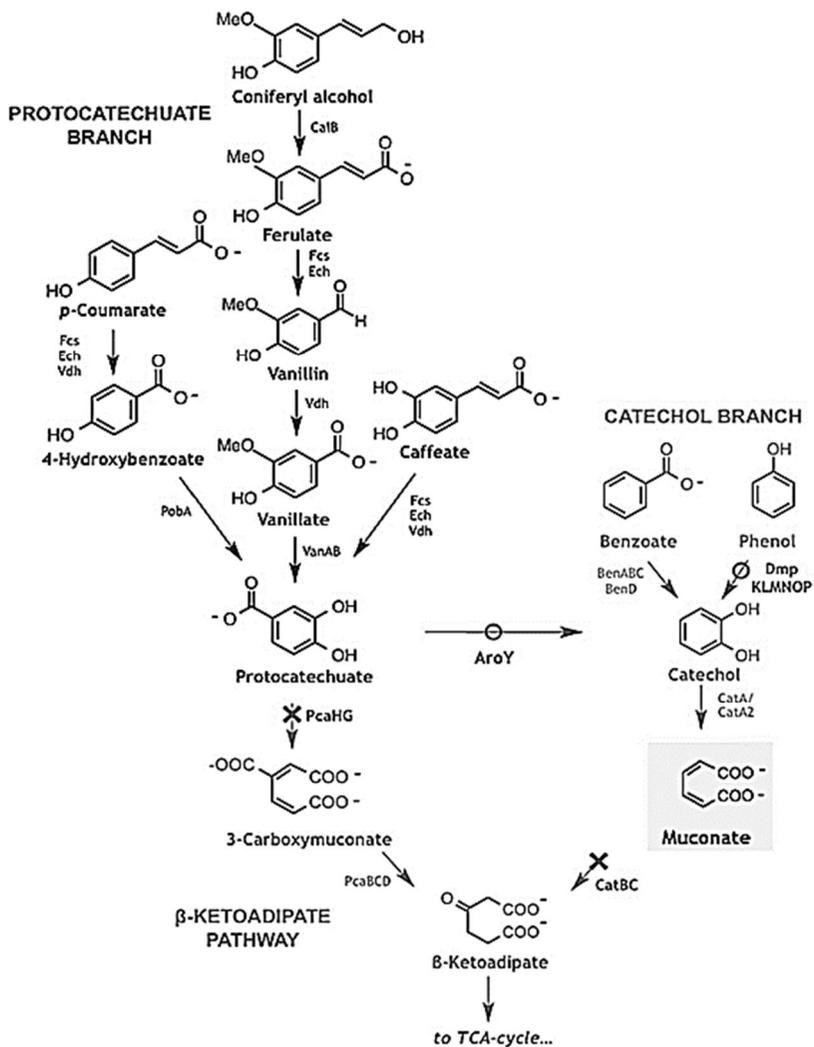


Figure 1-7. Biological funneling of lignin aromatics to muconate *P. putida* KT2440 (Polen et al., 2013).

3.4. Methods for fractionation of lignin and its extracts

Structure of lignin varies depending on its origin and extraction method used to obtain lignin. Lignin molecules are very heterogeneous, therefore, fractionation has become one of the best ways to gain specific lignins. The aim of fractionation is to obtain specific molecular weight fractions with defined properties. Therefore, it will be readily available for producing high value-added products.

Reducing molecular and structural heterogeneity of lignin by sequential extraction using organic solvents of increasing hydrogen-bonding capacity has been reported. The molecular weight of the isolated fractions increased with increase of the hydrogen bonding capacity and polarity of the solvents. Various technical lignins used for successive fractionation studies. Alcell lignin and kraft lignin using combination of solvents, like ethyl acetate-methanol, dichloromethane-acetone-methanol (Passoni et al., 2016; Thring et al., 1996). Small oligomeric lignins were extracted with hydrophobic solvents like hexane and diethyl ether in low yield (Wang et al., 2010). Significant differences have been observed in the yield of low-molecular weight (LMW) fractions in solvents with lower hydrogen binding capacity. For example, the yield of LMW lignin fractions extracted from kraft softwood lignins with dichloromethane was lower about 2.5-3 times than *n*-propanol in the case of a grass lignin (Gosselink et al., 2010). These results indicated that the performance of the isolated fractions in different applications, like wood adhesives, was closely related to their molecular weight and molecular properties (Gosselink et al., 2010; Yoshida et al., 1990).

Membrane technology is another emerging technology that it allows to obtain lignin fractions with defined molecular weight distributions by free-reagent treatment. The effectiveness of the fractionating process using

membrane technology lies on the selection of the proper cut-off (Arkell et al., 2014). Numerous studies have been carried out to investigate the ability of membranes to recover lignin precipitates from black liquor generated by pulping processes. One of the studies described that ceramic membrane coated with ZrO_2 was used for the continuous separation of a well-defined low-molecular weight lignin from softwood and hardwood kraft black liquors on a pilot scale (Keyoumu et al., 2004). Another study reported that black liquor from paper mill Billerud Gruvön were used to filtrate using ceramic membranes made of TiO_2 and ZrO_2 demonstrating cut-off and materials used as membrane were key factors for efficient separation of desired lignin fractions (Sevastyanova et al., 2014).

3.5. Chemical modification

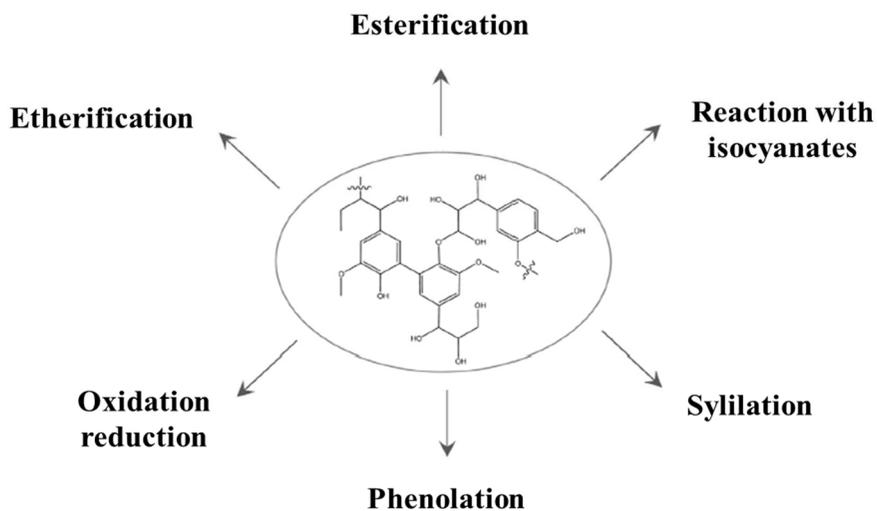
Lignin has various functional groups, which are comprised of hydroxyl, methoxyl, carbonyl, carboxylic structures. However, unmodified and macromolecule lignin is somehow limited in various applications because lignin has complex structure and different functional groups. For these reasons, several modification methods have been proposed to increase its chemical reactivity, improve the ease of processing the lignin, and also increase its solubility in organic solvents. Among the efforts, functionalization of hydroxyl groups has been extensively studied (Figure 1-8).

Etherification is the most interesting methods to substitute the functional group of lignin more reactively. Among the most well-known chemical modification attempted on lignin applications hydroxylation with propylene oxide (PO) is extensively studied (1984b; Wu and Glasser, 1984a). Oxypropylated lignins are generally prepared by dispersion of the solid lignin into neat propylene oxide in the presence of a base (usually KOH). Through an anionic ring-opening polymerization mechanism, the phenolic hydroxyl groups are extended with poly (propylene glycol) chains, possessing only one secondary hydroxyl group at each chain end (Hornof and Hombek, 1990). One of the studies reported that softwood and hard wood kraft lignin were oxypropylated with propylene oxide in aqueous NaOH. It described that the content of methoxyl groups affected on the formation of aliphatic hydroxyl group (Sadeghifar et al., 2012). Another research teams conducted production of polyols through the oxypropylation of auto-catalyzed ethanol-water lignin and wheat straw soda lignin. They concluded that the majority of phenolic hydroxyl groups are converted into aliphatic hydroxyl unit. These more reactive hydroxyl groups can be used in several polymerization reaction of lignin with bi-functional compounds (Ahvazi et al., 2011; Xue et al., 2013).

Among all of the reactions involving hydroxyl groups of lignin, esterification is probably the easiest way to carry out considering the reaction parameters and reactants used. Three reagents are commonly used: acidic compounds, acid anhydrides and chloride acids. The synthesis of lignin-based polyesters has been extensively studied by Gandini and coworkers (Gandini et al., 2002). Esterification has been performed on kraft lignin and organosolv lignin with either an aliphatic (sebacoyl chloride) or an aromatic (terephthaloyl chloride) acyl chloride. The reactivity of phenolic OH can be favored by the use of triethylamine as catalyst, whereas the use of pyridine promotes the reactivity of aliphatic OH groups (Guo et al., 1992). Because the lignin functionality is high, an insoluble network is formed in the presence of an excess of acyl chloride, with a maximum yield for COCl/OH ratios between 1.5 and 2 (Evtugin and Candini, 1996).

In presence of sufficient hydroxyl group, urethanization can be achieved by reaction with isocyanate groups. The polyurethane (PU) versatility offers the potential of preparing a wide range of products. Lignin-based PU is alternative products by increasing profitable value of lignin. Many approaches have been conducted to investigate performance evaluation of them. Sarkar and Huang synthesized a pre-polymer with hydroxyl terminated polybutadiene (HTPB) and 2,4 TDI with three molar ratios of NCO:OH (1.5, 2 and 2.5). Lignin was then reacted with the pre-polymer with a lignin content reaching 15 wt%. These experiments give flexible PUs with good mechanical properties (Huang and Zhang, 2002; Sarkar and Adhikari, 2007).

Functionalization of hydroxyl groups



Figures 1-8. Summary of the chemical reactions for the functionalization of lignin hydroxyl groups (Laurichesse and Avérous, 2014).

Chapter 2

Degradation characteristics of technical lignins
depending on peracetic acid concentration
at room temperature

1. Introduction

Lignin is a promising natural polymer with aromatic functionality, and thus it has been introduced as a key source for alternatives to petroleum-based materials and chemicals (Naseem et al., 2016; Stewart, 2008). However, the complexity and heterogeneity of lignin structures are considered big challenge to use (Beckham et al., 2016).

Lignin is a cross-linked phenolic polymer that acts as a structural material in vascular plants' support tissues. It is also covalently cross-linked to other main components such as polysaccharides, and therefore has a rigid structure (Boerjan et al., 2003; Zhong and Ye, 2015). In other words, lignin is a recalcitrant biopolymer, meaning that deconstructing lignin for various uses is difficult. Lignin's applicability can be improved, and various techniques for breaking the lignin structure, have been studied; examples include fast pyrolysis, and supercritical depolymerization (Bies et al., 2010; Erdocia et al., 2015; Gosselink et al., 2012; Nowakowski et al., 2010; Park et al., 2016). However, lignin degradation, needs a high temperature, which may be associated with reduced energy efficiency in terms of lignin bio-refinery.

Sometimes, lignin's hydrophobicity has to be considered an important aspect when dealing with the lignin polymer. Generally, organic solvents such as alcohols and dioxane are used to dissolve lignin (Schuerrch, 1952; Wang et al., 2010). Dissolution is a key step, which involves in reaction with other polymers. Depending on the lignin's characteristics, the solubility of the lignin may differ even in the same solvent. Therefore, it is necessary to find an appropriate solvent for objective.

Oxidation using peracetic acid (PAA) has recently been introduced as a promising technique for lignin degradation, since the deconstruction of wood components can be accomplished at a low-reaction temperature (Lee et al.,

2017; Wi et al., 2015). In addition, lignin can be well dissolved in a PAA solution, which may have advantages in lignin applications. Previous studies have reported the effects of PAA in lignin depolymerization for producing both dicarboxylic acids and monomeric aromatic compounds that are derived from various lignin sources (Ma et al., 2016; Popova et al., 2014) However, commercial PAA used in previous studies were commonly provided by several manufacturers from various countries, they show different solution properties despite reportedly being the the same concentration of PAA. Generally, a PAA solvent is comprised of acetic acid (AA), hydrogen peroxide (HP), and water with different concentrations. Therefore, it is difficult to grasp which properties of PAA are advantages for lignin depolymerization.

In this chapter, lignin degradation using PAA was conducted at room temperature without heating, and PAA was prepared directly by mixing with AA and HP at different mix ratios. Two types of technical lignins were used in the experiments to compare how the lignin degradation was affected by their structural differences.

2. Materials and methods

2.1. Materials

The structural changes of macromolecular lignin were investigated with PAA by using technical lignins as starting materials. Among the various technical lignins, this study used alkali lignin (AL) and kraft lignin (KL). Commercial AL was purchased from Sigma Aldrich Co., and KL was provided by Moorim P&P Co. located in the Republic of Korea. The chemical composition and properties of the two lignins are described in Table 2-1.

Table 2-1. Chemical compositions of AL and KL

	AL	KL
Total lignin content (%)	95.1 (± 0.01)	94.1 (± 0.61)
Klason lignin (%)	91.6 (± 0.01)	87.4 (± 0.93)
Acid soluble lignin (%)	3.5 (± 0.02)	6.7 (± 0.32)
Ash (%)	3.1 (± 0.18)	2.9 (± 0.13)
Carbohydrate (%)	1.5 (± 0.11)	1.9 (± 0.07)
M _w (Daltons)	3,391	2,813
C (wt%)	62.2	59.7
H (wt%)	5.8	5.3
O ¹ (wt%)	29.8	31.2
N (wt%)	0.7	0.4
S (wt%)	1.5	3.3

¹ Calculated by difference

2.2. Peracetic acid treatment

2.2.1. Peracetic acid preparation

Before the PAA treatments, AA and HP were used to create a PAA solution to examine the effects of PAA concentration on AL and KL lignin degradation. AA (98%) and HP (30%) were mixed at different ratios of 4:1, 1.5:1, 1:1, 1:1.5, and 1:4 (v/v), and the mixed solvent was stabilized for 1 h. AA and HP (separately) without mixing were used for controls. All chemical reagents used for the preparation of PAA were purchased from Sigma Aldrich.

2.2.2. Peracetic acid solution titration

PAA and HP concentrations in mixed solutions were measured by titration with modification (Hach, 2014; Solvay Chemicals, 2014; Tavast et al., 2011). First, 0.5 g of mixed solution with AA and HP was weighed in a 250 mL Erlenmeyer flask, and 50 mL of 1 N ice-cold sulfuric acid was added to the flask with stirring. And, two drops of ferroin indicator were added to the solution. The solution was titrated with 0.1 N ceric sulfate ($\text{Ce}(\text{SO}_4)_2$) until the salmon color disappeared and a light blue color appeared. Ten milliliters of 2.5 N potassium iodide (KI) was put into the solution and this was titrated with 0.1 N sodium thiosulfate ($\text{Na}_2(\text{SO}_2)\text{O}_3$) until light brown-yellow color appeared.

The concentration of PAA and HP in the mixed solvent was calculated with the following equation.

$$\% \text{H}_2\text{O}_2 = \frac{V_1 \times N_1 \times \text{meqH}_2\text{O}_2 \times F \times 100}{m}$$

V_1 = Volume of $Ce(SO_4)_2$ solution consumed for the H_2O_2 titration (mL)

N_1 = Normality of $Ce(SO_4)_2$

meq H_2O_2 = Milliequivalent of H_2O_2 divided by the number of electrons exchanged in the oxidation-reduction reaction, divided by 1,000.

$$\frac{34g/mol}{2e^-(1000)} = 0.017g H_2O_2 /milliequivalent$$

F = Dilution factor (1 for undiluted samples)

m = Sample amount (g)

If $N_1 = 0.1$ and $F = 1$,

$$\text{Then \% } H_2O_2 = \frac{0.17 \times V_1}{m}$$

$$\%PAA = \frac{V_2 \times N_2 \times meqPAA \times F \times 100}{m}$$

V_2 = volume of $Na_2(SO_2)O_3$ solution consumed for the PAA titration (mL)

N_2 = Normality of $Na_2(SO_2)O_3$

meq PAA = Milliequivalent of PAA divided by the number of electrons exchanged in the oxidation-reduction reaction, divided by 1000.

$$\frac{76g/mol}{2e^-(1000)} = 0.038g H_2O_2 /milliequivalent$$

F = Dilution factor (1 for undiluted samples)

m = Sample amount (g)

If $N_2 = 0.1$ and $F = 1$,

$$\text{Then \% PAA} = \frac{0.38 \times V_2}{m}$$

2.2.3. Peracetic acid treatment

Reaction with PAA was carried out to induce the structural changes in the lignins. 0.1 g of lignin was put into a glass bomb with 3 mL PAA solution (prepared in advance). The reaction was conducted in a dry block (MG-2200,

EYELA, Japan) at room temperature ($25\pm 1^\circ\text{C}$) for 1, 4, and 8 h. The solid samples were partially degraded after the reaction finished. The solid and liquid phases were separated by first diluting the PAA-treated samples with 10-fold distilled water to adjust the solution's pH for further experiments. Then, solid and liquid fractions were divided by centrifugation. Dried-solid samples were obtained by lyophilization and liquid samples were stored in vials and kept in a refrigerator.

2.3. Solid fraction analysis

2.3.1. Nitrobenzene oxidation (NBO)

NBO was performed to identify the oxidation products by cleavage of β -O-4 linkages in solid fractions according to the method described by Iiyama (Iiyama and Wallis, 1990). 40 mg of each solid sample was placed in a 10 mL glass bomb with 4 mL of 2 M aqueous sodium hydroxide and 250 μ L of nitrobenzene at 170°C for 2 h. After the reaction, 20 μ L of 3-ethoxy-4-hydroxybenzaldehyde (0.5 g dissolved in 10 mL of methanol) was added as an internal standard. The oxidized samples were extracted twice using 20 mL of dichloromethane (DCM), and 4 M HCl was added to the solution until the pH value fell below 2. The acidified sample was extracted twice again by DCM and then the filtrate was evaporated. For identifying the degradation products, extracted samples were silylated using 100 μ L pyridine and 100 μ L *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) at 105°C for 2 h. After that, the silylated sample was analyzed using a gas chromatography-mass spectrometry (GC/MS) instrument. Product separation was set at a split ratio of 1:20. The injector and detector temperature were set at 200°C and 250°C, respectively. The oven temperature program consisted of 120°C for 10 min, followed by heating at a rate of 10°C/min to 280°C, where the final temperature was maintained for 20 min.

2.3.2. Phosphorus-31 nuclear magnetic resonance (^{31}P -NMR)

The hydroxyl group contents in solid sample generated after PAA treatment was determined by conducting ^{31}P -NMR analysis in accordance

with established literature methods (Argyropoulos, 1994; Pu et al., 2011) . A solvent solution of 1.6:1 (v/v) of pyridine to chloroform-d₃ was prepared. The solvent solution (1 mL) was used to prepare a mixture solution containing 4 mg of cyclohexanol (internal standard) and 3.6 mg of chromium acetylacetonate (relaxation reagent). And then, an accurately known amount of 20~25 mg of a dried lignin sample was introduced into a 4 mL vial with a magnetic spin bar. And, the sample was dissolved in 400 μ L of solvent solution and 150 μ L of mixture solution and were mixed for a few minutes. Lastly, 70 μ L of phosphorylating reagent (2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane) was added and transferred into a 5 mm NMR tube for subsequent NMR acquisition. ¹³P-NMR spectra of the prepared samples was obtained using a NMR spectrometer (JEOL JNM-LA400 with LFG, JEOL, Japan).

2.3.3. Gel permeation chromatography (GPC)

GPC was performed to analyze the molecular weight (M_w) distribution and polydispersity index (PDI) of AL and KL-derived products in solid and liquid fractions using a 1260 Infinity II refracting index detector (Agilent Technologies, Santa Clara, CA, USA). Solid samples in the study were well dissolved in tetrahydrofuran (THF) as mobile phase. Therefore, 2-3 mg of solid samples were dissolved in THF (2 mL) and used for the analysis. The sample was introduced into GPC instrument equipped with a PLgel5 μ m MIXED-C column ($300 \times 7.5 \text{ mm}^2$, VARIAN). The rate of mobile phase (THF) flow was set at 1 mL/min. Several polystyrene standards were used to calibrate the molecular weight, and the range was from 266 to 60,000 Daltons. The injection volume was 20 μ L

2.4. Liquid fraction analysis

2.4.1. Solvent extraction

Solvent extraction was performed using ethyl acetate (EA) as an organic solvent. Each liquid fraction (30 mL) and 60 mL of the solvent were poured into a 250 mL funnel, the solution was mixed using a shaking extractor. The extraction step was repeated twice to sufficiently dissolve the lignin-derived compounds.

2.4.2. Chemical properties of lignin compounds

2.4.2.1. Gas chromatography-Mass spectrometry (GC/MS)

Monomeric degradation products generated by PAA treatment were identified using a gas chromatograph (Agilent 7890B, Agilent Technologies, CA, USA) and mass selective detector (Agilent 5975A, Agilent Technologies, CA, USA) For analysis of the degradation products, the solutes dissolved in ethyl acetate were silylated using 100 μ L pyridine and 100 μ L *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA) at 105°C for 2 h. The product separation for degradation product analysis was at a split ratio of 1:15 using a DP-5 capillary column (60 m \times 0.25 mm \times 0.25 μ m film thickness). Helium (99.9% purity) was used as a carrier gas. Inlet injector and flame ionization detector (FID) temperatures were set at 220°C and 300°C, respectively. The oven temperature program was consisted of 50°C for 5 min, followed by

heating at a rate of 3°C/min to 300°C, and the final temperature was maintained for 10 min. Mass data of degradation products were identified by NIST MS Search software (NIST/EPA/NIH Mass Spectral Database (NIST11)).

2.4.2.2. Ultraviolet (UV)-visible spectroscopy

Relative phenolic hydroxyl group contents of EA-soluble products was determined by UV-visible spectrophotometer (UV-1601PC, Shimadzu, Japan). This method is based on the difference in absorption at 200-300 nm between phenolic units of the solutes in EA. 100 µL of aliquot (dissolved in 10 mL of EA) of each sample were added to 2900 µL of EA in a quartz cell. Detection wavelength ranges were set from 200 to 500 nm. EA was used as reference in order to obtain the baseline.

2.4.2.3. Gel permeation chromatography (GPC)

The molecular weights and polydispersity index (PDI) of lignin-derived compounds extracted by EA was determined by GPC. The solutes (about 2-3 mg) were purged by nitrogen gas to remove the solvent and were dissolved in tetrahydrofuran. The dissolved samples were analyzed by the same instrument according to the methods described in Section 2.3.3.

3. Results and discussion

3.1. Concentration of PAA solution depending on the mix ratio

PAA can be formed by mixing AA and HP. Commercial PAA is generally comprised of AA, HP, PAA, and water. In addition, catalysts such as sulfuric acid are commonly added to the solution to facilitate its reaction equilibrium (Zhao et. al., 2008). In this chapter, AA and HP were separately used to investigate how the degradation behavior of lignins depended on the concentration of PAA solutions without a catalyst.

AA and HP were mixed according to various solvent mix ratios (4:1, 1.5:1, 1:1, 1:1.5, 1:4, v/v), which were used to degrade solid lignin at room temperature. The concentration of PAA solutions was determined by titration methods, and Table 2-2 shows how the concentration depended on the mix ratio. The concentration of HP in the solution increased in proportion to the HP input amount; however, the PAA concentration did not show a proportional increase. The highest concentration of PAA was 3.0% at 1:4 (v/v), while that of the HP was 20.1%.

Generally, the concentrations of commercial PAA provided by global corporations are 20-40%. Added mineral acids such as sulfuric and formic acid can provide a higher concentration of PAA can be obtained. However, the PAA solutions used in this study were prepared without any catalyst, and so

the concentration of PAA was lower than those of PAA solutions described in other studies (Barros et al., 2010; Ma et al., 2016; Sun et al., 2000).

Table 2-2. PAA and HP concentrations depending on the solvent mix ratios

Mix ratio (v/v)	Concentration (%)	
	PAA	HP
AA:HP ¹		
AA	-	-
4:1	1.5	5.8
1.5:1	1.5	12.6
1:1	1.5	15.0
1:1.5	2.3	15.6
1:4	3.0	20.1
HP	-	30

¹ Mix ratio (v/v) of acetic acid (AA) and hydrogen peroxide (HP)

3.2. Degradation rate during peracetic treatment

PAA is known as a powerful oxidizing agent, and it has been applied to depolymerize lignin macromolecules under various treatment conditions. PAA can depolymerize lignin at relatively lower temperature compared to most thermal oxidation techniques. Although the reaction temperature is an important factor in lignin degradation, the concentrations of PAA and HP are been considered key factors during PAA treatment.

This study used two types of technical lignins (AL and KL) to investigate the conversion characteristics by PAA, depending on the concentration at room temperature. At a low-reaction temperature (25°C), lignins were partially degraded. However, the degradation rates (% g/g of initial lignin) of the two types of lignin were significantly different.

Figure 2-1 shows the degradation rates of AL by PAA increased as the reaction time increased except at the 1:4 (v/v). Among solvent mix ratios, the 1:1.5 (v/v) showed the highest degradation rate of 29.0%. In the case of KL (Figure 2-2), the highest degradation rate (65.3%) was also observed at 1:1.5 (v/v). Except for 1:4 (v/v), more than half of the initial lignin content was reduced in the 8 h reaction using PAA.

Interestingly, the degradation rates of the two types of lignins were significantly different. These results can be explained by lignin structures depending on species. AL was produced from softwood, and KL was produced from hardwood. Softwood and hardwood have different structural characteristics; generally, softwood is mainly comprised of guaiacyl (G) units,

while hardwood consists of guaiacyl (G) and syringyl (S) units in various proportions depending on the wood species. These structural characteristics have different chemical reactivities during oxidation. An acidic condition can result in the dominant cleavage of the β -O-4 bonds. In addition, it is well-known that hardwoods that contain syringyl units can be cleaved more readily than softwoods due to their different reactivity (Santos et al., 2013; Shimizu et al., 2012). Therefore, it was concluded that the degradation of KL during PAA treatment at room temperature occurs more rapidly than that of AL.

Additional experiments were conducted to compare the degradation behavior when using AA or HP. AA and HP also affected lignin degradation at room temperature. However, the degradation rate did not show a big difference as the reaction time increased, which means that the formation of PAA is a crucial factor in lignin degradation. Based on the result of the PAA solutions in Section 3.1, all solutions of AA and HP formed PAA, and its concentration was the highest at 1:4 (v/v); however, the highest lignin degradation rate was observed at 1:1.5 (v/v). These results demonstrated that the HP concentration is another key factor in lignin degradation at room temperature; in other words, using too much HP when making a PAA solution negatively affects lignin degradation.

AL

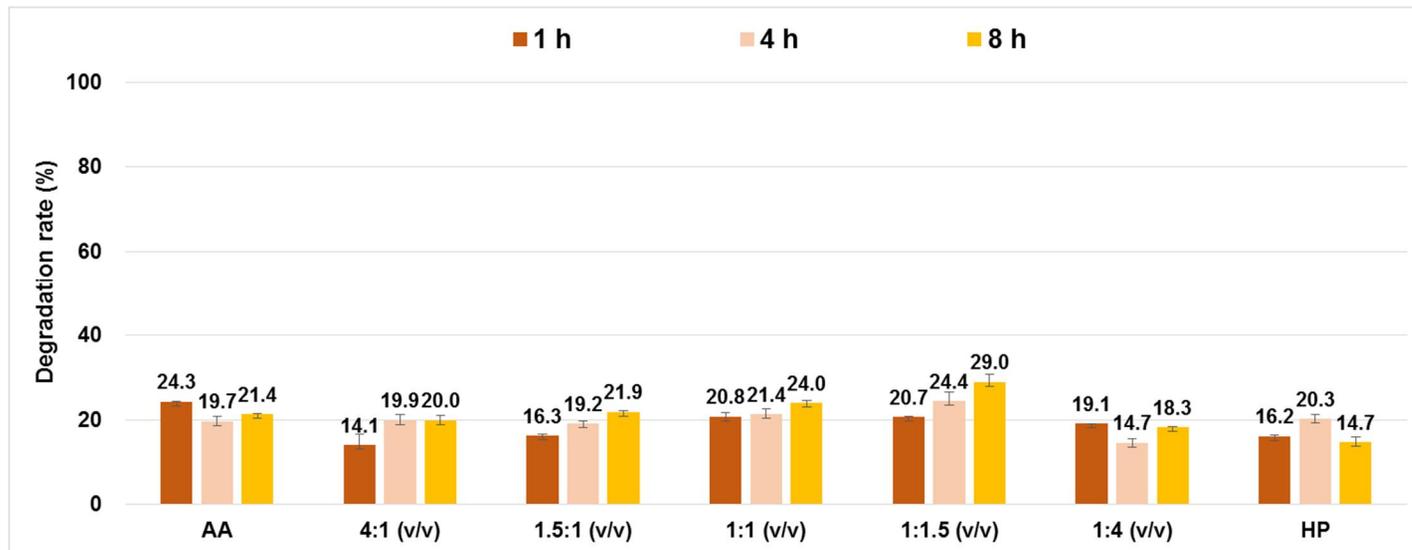


Figure 2-1. Degradation rates (%) after the PAA treatment of AL.

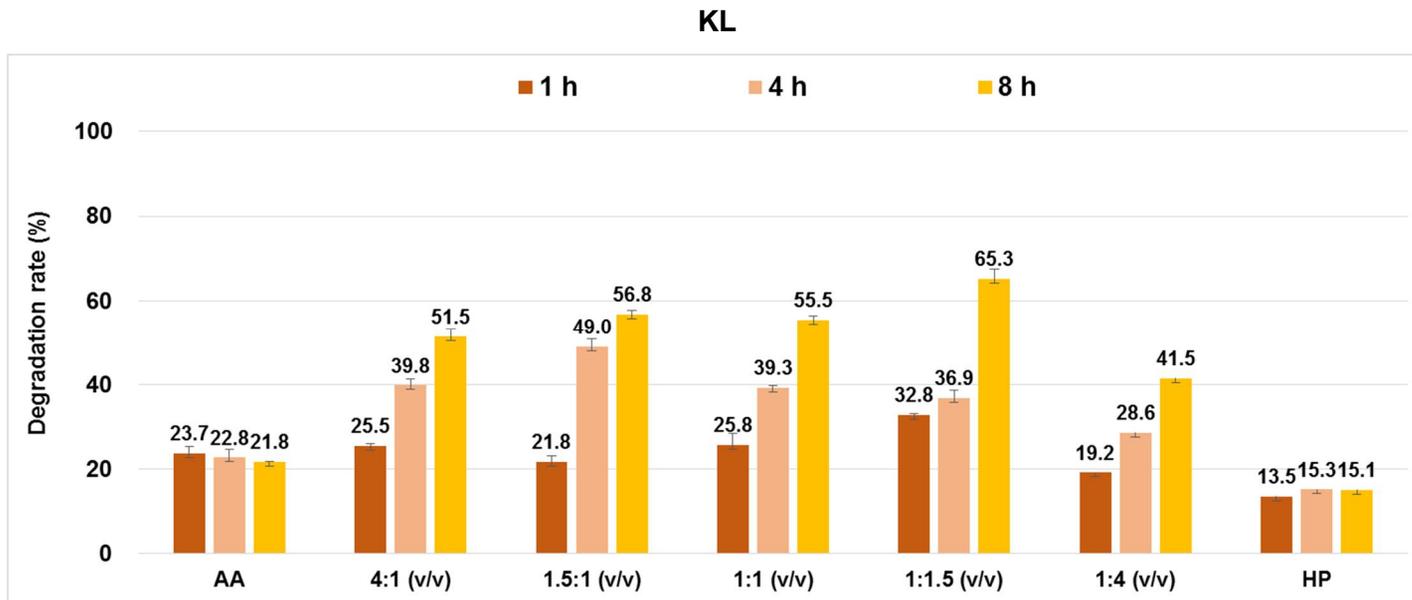


Figure 2-2. Degradation rates (%) after the PAA treatment of KL.

3.3. Solid fraction analysis

3.3.1. Nitrobenzene oxidation products

A PAA solution with AA and HP degraded AL and KL to low-molecular weight lignin dissolved in liquid fractions. However, a large amount of solids remained until after 8 h of reaction. Therefore, nitrobenzene oxidation (NBO) products were analyzed to investigate the changed structures of solid samples after PAA treatment.

The total amounts of oxidized compounds from AL and KL were 802.1 and 572.0 $\mu\text{mol/g}$, respectively, as shown in Tables 2-3 and 2-4. AL used in this study was mostly comprised of G units. The total amounts of S and G units were 7.6 and 779.2 $\mu\text{mol/g}$, respectively, confirming that softwood species produced AL. Meanwhile, the total amounts of S and G units from KL were 336.5 and 235.5 $\mu\text{mol/g}$ respectively, and the S/G ratio was 1.43; these results demonstrated that KL was produced from hardwood. Small quantities of H units were also present in the total amounts of NBO products. The NBO product yields depended on both the conditions of the isolation processing and the lignin source. KL is produced by the kraft cooking process, which needs a higher temperature compared to alkali pulping, with the result that the process may increase the condensation of lignin (Laurichesse and Avérous, 2014; Tarabanko and Tarabanko, 2017). Therefore, total content of NBO products for AL and KL was significantly different.

AA and HP solutions were used to demonstrate the effect of PAA for lignin degradation. However, the degradation rates of AL treated by AA and HP (as described in Section 3.2) were lower than those of other mixed solutions even though the reaction time was longer. This meant that the total

amounts of NBO products in the solid samples treated with AA and HP slightly decreased, compared to those of NBO products in the untreated AL.

The total contents of NBO products in KL solid fractions after PAA treatment decreased as the reaction time increased in all solutions with AA and HP similar to the results of AL. Interestingly, the amounts of S units in PAA-treated AL drastically decreased as the reaction time increased. The G units were similar regardless of the reaction severity, meaning that the S/G ratio was significantly decreased to <1.0 . These resulted from the different reactivities of S and G units during degradation. As mentioned in Section 3.2, S units were more rapidly cleaved than G units, and significant decrease in the total amount of NBO products was therefore found in KL.

In the case of AA and HP without mixing, their total content did not show the any large differences as the reaction time increased. In particular, HP without mixing showed the highest content of S units among all conditions. It was found that HP did not degrade lignin at room temperature even when the reaction continued for 8 h. Therefore, the total amount of oxidized products was similar to the untreated KL.

Table 2-3. Nitrobenzene oxidation products ($\mu\text{mol/g}$) in the solid fractions from AL with PAA treatment

	AA				HP							
Time (h)	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio				
1	5.0 (± 0.1)	768.1 (± 10.4)	788.6 (± 10.6)	0.01	5.2 (± 0.5)	678.1 (± 37.2)	695.4 (± 38.8)	0.01				
4	4.5 (± 0.0)	579.9 (± 0.7)	595.2 (± 0.6)	0.01	5.8 (± 0.0)	724.5 (± 7.7)	745.0 (± 5.9)	0.01				
8	4.8 (± 0.2)	665.3 (± 6.2)	683.2 (± 7.6)	0.01	5.4 (± 0.4)	694.2 (± 30.4)	713.8 (± 31.6)	0.01				
	4:1 (v/v)				1.5:1 (v/v)				1:1 (v/v)			
Time (h)	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio
1	3.9 (± 0.0)	597.6 (± 15.4)	611.7 (± 16.0)	0.01	4.1 (± 0.1)	571.9 (± 14.1)	587.8 (± 14.6)	0.01	3.9 (± 0.2)	583.9 (± 26.8)	599.4 (± 28.1)	0.01
4	3.2 (± 0.2)	569.5 (± 40.7)	580.4 (± 41.0)	0.01	3.1 (± 0.1)	567.2 (± 12.3)	580.0 (± 12.1)	0.01	3.3 (± 0.1)	552.9 (± 0.6)	565.3 (± 1.1)	0.01
8	2.6 (± 0.2)	560.2 (± 1.4)	570.1 (± 1.5)	0.01	2.1 (± 0.2)	512.3 (± 24.7)	523.0 (± 26.2)	0.01	2.3 (± 0.2)	541.8 (± 16.6)	552.7 (± 16.8)	0.01
	1:1.5 (v/v)				1:4 (v/v)				Control (AL)			
Time (h)	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio
1	3.7 (± 0.1)	603.3 (± 10.3)	619.1 (± 11.4)	0.01	4.4 (± 0.2)	614.8 (± 20.5)	630.2 (± 20.8)	0.01	7.6 (± 0.2)	779.2 (± 1.4)	802.1 (± 0.7)	0.01
4	3.7 (± 0.0)	558.8 (± 2.5)	571.7 (± 2.9)	0.01	4.3 (± 0.2)	608.9 (± 13.7)	624.8 (± 13.3)	0.01				
8	3.5 (± 0.1)	522.0 (± 14.8)	532.1 (± 14.9)	0.01	4.1 (± 0.2)	593.5 (± 15.7)	607.5 (± 16.2)	0.01				

Table 2-4. Nitrobenzene oxidation products ($\mu\text{mol/g}$) in the solid fractions from KL with PAA treatment

	AA				HP							
Time (h)	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio				
1	259.2 (±1.2)	200.1 (±2.8)	459.3 (±4.0)	1.30	318.6 (±13.6)	224.5 (±7.6)	543.1 (±21.2)	1.42				
4	255.9 (±0.1)	198.3 (±1.9)	454.2 (±1.9)	1.29	283.8 (±6.2)	204.4 (±7.2)	488.2 (±13.4)	1.39				
8	250.4 (±7.4)	196.7 (±8.3)	447.1 (±15.7)	1.27	296.3 (±0.4)	215.6 (±1.1)	511.9 (±1.4)	1.37				
	4:1 (v/v)				1.5:1 (v/v)				1:1 (v/v)			
Time (h)	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio
1	271.5 (±2.1)	227.3 (±0.4)	498.9 (±2.5)	1.19	270.0 (±3.4)	233.1 (±2.3)	503.1 (±5.7)	1.16	278.2 (±9.5)	225.4 (±2.5)	503.6 (±12.0)	1.23
4	207.4 (±8.1)	239.5 (±8.2)	447.0 (±16.3)	0.87	192.0 (±5.5)	238.0 (±8.1)	430.1 (±13.6)	0.81	227.3 (±3.2)	238.8 (±2.8)	466.1 (±6.0)	0.95
8	167.3 (±4.1)	230.8 (±2.6)	398.1 (±6.7)	0.73	154.5 (±2.1)	225.2 (±0.4)	379.7 (±2.5)	0.69	164.5 (±7.1)	227.4 (±5.4)	391.9 (±12.6)	0.72
	1:1.5 (v/v)				1:4 (v/v)				Control (KL)			
Time (h)	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio	Sum S unit	Sum G unit	Total (S, G, H)	S/G ratio
1	295.3 (±5.9)	231.6 (±3.9)	526.9 (±9.8)	1.28	315.3 (±1.1)	237.7 (±3.4)	553.0 (±4.5)	1.33	336.5 (±4.9)	235.5 (±6.4)	572.0 (±11.3)	1.43
4	222.4 (±1.0)	228.8 (±5.8)	451.3 (±6.8)	0.97	252.6 (±0.8)	210.9 (±1.4)	463.5 (±2.2)	1.20				
8	117.2 (±0.5)	206.3 (±0.6)	323.5 (±1.1)	0.57	225.1 (±9.3)	226.5 (±7.3)	451.6 (±16.6)	0.99				

3.3.2. Hydroxyl group contents

The concentrations of different hydroxyl groups were calculated in relation to the peak area of the derivative hydroxyl groups of the internal standard. The results of the hydroxyl group contents of each solid fraction after PAA treatment are shown in Tables 2-5 and 2-6. PAA oxidized the lignin and induced-degradation, which was attributed to changes in functional groups.

The contents of the hydroxyl groups in the untreated AL and KL showed different values; this can be due to the raw material, and different pulping processes. Previous research reported structural differences between kraft lignin and soda lignin and mentioned that kraft lignin has a lower number of phenolic and aliphatic groups. This is because kraft pulping can be attribute to the process of repolymerizing lignin fragments (Mansouri et al., 2011). However, the KL used in our study showed relatively higher contents of phenolic groups, which may be due to raw materials and other isolation processes.

The contents of aliphatic, phenolic and carboxylic groups changed during PAA treatment. In both AL and KL, phenolic-OH group proportionally decreased as the reaction time increased. This was because side chain oxidation accelerated due to β -O-4 cleavage by PAA oxidation as the reaction time increased. Cleaved compounds became dissolved in the liquid fraction, and the more condensed and polymerized lignin remained in solid form after PAA treatment.

Only a minor amount of the carboxylic-OH groups was found in the AL samples. Meanwhile, KL showed a proportional increase of carboxylic-OH groups. These results demonstrated that KL was more degraded and oxidized during PAA treatment. Therefore, the carboxylic-OH contents of KL solid samples increased more than those of the untreated AL solid samples as the

reaction time increased.

Table 2-5. Hydroxyl group contents ($\mu\text{mol/g}$) in the solid fractions of PAA-treated AL

	AA			HP					
Time (h)	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic			
1	1.64	2.99	0.03	1.75	3.14	0.05			
4	1.70	2.98	0.03	1.90	3.17	0.08			
8	1.70	2.72	0.10	1.59	3.10	0.03			
	4:1 (v/v)			1.5:1 (v/v)			1:1 (v/v)		
Time (h)	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic
1	1.61	2.80	0.02	1.06	1.86	0.05	1.07	2.05	0.05
4	1.57	2.45	0.06	0.91	1.72	0.19	1.10	1.77	0.03
8	1.35	2.09	0.15	0.89	1.40	0.13	1.04	1.70	0.07
	1:1.5 (v/v)			1:4 (v/v)			Control (AL)		
Time (h)	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic
1	0.60	1.06	0.03	1.18	2.17	0.13	2.05	3.30	0.12
4	1.10	1.88	0.04	1.17	2.13	0.08			
8	1.18	1.80	0.04	1.14	2.10	0.02			

Table 2-6. Hydroxyl group contents ($\mu\text{mol/g}$) in the solid fractions of PAA-treated KL

	AA			HP					
Time (h)	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic			
1	0.83	3.69	0.56	0.74	4.33	0.34			
4	0.75	3.07	0.48	0.78	3.93	0.42			
8	0.63	3.78	0.46	0.75	3.42	0.35			
	4:1 (v/v)			1.5:1 (v/v)			1:1 (v/v)		
Time (h)	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic
1	0.77	2.98	0.35	0.82	3.02	0.35	0.84	3.30	0.38
4	0.97	2.68	0.40	1.08	2.92	0.48	1.01	2.98	0.40
8	0.97	2.52	0.46	1.07	2.62	0.51	1.52	2.77	0.67
	1:1.5 (v/v)			1:4 (v/v)			Control (KL)		
Time (h)	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic	Aliphatic	Phenolic	Carboxylic
1	0.86	4.07	0.22	0.66	3.21	0.12	0.60	4.64	0.27
4	0.70	3.02	0.28	0.78	3.41	0.23			
8	0.72	2.03	0.48	0.76	3.07	0.28			

3.3.3. Molecular weight distribution

The molecular weight distributions of solid fractions after PAA treatment were observed to vary according to the experimental conditions. The results are given in Table 2-7 and 2-8. The untreated AL had an M_w of 3,391 Da and M_n of 1806 Da, while KL showed an M_w of 2,813 Da and M_n of 970 Da. The polydispersity indexes (PDI) were 1.88 and 2.90 for AL and KL, respectively.

As listed in Table 2-7, the molecular weights of solid samples after PAA treatment as the reaction time increased; the PDI of most samples also increased. Except for HP, M_w values increased compared to the untreated AL, and the values were 2,397-4,286 Da. The high molecular lignin remained in a solid state, while low-molecular lignin fragments were generated and dissolved in the liquid fraction. Based on previous results, lignin degradation was observed and low-molecular fragments became dissolved in liquid fractions. Therefore, the M_w of KL solid fractions was clearly increased to a higher value than that of the untreated KL.

Meanwhile, the molecular weight distributions of the remaining solid fractions of KL showed similar tendencies to those of AL. As shown in Table 2-8, the M_w of all solid samples was increased compared to that of the untreated KL. As the reaction time increased, the values were 2,654-3,612 Da. Similar to the result of AL, the PDI of KL solid samples increased as the reaction time increased, indicating that the remaining solid samples had more condensed structures.

Table 2-7. Molecular weight (Dalton) of lignin products in the solid fractions from PAA treated-AL

AA:HP ratio (v/v) ¹	Reaction time (h)								
	1 h			4 h			8 h		
	M _w	M _n	PDI	M _w	M _n	PDI	M _w	M _n	PDI
AA	3406	1232	2.76	3623	1226	2.96	3555	1240	2.87
4:1	3648	1340	2.72	3860	1298	2.97	4286	1326	3.23
1.5:1	3907	1405	2.78	3860	1256	3.07	4154	1362	3.05
1:1	3500	1317	2.66	3473	1265	2.75	3544	1264	2.87
1:1.5	3840	1383	2.78	4001	1393	2.87	4095	1322	3.10
1:4	3450	1268	2.72	3557	1341	2.65	3743	1405	2.66
HP	2397	1040	2.30	2570	1054	2.44	3669	1374	2.67
Control ²	3391	1806	1.88						

¹ Mix ratio of acetic acid and hydrogen peroxide

² Untreated alkali lignin

Table 2-8. Molecular weight (Dalton) of lignin products in the solid fractions from PAA treated-KL

AA:HP ratio (v/v) ¹	Reaction time (h)								
	1 h			4 h			8 h		
	M _w	M _n	PDI	M _w	M _n	PDI	M _w	M _n	PDI
AA	3397	1243	2.73	3612	1273	2.84	3294	1217	2.71
4:1	2803	1148	2.44	3048	1167	2.61	3186	1200	2.66
1.5:1	2783	1106	2.52	2998	1103	2.72	3120	1147	2.72
1:1	2654	1128	2.35	2958	1121	2.64	3093	1112	2.78
1:1.5	2736	1092	2.51	2878	1022	2.82	3053	1055	2.89
1:4	2659	1063	2.50	2851	1123	2.54	3147	1214	2.59
HP	2695	1042	2.59	2751	1125	2.45	2721	1146	2.37
Control ²	2813	970	2.90						

¹ Mix ratio of acetic acid and hydrogen peroxide

² Untreated kraft lignin

3.4. Liquid fraction analysis

3.4.1. Lignin-derived compound distribution

PAA converted lignin into small fragments, which were dissolved in the liquid fraction. Generally, low-molecular compounds are worthwhile value-added chemicals (such as vanillin, and phenol derivatives) for further applications. Therefore, GC/MS was conducted to investigate the possibility of producing degraded compounds and comparing the compound distribution depending on mix ratio.

Table 2-9 shows that 17 monomeric compound species were produced from PAA-treated AL. Acetic acid was also detected a degradation product. Acetic acid is a component of the PAA solution. Thus, acetic acid was omitted from the table list. The lignin-derived compounds were divided into two groups: dicarboxylic acids (DCA) and aromatics. The aromatic compounds were mainly comprised of vanillic acid, protocatechuic acid and 1,4-dihydroxy-2,6-dimethoxybenzene. Meanwhile, various types of DCAs were produced by the ring-opening of aromatic structures in lignin. Glycolic, oxalic, maleic, and succinic acid were mainly generated as DCAs (Farrand and Johnson, 1971; Linger et al., 2014; Ma et al., 2014). Interestingly, these DCAs were only produced at 1:1.5 and 1:4 (v/v). These results clearly indicate that increasing the HP concentration when PAA solutions induces the accelerated production of DCA compounds.

The total contents of the various lignin-derived compounds were calculated based on the contents of the internal standard. However, the highest contents of lignin-derived compounds were below 0.1% of the initial AL. These results indicated that the degradation compounds from AL were mainly

present as larger molecules rather than monomers.

Compared to AL, there were 9 monomeric compound species from KL as listed in Table 2-10. There were also also some dicarboxylic acids and aromatic compounds. Aromatics were mainly comprised of vanillic acid, syringic acid, and 1,4-dihydroxy-2,6-dimethoxybenzene. In the case of the KL, dicarboxylic acids were only detected at 1:1.5 (v/v). This meant that the ring-opening reaction started dominantly at 1:1.5 (v/v).

Meanwhile, the total contents of KL-derived compounds were small quantities, the same as AL. The highest contents were detected as 0.08% of the initial KL. Similar tendencies were observed for AL and KL, which demonstrated that PAA oxidation had less of an effect on the production of monomeric compounds at room temperature (although lignin degradation occurred). Previous studies reported that PAA can produce a much larger amount of monomeric lignin compounds from corn-stover lignin treated with diluted acid. The highest obtained yield of monomeric phenols from lignin was 22% after 60 min of PAA treatment at 60°C. However, when the reaction was conducted at 30°C, the monomeric phenol yield decreased to about 5% (Ma et al., 2016). This meant that the reaction temperature during PAA treatment was an important factor when producing lignin-derived monomeric compounds.

Table 2-9. Monomeric compounds in the liquid fractions of PAA treated-AL detected by GC/MS

No.	RT ¹	Product	Group	Mix ratio (v/v)						
				AA	4:1	1.5:1	1:1	1:1.5	1:4	HP
1	16.082	Glycolic acid	DCA ²					v	v	
2	17.702	2-Hydroxybutyric acid	DCA					v	v	
3	17.937	Oxalic acid	DCA					v	v	
4	18.165	Hydracrylic acid	DCA					v	v	
5	19.098	2-Ketobutyric acid	DCA					v	v	
6	22.783	Maleic acid	DCA					v	v	
7	22.926	Succinic acid	DCA					v	v	v
8	23.287	Methylsuccinic acid	DCA					v	v	
9	23.504	Glyceric acid	DCA					v	v	
10	23.823	Fumaric acid	DCA					v	v	
11	25.318	Hydroquinone	Ar ³		v	v	v	v		
12	27.464	Malic acid	DCA					v	v	
13	28.614	4-Hydroxybenzoic acid	Ar	v ⁴	v	v	v	v		
14	30.491	1,4-Dihydroxy-2,6-dimethoxybenzene	Ar	v	v	v	v	v		
15	32.614	Catechol	Ar	v	v	v	v	v		
16	33.403	Vanillic acid	Ar	v	v	v	v	v		
17	34.502	Protocatechuic acid	Ar	v	v	v	v	v		

¹RT: Retention time

²DCA: Dicarboxylic acid

³Ar: Aromatics

⁴v: Detected in GC/MS

Table 2-10. Monomeric compounds in the liquid fractions of PAA treated-KL detected by GC/MS

No.	RT ¹	Product	Group	Mix ratio (v/v)						
				AA	4:1	1.5:1	1:1	1:1.5	1:4	HP
1	16.082	Glycolic acid	DCA ²		v	v	v	v	v	
2	22.783	Maleic acid	DCA					v		
3	22.926	Succinic acid	DCA					v		
4	23.287	Methylsuccinic acid	DCA					v		

5	23.504	Glyceric acid	DCA						v
6	23.823	Fumaric acid	DCA						v
7	30.491	1,4-Dihydroxy-2,6-dimethoxybenzene	Ar ³		v	v	v	v	v
8	33.403	Vanillic acid	Ar	v ⁴	v	v	v	v	v
9	38.035	Syringic acid	Ar	v	v	v	v	v	v

¹RT: Retention time

²DCA: Dicarboxylic acid

³Ar: Aromatics

⁴v: Detected in GC/MS

3.4.2. Phenolic hydroxyl group distribution

Lignin is complexly linked to the C-O, C-H, and O-H bonds of hydroxyl and methyl groups. In particular, the hydroxyl group is an important functional group involved in lignin functionality. Therefore, the UV absorbance difference was observed to compare the relative content of the hydroxyl group in EA- soluble products.

Distinct regions in the UV spectra can be divided into two parts. First, absorbance peaks were observed at 270-280 nm, which is assigned to $n \rightarrow \pi^*$ electronic transitions. The symbol “ n ” refers to non-bonding electrons, which correspond to the lone electron pairs of the ether (-O-) bond and the hydroxyl (-OH) bond. In addition, the strong peak at 270 nm is assigned to the $\pi \rightarrow \pi^*$ electronic transition of aromatics (Song et al., 2015; Yáñez-S et al., 2014). These absorbance peaks were attributed to degraded products from the initial solid AL and KL. Meanwhile, those below 200 nm were regarded as noise peaks because these are irrelevant peaks in lignin structures.

In the UV spectra of EA-soluble products generated after PAA treatment depending on the solvent mix ratio, the absorbance peaks at 270-280 nm increased as the reaction time. These tendencies were observed similarly at AL and KL as shown in Figure 2-3 and 2-4. The absorbance differences were attributed to relative content of the lignin-derived compounds dissolved in liquid fractions. Among solvent mixed conditions, the highest absorbance at 270-280 nm was observed at 1:1.5 (v/v) in the case of AL, while that was observed at 4:1 (v/v) in the case of KL. More aromatic compounds related to ether and hydroxyl bonds in lignin were found at 4:1 (v/v). As mentioned in the GC/MS results above, aromatic lignin compounds were mostly detected as lignin-derived compounds at 4:1 (v/v) with KL, while dicarboxylic acids were produced at high HP concentrations. Dicarboxylic acids can be generated by the ring-opening of lignin compounds, and thus the

absorbance peaks attributed to lignin structures were relatively decreased.

EA-soluble fractions from AL and KL showed the highest content of the phenolic hydroxyl groups at different conditions because they had different amounts of lignin-derived compounds. Based on solid degradation rates, the two types of lignins generated phenolic or dicarboxylic groups differently even though the same PAA was used.

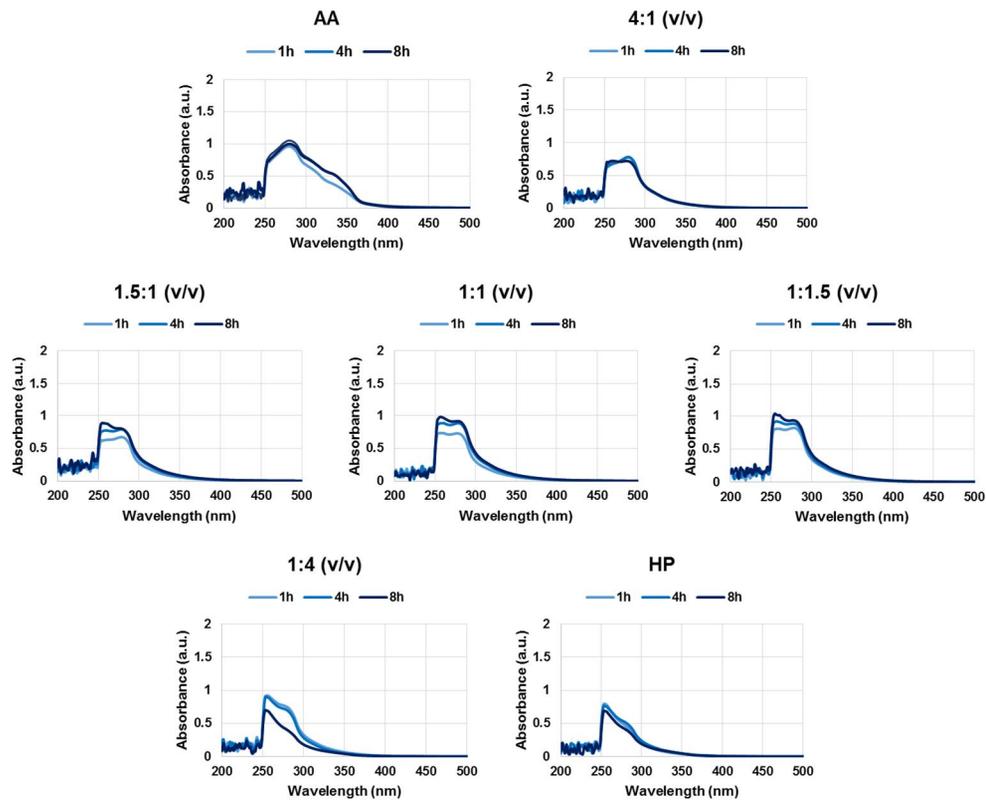


Figure 2-3. UV absorbance differences depending on the AA and HP (AL) mix ratios.

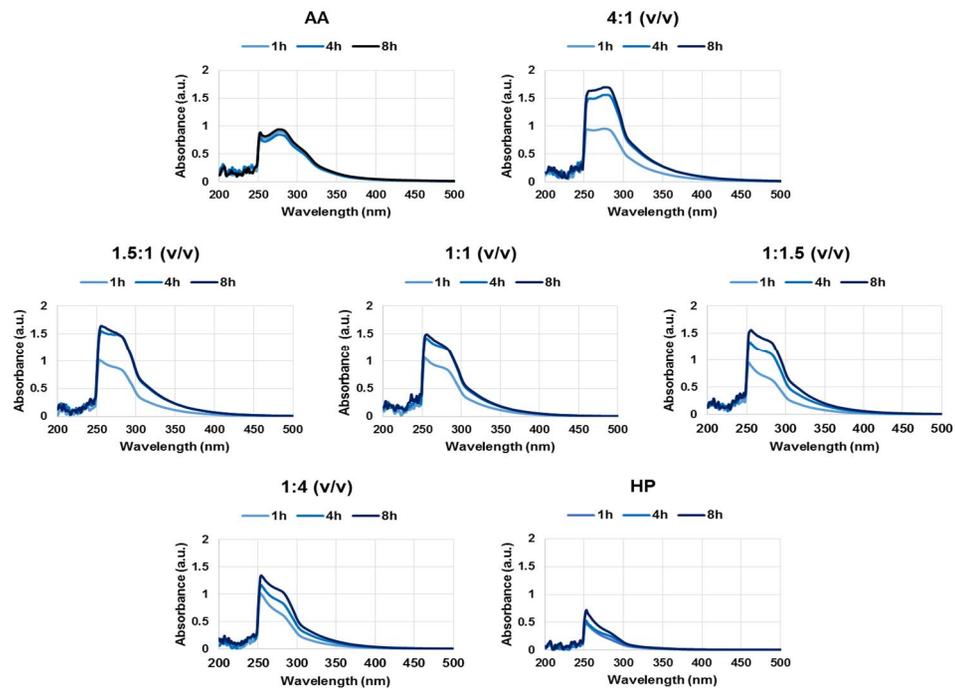


Figure 2-4. UV absorbance differences depending on the AA and HP (KL) mix ratios.

3.4.3. Molecular weight distribution

The molecular weight distributions of liquid fractions from AL and KL were observed after PAA treatment, and the results are described in Tables 2-11 and 2-12.

In all conditions, the molecular weights of AL and KL-derived compounds in liquid fractions showed lower values after PAA treatment. In addition, the PDI of most samples also decreased. The M_w values of AL-derived compounds in the liquid fractions were 625-1,048 Da, while those from KL were 829-1,204 Da. It was possible to generate lower molecular compounds than the initial lignin using PAA at room temperature. According to the results of GC/MS analysis, monomeric compounds were detected in very small amounts. However, low-molecular compounds in the liquid fractions showed narrower molecular distributions than the initial lignin.

Table 2-11. Molecular weight (Dalton) of AL-derived products in the liquid fractions

AA:HP ratio (v/v) ¹	Reaction time								
	1 h			4 h			8 h		
	M _w	M _n	PDI	M _w	M _n	PDI	M _w	M _n	PDI
AA	1101	656	1.68	1098	662	1.66	1048	649	1.61
4:1	995	554	1.80	1072	622	1.72	1056	605	1.75
1.5:1	988	575	1.72	1082	629	1.72	1069	616	1.74
1:1	836	525	1.59	980	600	1.63	1101	641	1.72
1:1.5	900	551	1.63	949	559	1.70	979	586	1.67
1:4	643	432	1.49	625	436	1.43	671	460	1.46
HP	646	435	1.49	850	537	1.58	718	468	1.53
Control ²	3391	1806	1.88						

¹ Mix ratio of acetic acid (AA) and hydrogen peroxide (HP)

² Untreated alkali lignin

Table 2-12. Molecular weight (Dalton) of KL-derived products in the liquid fractions

AA:HP ratio (v/v) ¹	Reaction time								
	1 h			4 h			8 h		
	M _w	M _n	PDI	M _w	M _n	PDI	M _w	M _n	PDI
AA	898	586	1.53	867	560	1.55	896	585	1.53
4:1	1067	641	1.66	1204	708	1.70	1090	638	1.71
1.5:1	901	558	1.61	1028	618	1.66	1013	615	1.65
1:1	924	580	1.59	894	538	1.66	949	582	1.63
1:1.5	829	531	1.56	907	560	1.62	969	595	1.63
1:4	950	597	1.59	981	606	1.62	1006	633	1.60
HP	- ³	-	-	-	-	-	-	-	-
Control ²	2813	970	2.90						

¹ Mix ratio of acetic acid (AA) and hydrogen peroxide (HP)

² Untreated kraft lignin

³ Not detected

4. Summary

This study carried out lignin oxidation at room temperature using PAA solutions with different mix ratios. Although the PAA concentration was lower than that of commercial PAA, lignin degradation was even possible at room temperature. AL and KL showed the highest respective degradation rates of 29.0% and 65.3% at 1:1.5 (v/v). The solid lignin degradation rate tended to increase as the HP concentration increased. However, at 1:4 (v/v), the degradation rate decreased even though PAA concentration was highest in that solution. Therefore, it was concluded that the concentrations of both PAA and HP (present in the PAA solutions) were important factors in lignin degradation.

The characteristics of the lignin compounds in the liquid fractions produced by PAA treatment were investigated. It was observed that the molecular weight and PDI showed lower values than the initial lignins, which meant that low-molecular weight compounds were produced from solid lignin, suggesting that it is possible to produce lignin that has a more uniform structure.

Chapter 3

Peracetic acid-induced depolymerization of
technical lignins and
its chemical properties

1. Introduction

Lignin is both a biodegradable polymer and an abundant natural material. It also includes a bundle of aromatic structures, which makes it considered promising source in bio-based industries (Hatakeyama and Hatakeyama, 2009; Lora and Glasser, 2002). Lignin is a common waste product in the paper industry and residue in bioethanol processes, and its production increasing rapidly. Although it is mostly used for power generation, significant efforts has concentrated on making better uses of lignin as a high value- chemical (Guo et al., 2013; Mansouri and Salvadó, 2006; Yang and Wyman, 2008).

Lignin-based biorefineries have focused on two main strategies for lignin valorization. 1) Poly-functional monomeric compound production by oxidative reactions that can be uses as feedstock for the polymer industry as an alternative to petroleum-based building blocks (Werhan et al., 2011; Lange et al., 2013) . Various technologies have been introduced for lignin depolymerization. But there are still difficulties generating monomeric compounds, completely. Carbon-carbon linkages in lignin structures are difficult to cleave, which is challenging for lignin uses (Shuai and Saha, 2017). Most high molecular lignin polymers, such as oligomers, remain in depolymerized products, although the molecular weight was decreased. Thus, direct use of the lignin polymer is required.

2) Previous studies proposed lignin polymer selective functionalization to improve compatibility and performance in composite and copolymer materials (Crestini et al., 2010; Laurichesse and Avérous, 2014; Ludmila et al., 2015). Since lignin has a large number of functional groups, direct use of the lignin polymer has also been. However, high-end lignin uses have not been

achieved due to its structural complexity and heterogeneity. In particular, technical lignins show high structural diversity and broad molecular distribution that makes its commercial use difficult.

Oxidation technology using peracetic acid (PAA) to produce more homogeneous products from lignin has been recently employed in lignin fragmentation studies. PAA contains a perhydroxyl group (-OOH) in the place of the hydroxyl group of its parent acid. The hydroxonium ion (HO⁺) produced from the heterolytic cleavage of the peroxy bond is a strong electrophilic species that can readily attack lignin, which has several electron-rich sites. Therefore, peroxy acids are available for deconstruction of the macromolecule lignin under low-temperature conditions (Barros et al., 2010; Ma et al., 2016; Ma et al., 2014; Sun et al., 2000). However, there has been little effort toward applying PAA chemistry to convert lignin into valued products thus far.

This chapter conducted PAA treatment with heating to depolymerize lignin. Two technical lignins were used as starting materials depending on the species. And, PAA solutions were also prepared at different concentrations to compare their effects on lignin degradation. Chemical properties of the degradation compounds from initial lignin were analyzed.

2. Materials and methods

2.1. Materials

This study used two types of technical lignins as starting materials. Alkali lignin (softwood) was purchased from Sigma Aldrich, while kraft lignin (hardwood) was obtained from Moorim P&P Co. in Republic of Korea. Chemical compositions of the materials are described in Section 2.1 in Chapter 2.

2.2. Peracetic acid liquefaction

2.2.1. Peracetic acid preparation

PAA was used for lignin depolymerization. PAA solutions with mixing ratios 4:1, 1:1, and 1:4 (v/v) were prepared according to the method described in Section 2.2.1 in Chapter 2.

2.2.2. Peracetic acid solution titration

Prepared PAA solution concentration was determined by titration according to the procedure described in Section 2.2.2 in Chapter 2.

2.2.3. Peracetic acid liquefaction

PAA treatment was performed to induce lignin structural changes. Lignin (0.1 g) was placed into a glass bomb with prepared PAA solution (3 mL) and the reaction was conducted in a dry block at 80°C for 1, 4, and 8 h. When the reaction had finished, the liquefied samples were cooled and diluted with 10-fold with distilled water to increase pH for further experiments. Structural changes of lignin-derived compounds after PAA treatment were identified by solvent extraction following the procedure in Section 2.4.1 in Chapter 2. Samples were centrifuged to separate liquid and solid fractions, respectively. Solid samples were obtained by lyophilization and stored in

glass vials.

2.3. Liquefied lignin analysis

2.3.1. Solvent extraction

Solvent extraction was performed using ethyl acetate (EA) to investigate chemical properties of lignin-derived compounds after PAA liquefaction following the methods described in Section 2.4.1 in Chapter 2.

2.3.2. Chemical properties of lignin compounds in ethyl acetate fractions

Chemical properties of AL and KL-derived compounds in EA fractions were identified by GC/MS, UV-vis spectroscopy, and GPC following the methods in Section 2.4.2 in Chapter 2.

2.3.3. Residual fractions

2.3.3.1. Fourier-transform infrared spectroscopy (FT-IR)

After PAA liquefaction, only a fraction was extracted by EA, and the remaining products retained in aqueous phase. FT-IR spectra for lignin-derived were obtained from the residual fraction by freeze-drying. 32 scans were recorded over 4,000-650 cm^{-1} at a spectral resolution of 4 cm^{-1} using a FT-IR spectrometer (Nicolet 6700, Thermo Scientific, USA). As a reference,

the background spectrum of air was collected, and the lignin samples were measured directly on the ZnSe ATR crystal. Spectra were analyzed using OMNIC 9.2 software (Thermo Scientific, USA).

3. Results and discussion

3.1. Solid degradation rate

PAA treatment was performed at 80°C to completely liquefy both technical lignins. Complete lignin degradation was possible under all conditions when the samples were reacted with AA-HP mix solutions except at 1:4 (v/v) AL. Lignin decomposition generally requires high temperature, but the PAA treatment completely degraded solid lignin at relatively low temperature (<100°C).

Solid lignin degradation rate (% g/g of initial lignin) increased in proportion with the reaction time even when only HP was used. In particular, KL showed higher degradation rate than AL, with maximum degradation rate 94.2% after for 8 h without PAA (Figure 3-2). However, HP-treated AL degradation rates were lower than those of KL (Figure 3-1), due to the lignin species and characteristics used as starting material. AA had no effect on complete dissolution.

This chapter investigated complete lignin liquefaction and characteristics analysis. Therefore, further analysis focused on completely liquefied samples obtained from PAA treatment.

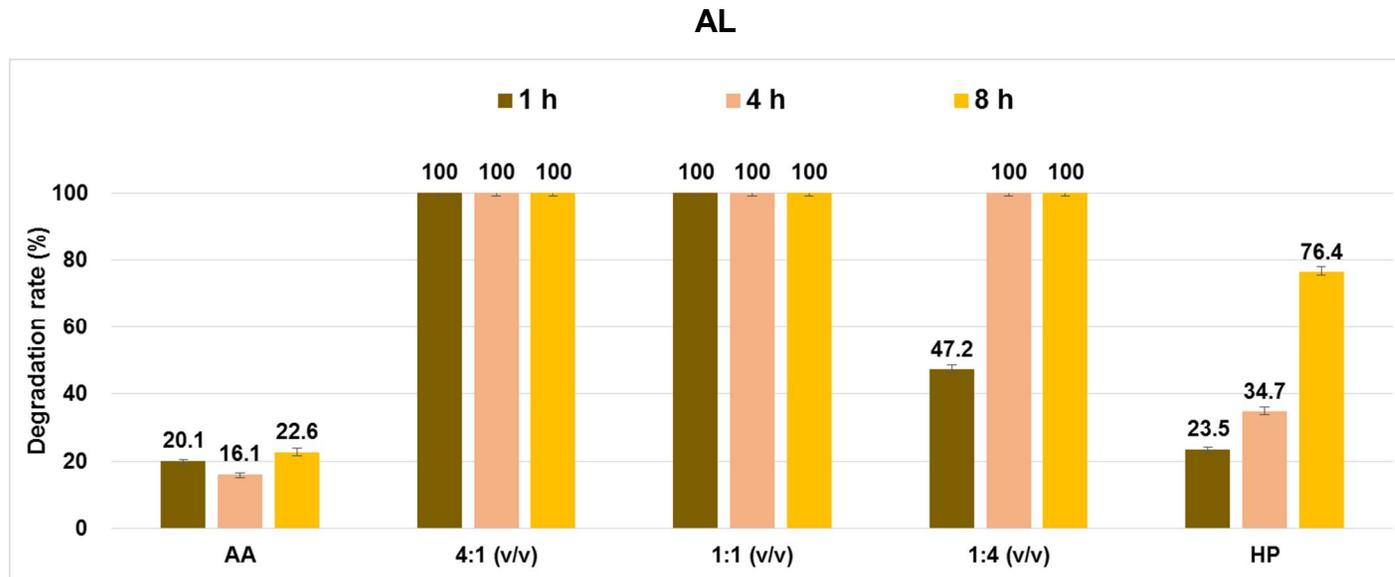


Figure 3-1. Degradation rates (%) after PAA treatment of AL.

KL

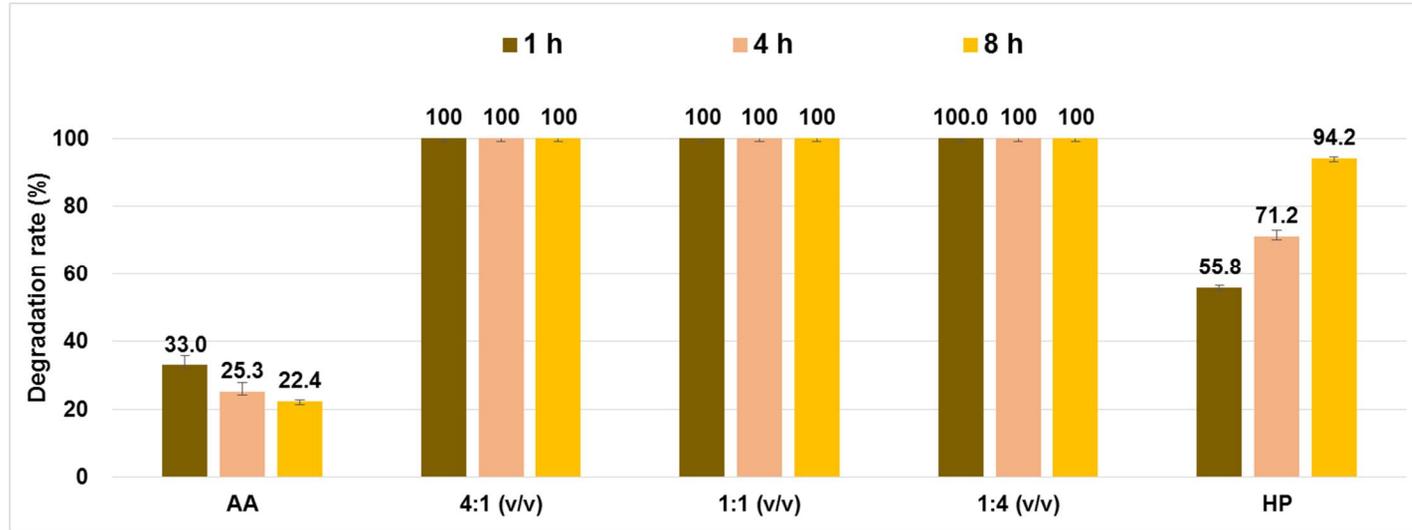


Figure 3-2. Degradation rates (%) after PAA treatment of KL.

3.2. Chemical properties of liquefied lignin

3.2.1. Relative portions of EA and the residual fractions in liquefied lignin

The AL and KL samples were completely dissolved in PAA solution after the thermal reaction. Lignin is known to be a hydrophobic polymer and, hence non-polar solvents are normally employed to dissolve it for later use. Lignin-derived products were isolated after EA extraction, since only a fraction of the total untreated lignin was extracted by EA. Figure 3-3 shows relative EA and residual fraction. The highest EA portion was obtained at 4:1 (v/v) for both AL and KL. When reacted with the highest HP concentration in solution, product amounts in the EA fraction were lower than for other conditions. Hence, it was assumed that the amount of HP in PAA solution is a crucial factor to change lignin solubility. HP can be decomposed by self-decomposition at elevated temperatures, and HP and PAA generate oxygen during the PAA reaction, which accelerates lignin degradation (Kumoro et al., 2017; Kunick et al., 2012; Yuan et al., 1997).

These phenomena show that the modified dissolution behavior in lignin by PAA oxidation can be explained by the increase in the amount of hydrophilic functional groups. The mechanism leading lignin hydrophilic properties follows β -aryl ether bond cleavage, benzyl alcohol side chain oxidative cleavage, quinone oxidation of the aromatic nucleus, and muconic acid ester formation by aromatic nucleus hydrolysis. Previous studies have also shown that lignin demethoxylation of ring methoxyl groups and aromatic hydroxylation are promoted by PAA oxidation (Sakai and Kondo, 1972; Sakai and Kondo, 1975).

KL showed higher residual fraction portion than AL (83.6% and 78.1% maximum portion, respectively, for 1:4 (v/v)), since hardwood lignin is more

susceptible to solubilization than softwood lignin. Syringyl nuclei leak faster than guaiacyl nuclei because syringyl unit solubility is higher than for the guaiacyl unit. These results are consistent with previous studies that reported syringyl nuclei reaction rate is 2-2.5 times faster than that of guaiacyl nuclei during PAA oxidation (Sakai, 1974). Lignin β -O-4 bond are more easily chemically cleaved than those of guaiacyl lignin (Shimizu et al., 2012).

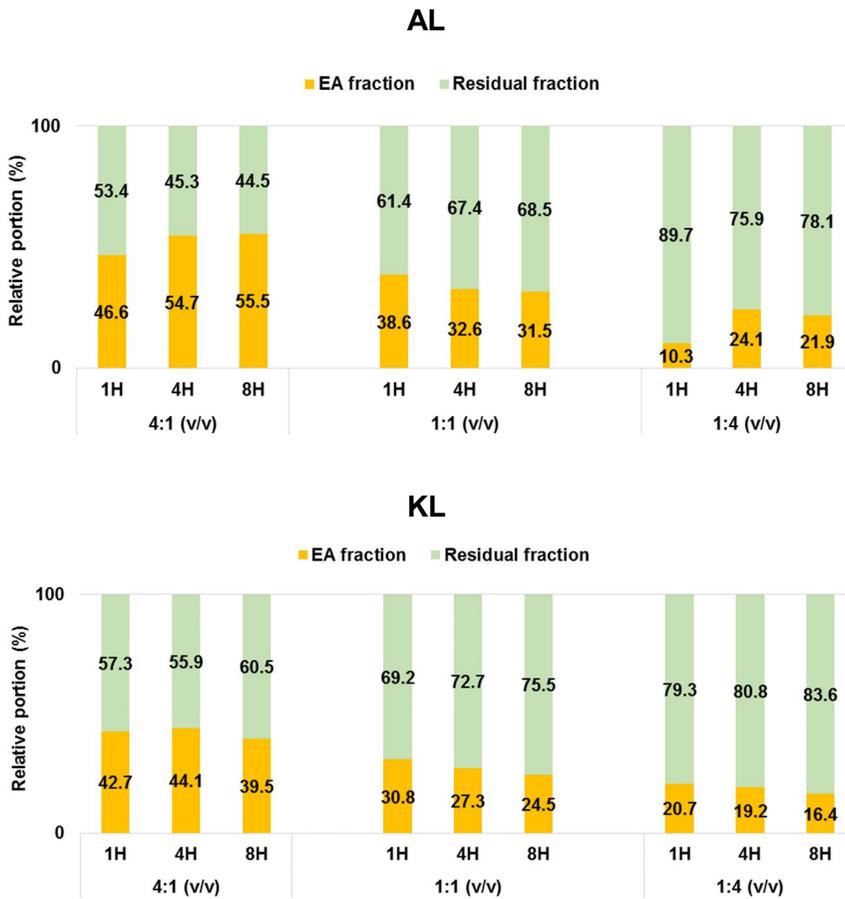


Figure 3-3. Relative portion of EA and residual fractions after PAA treatment for AL and KL.

3.2.2. EA fraction

3.2.2.1. Lignin-derived monomeric compounds

3.2.2.1.1. Monomeric compound distribution

The technical lignins used in this study were completely liquefied by PAA solutions at 80°C. GC/MS analysis was conducted to investigate the degraded compounds from the lignins after PAA treatment. PAA liquefaction produced many monomeric compounds from AL and KL were produced as listed in Table 3-1 and 3-2. Table 3-1 shows that 17 monomeric compounds species were produced from AL, comprising dicarboxylic acids (DCAs) and aromatics; whereas 15 monomeric compounds species were produced from KL (Table 3-2). Previous studies have also reported simultaneous DCAs and aromatics production after PAA oxidation (Ma et al., 2014; Popova et al., 2014). Interestingly, Table 3-1 and 3-2 show different degraded compound distributions depending on AA and HP mix conditions. Aromatic compounds such as vanillic acid and catechol, were mainly produced at 4:1 (v/v), and small amounts of DCAs, such as succinic and fumaric acid, were generated. However, as the HP portion in PAA solution increased, aromatic compounds became undetectable. PAA is a promising agent because it can selectively degrade macromolecular lignin into monomeric aromatics and DCAs. Thus, controlling PAA solution concentration can selectively produce the desired compounds.

It was demonstrated that DCAs production can be accelerated by increasing HP concentration in PAA solution. HP is also self-decomposed by elevated temperature, generating oxygen. Much oxygen is produced with higher HP concentration, due to accelerated lignin degradation. In particular, HP accelerates the ring-opening reaction with heat, converting aromatic

compounds into DCAs. Figure 3-4 shows that lignin could degrade to DCAs through several reaction pathways including demethoxylation, decarboxylation, and hydroxylation (Criquet and Leitner, 2015; Johnson et al., 2016; Santos et al., 2016).

Table 3-2. Monomeric compounds in the EA fractions of PAA treated-KL detected by GC/MS

No.	RT ¹	Product	Group	Mix ratio (v/v)		
				4:1	1:1	1:4
1	16.082	Glycolic acid	DCA ²	v ⁴	v	v
2	17.937	Oxalic acid	DCA		v	v
3	18.165	Hydracrylic acid	DCA		v	v
4	19.869	Malonic acid	DCA		v	v
5	20.145	Methylmalonic acid	DCA		v	
6	22.926	Succinic acid	DCA	v	v	v
7	23.287	Methylsuccinic acid	DCA	v	v	v
8	23.504	Glyceric acid	DCA	v	v	v
9	23.823	Fumaric acid	DCA	v	v	v
10	25.084	Hydroquinone	Ar ³	v		
11	25.196	Glutaric acid	DCA		v	v
12	27.464	Malic acid	DCA	v	v	v
13	28.614	4-Hydroxybenzoic acid	Ar	v	v	v
14	30.491	1,4-Dihydroxy-2,6-dimethoxybenzene	Ar	v		
15	32.614	Catechol	Ar	v		
16	33.403	Vanillic acid	Ar	v	v	v
17	34.502	Protocatechuic acid	Ar	v	v	v

¹ RT: Retention time

² DCA: Dicarboxylic acid

³ Ar: Aromatics

⁴ v: Detected in GC/MS

No.	RT ¹	Product	Group	Mix ratio (v/v)		
				4:1	1:1	1:4
1	16.082	Glycolic acid	DCA ²	v ⁴	v	v
2	17.702	2-Hydroxybutyric acid	DCA	v	v	v
3	17.937	Oxalic acid	DCA		v	v
4	18.165	Hydracrylic acid	DCA		v	v
5	22.783	Maleic acid	DCA	v	v	v
6	22.926	Succinic acid	DCA	v	v	v
7	23.287	Methylsuccinic acid	DCA	v	v	v
8	23.504	Glyceric acid	DCA	v	v	v
9	23.823	Fumaric acid	DCA	v	v	v
10	27.464	Malic acid	DCA	v	v	v
11	28.614	4-Hydroxybenzoic acid	Ar ³	v	v	
12	30.491	1,4-Dihydroxy-2,6-dimethoxybenzene	Ar	v		
13	32.614	Catechol	Ar	v		
14	33.403	Vanillic acid	Ar	v		
15	34.502	Protocatechuic acid	Ar	v		

¹ RT: Retention time

² DCA: Dicarboxylic acid

³ Ar: Aromatics

⁴ v: Detected in GC/MS

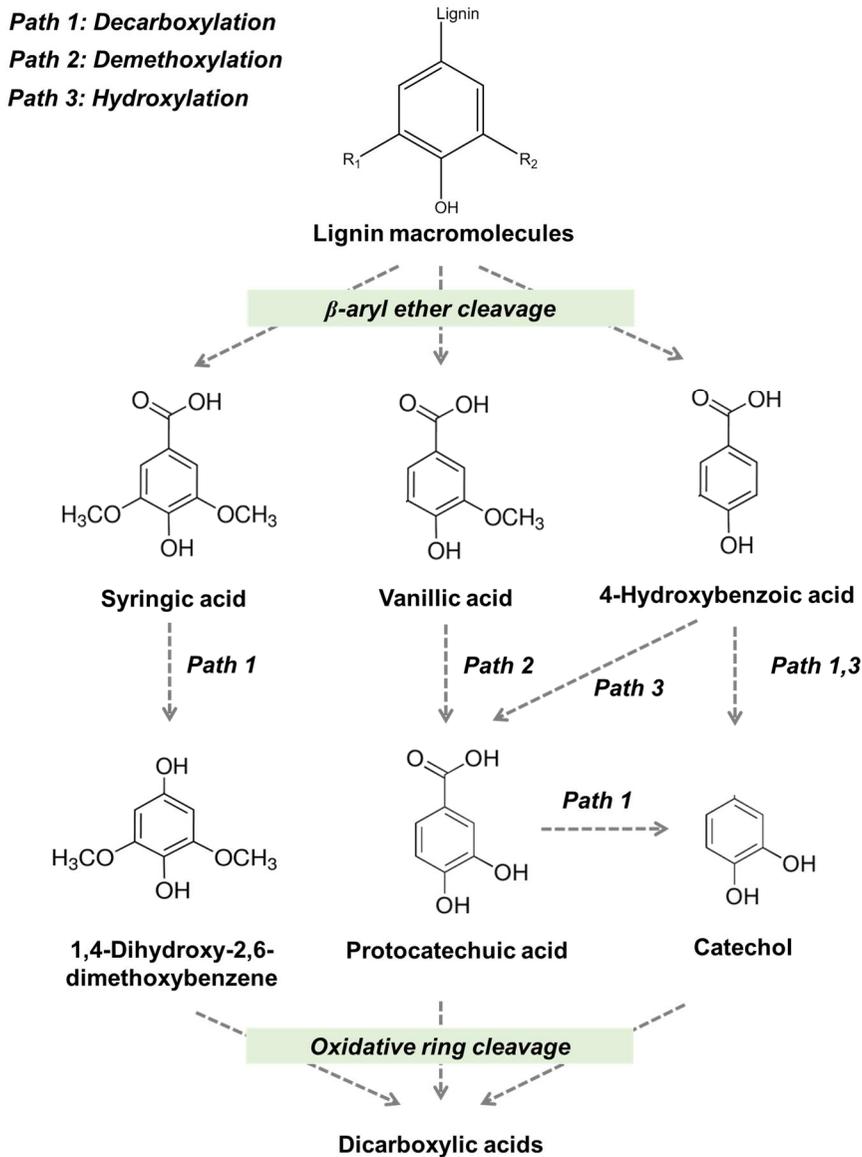


Figure 3-4. Plausible degradation pathways for lignin-derived monomeric compounds.

3.2.2.1.2. Monomeric compound yields

Various monomeric compounds (aromatics and DCAs) were produced after PAA liquefaction with significantly different distributions depending on the reaction conditions as mentioned in Section 3.2.2.1.1.

Maximum monomeric compound quantities for both AL and KL were obtained under 4:1 (v/v) (14.1 and 14.6 mg/g of initial lignin). Figure 3-5 shows that aromatic compounds were mainly produced as degraded products at 4:1 (v/v), hence total contents appeared to be dependent on aromatic compounds. AL produced 8.2-9.8 mg/g of aromatics, whereas KL produced 8.4-10.0 mg/g. DCA content was lower than that of aromatics, 2.4-4.3 and 4.6-5.0 mg/g for AL and KL, respectively.

Degraded compound distributions differed slightly under 1:1 and 1:4 (v/v) compared with 4:1 (v/v). DCAs were the main compound produced for both AL and KL, accounting for the majority of the total content. In particular, aromatic compounds were drastically decreased as the reaction time increased, implying that aromatic compounds were converted to DCAs at high HP concentration for longer reaction times. Total degraded product content under 1:1 and 1:4 (v/v) was lower than for 4:1 (v/v). However, DCA content for AL and KL at 1:1 and 1:4 (v/v) were higher than at 4:1 (v/v), with maximum contents 11.6 and 12.1 mg/mg, respectively.

Importantly, monomeric compound yields from AL and KL by PAA were very low compared to previous lignin depolymerization studies. Maximum monomeric compound yields were <1.5 % of untreated lignin in this study. It means that large quantities of high molecular lignin fragments remain in the liquid fractions. In summary, it was assumed that the PAA used in this study could cleave lignin into smaller fragments with a narrow molecular weight distribution. However, it seemed that PAA had small

monomeric compound production.

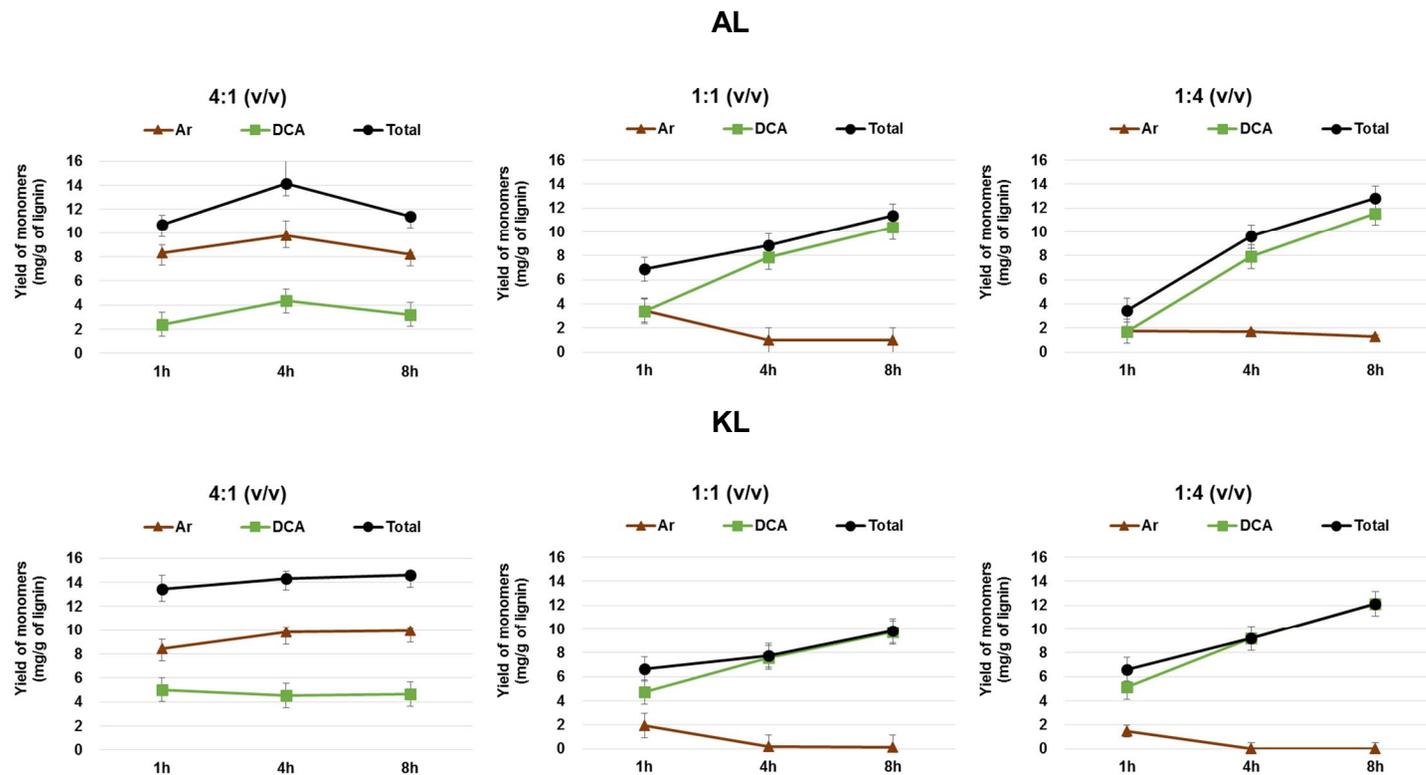


Figure 3-5. Monomeric compound yields derived from AL and KL after PAA liquefaction (Ar: aromatics; DCA: dicarboxylic acid).

3.2.2.2. Phenolic hydroxyl group distribution

UV-vis spectroscopy was used to detect relative amounts of phenolic hydroxyl group dissolved in EA fractions. Figure 3-6 shows UV absorption peaks at 250-450 nm as a shoulder curve. In particular, there are distinct peaks attributed to the phenolic structure containing at 270~280 nm, which can be assigned to $\pi \rightarrow \pi^*$ electronic transition of aromatics (Alexiou et al., 2015; Sisa et al., 2010; Song et al., 2015). Peaks 200-250 nm were considered to be noise because this region was irrelevant to lignin structures. Therefore, discussion was focused on the bands starting at 250 nm.

UV absorption bands showed different shapes between reaction conditions with different AA and HP ratios. Broad bands 250-300 nm for the EA fractions obtained from AL and KL at 4:1 (v/v), which indicate the presence of lignin products. However, the reaction conditions under 1:1 and 1:4 (v/v) showed sharply reduced absorption 250-300 nm. Band absorption depletion also reduced with increasing reaction time, which was consistent with GC/MS outcomes (Section 3.2.2.1). DCA production increased as HP portion in the PAA solutions increased, because ring-opening reaction of the lignin-derived compounds was accelerated. Higher HP and PAA concentration also affected the degraded compounds species due to accelerating self-decomposition. Therefore, considerable oxygen was generated depending on HP and PAA concentrations, resulting in higher DCA production.

The portion of EA-soluble products in the liquid fraction also decreased with increasing HP portion in the PAA solution. Therefore, the relative amount of lignin compounds in EA fractions also decreased as the HP ratio increased in solvent for both AL and KL.

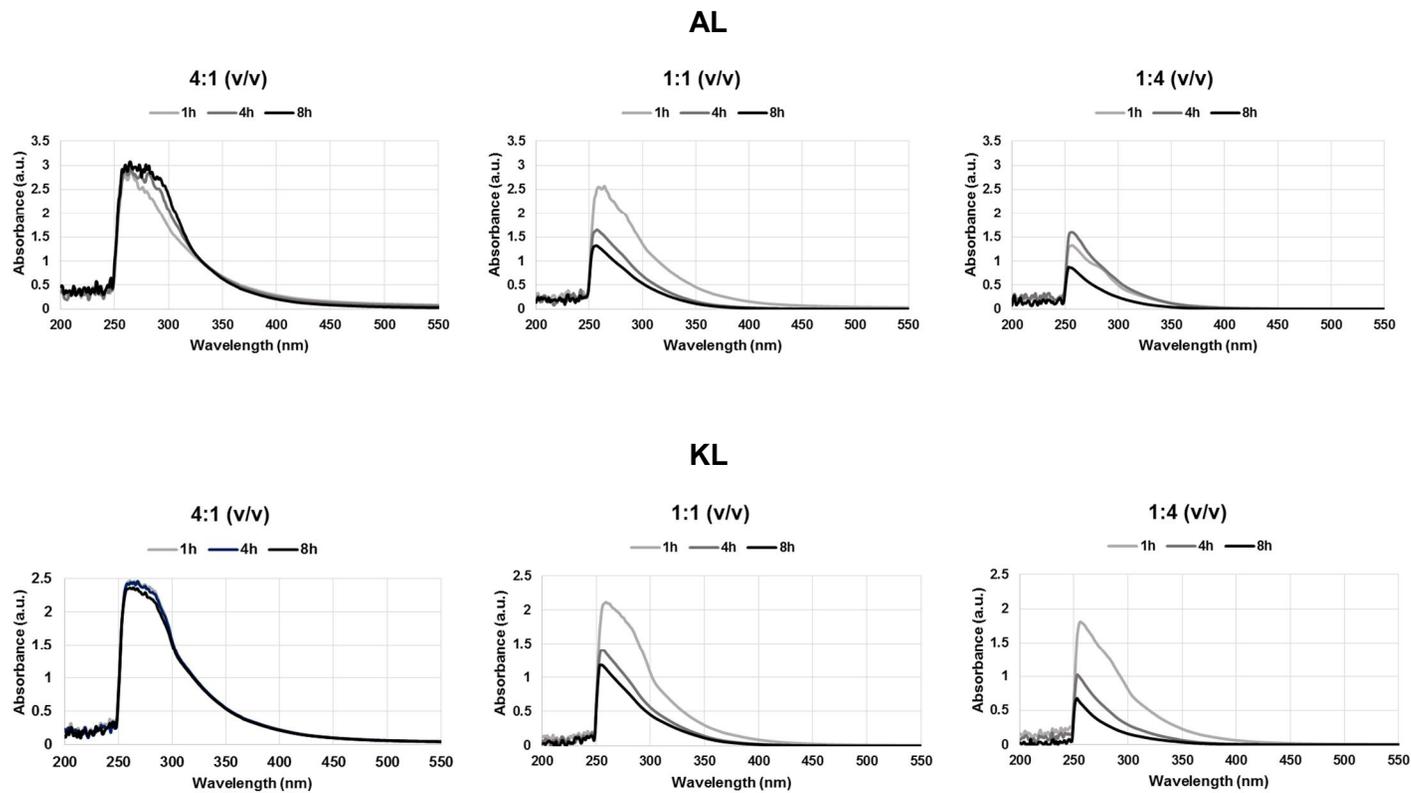


Figure 3-6. UV-absorbance spectra for lignin-derived compounds in the EA fractions.

3.2.2.3. Molecular weight distribution

Molecular weight (M_w) and polydispersity index (PDI, M_w/M_n) were compared for AL and KL after PAA liquefaction, as shown in Table 3-3, to investigate possible lignin depolymerization. Molecular weights for untreated AL and KL 3,391 and 2,813 Da, respectively. In EA fractions, the molecular weight and PDI of both AL and KL decreased as the reaction time increased. The range of the molecular weight was 823-2,031 and 769-1,546 Da in AL and KL, respectively. In particular, EA-soluble products under 1:4 (v/v) after 8 h exhibited lowest molecular weight for both AL and KL, and PDI decreased proportionally as reaction time increased.

PAA at 80°C completely liquefied both AL and KL, and degraded macromolecule lignins into lower molecular fragments. Lignin depolymerization to produce valued-products is generally conducted under high-temperature and pressure conditions. The thermal reaction forms solid (char) and liquid (lignin-oil) (Huber et al., 2006; Roberts et al., 2011). Lignin-oils produced by thermal decomposition have significantly lower molecular weights with narrow distribution compared to the initial materials (Cheng et al., 2010; Erdocia et al., 2015; Yuan et al., 1997). Although PAA liquefaction was performed at a lower temperature compared to typical thermal reactions, the process retained the positive effect of low-molecularization of lignins, turning macromolecules into smaller fragments. The 1:4 (v/v) ratio achieved superior lignin low-molecularization, with narrower molecular weight distribution.

Interestingly, EA-soluble products exhibited bimodal curves in GPC diagrams for all conditions (Figure 3-7), consistent with previous studies (Kim et al., 2017; Mansoure et al., 2011). Hence, lignin-derived compounds were divided into two groups with approximately 8.6 ml retention volume on

both sides. High molecular compounds accounted for almost the entire amount of EA-soluble product. The left side of each GPC diagram showed a broad area under all conditions, indicating that PAA can degrade lignin to produce lower the molecular weight while retaining oligomeric rather than monomeric.

The EA fraction yields were decreased further when lignin was treated with PAA solution higher HP portion. EA fraction yield was lowest for 1:4 (v/v) in all treatment conditions, as described in Section 3.2.1. Most compounds in the liquid fractions were changed to water-soluble compounds, resulting in lignin functionality changes. Although molecular weights of EA-soluble products decreased, the amount of lignin-derived compounds produced from EA fraction should also be considered.

Table 3-3. Molecular weight (Dalton) of lignin products in the EA fractions

AA:HP ratio (v/v) ¹		Reaction time								
		1 h			4 h			8 h		
		M _w	M _n	PDI	M _w	M _n	PDI	M _w	M _n	PDI
AL	4:1	1948	801	2.43	2001	819	2.44	2031	835	2.43
	1:1	1404	706	2.00	1204	653	1.84	1108	625	1.77
	1:4	1151	628	1.83	936	578	1.62	823	545	1.51
	Control ²	3391	1806	1.88						
AA:HP ratio (v/v) ¹		Reaction time								
		1 h			4 h			8 h		
		M _w	M _n	PDI	M _w	M _n	PDI	M _w	M _n	PDI
KL	4:1	1546	801	1.93	1520	770	1.97	1440	779	1.85
	1:1	1189	685	1.76	1037	647	1.60	945	602	1.60
	1:4	885	550	1.61	815	534	1.53	769	521	1.48
	Control ²	2813	970	2.90						

¹ Mix ratio of acetic acid (AA) and hydrogen peroxide (HP)

² Untreated lignin

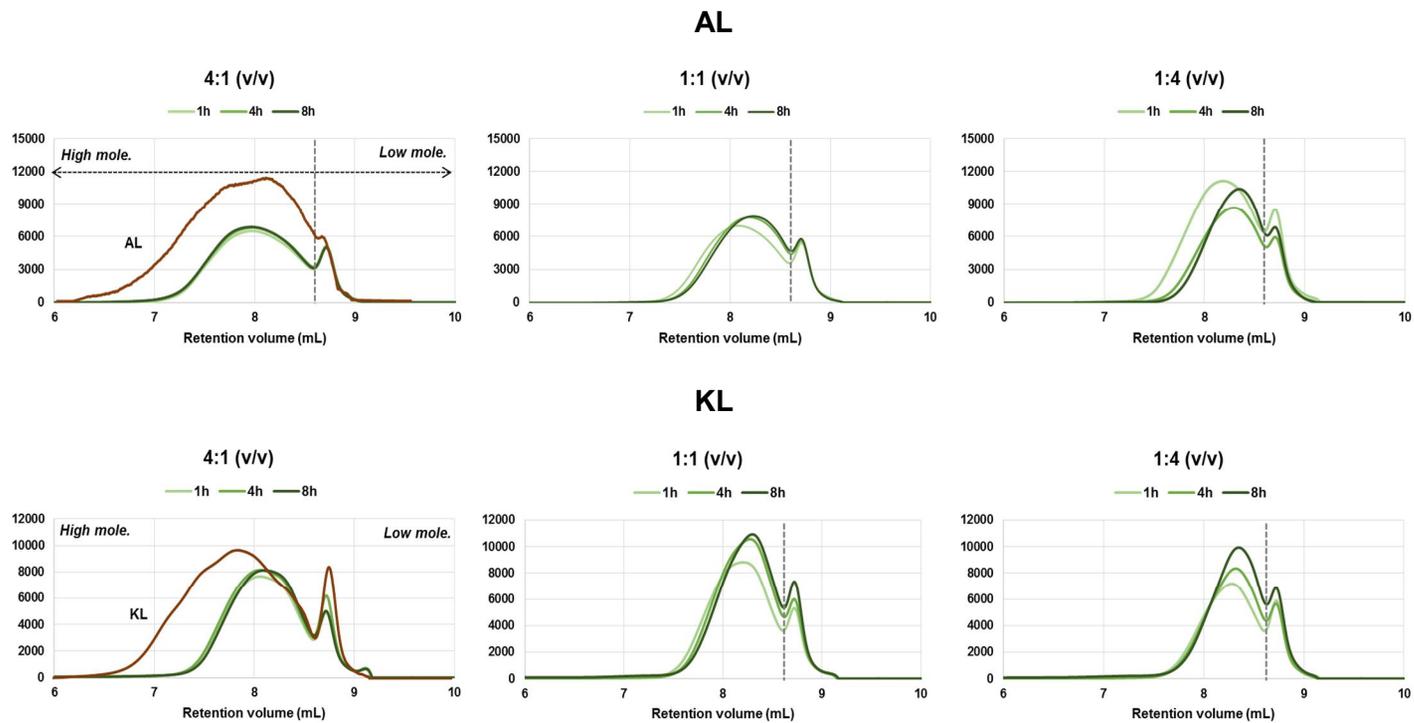


Figure 3-7. Chromatograms of AL and KL-derived products in EA fractions after PAA liquefaction.

3.2.3. Residual fractions

3.2.3.1. Structural changes

After PAA treatment, EA extraction was conducted to analyze lignin-derived products. However, only a fraction of the untreated lignin content was extracted as EA-soluble products, and a significant amount of residual fractions remained. Therefore, FT-IR spectroscopy was performed to confirm residual product structures.

Figure 3-8 shows typical FT-IR spectra, indicating peaks originating from residual product structures. Typical AL and KL absorptions occurred at the top of the figure. In particular, vibration bands were assigned at 1,598, 1,513, and 1,455 cm^{-1} , originating from the aromatic skeleton, including C=C. C-O bands related to ether bonds in syringyl and guaiacyl groups occurred from 1,200-1,000 cm^{-1} . Band assignments were similar to typical lignin structure peaks from previous studies (Lisperguer et al., 2009; Sills and Gossett, 2012; Zhou et al., 2011).

There were large band absorption differences for residual fractions after PAA treatment, but it is unclear how typical absorbance changes are related to lignin structures. The band at 1,719 cm^{-1} increased significantly, which was attributed to increased C=O bond in carboxyl (COOH) groups in organic materials (C=O bands increased for increased reaction time). C=O absorptions occurred more significantly for 1:4 (v/v), with intense bands at approximately 1,200 and 1,100 cm^{-1} . The absorption differed for peak assignments originating from untreated AL and KL. This was due to PAA generating more dicarboxylic acids under 1:4 (v/v), as mentioned previously, modifying the original structure to induce large amounts of carboxyl groups. Peaks at 1,210, and 1,043 cm^{-1} for AL and at 1,166, and 1,046 cm^{-1} for KL were intensified under 1:4 (v/v). These bands can be related to C-O stretching bands in

carboxylic acids and methoxy-containing compounds, consistent with previous studies (Jang and Wilkie, 2004; Khajavi and Berendjchi, 2014; Max and Chapados, 2004). Different band assignments between AL and KL may be attributed to different amounts of various dicarboxylic acids were produced due to species differences.

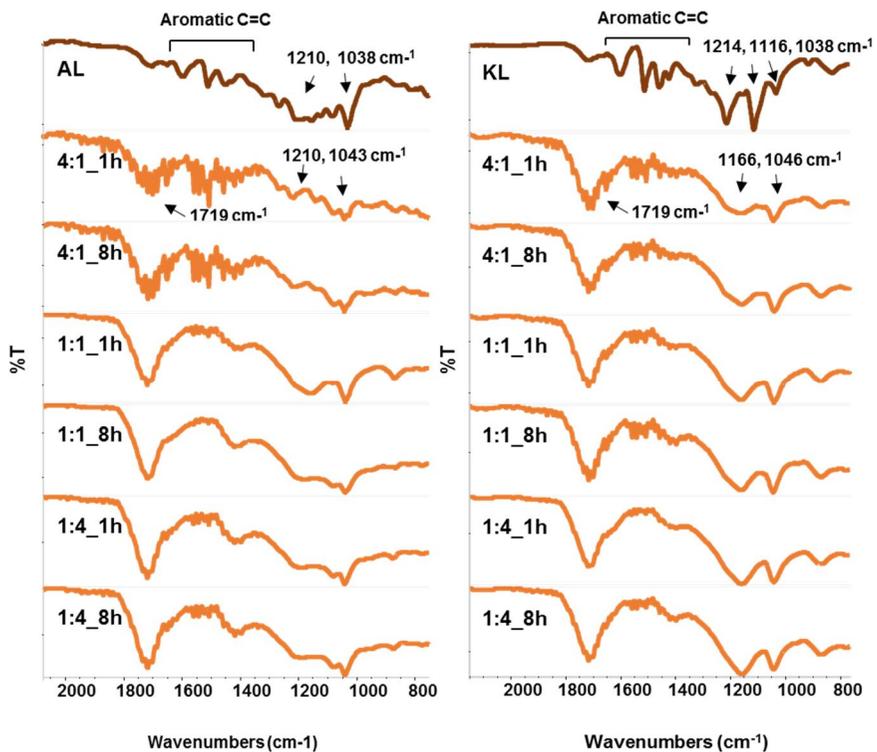


Figure 3-8. FT-IR spectra for the residual fractions after PAA treatment.

4. Summary

In this chapter, degradation characteristics of technical lignins were investigated using PAA solution at 80°C. PAA completely liquefied lignin even under relatively low-temperature conditions, and showed positive effects for molecular weight reduction of lignin-derived products. When reacting with high AA concentration in a solvent, lignin tends to decompose while maintaining its aromatic structure. However, DCA production was accelerated by the ring opening reaction when reaction with high HP concentration in the solvent. Therefore, different degraded product types were generated depending on AA, HP, and PAA concentrations. Thus, production of targeted compounds could be possible by controlling these concentrations.

However, maximum monomeric compound contents were obtained as small quantities (approximately 1.5 % of initial lignin) for both AL and KL, i.e., PAA mainly converted the lignin into relatively higher molecular fragments rather than monomeric compounds. In summary, although PAA mainly did not produce lignin as a monomeric compound, it produced more homogeneous structures. Therefore, it is expected that more homogeneous lignin may be available for application additives and chemicals in various industries.

Chapter 4

Open-column fractionation of
peracetic acid-treated kraft lignin and
its characterization for value-added applications

1. Introduction

Various technical lignins have been considered as potentially interesting raw materials, since they are produced in processes that deal with the treatment of lignocellulosic biomass and are readily available in large amounts. In particular, the method of isolating lignin from lignocellulosic biomass can be a crucial factor in consideration of its end-uses (Ragauskas et al., 2014; Strassberger et al., 2014). However, widely used lignins are mostly produced in paper and pulp industries, and many lignin upgrading processes for making high value-products have been attempted to improve economic value. Thus, demand for lignin and lignin-containing residues is steadily increasing (Doherty et al., 2011; Leskinen et al., 2015). Among technical lignins, kraft lignin is a commonly available commercial lignins and its applications have been promoted for more appropriate. However, the utilization of kraft lignin is restricted by condensed inter-unit linkages due to a complex degradation and condensation during the kraft pulping process. Use of the lignin is limited by its structural diversity in terms of high polydispersity and non-uniform reactivity. Furthermore, the diversity of the various functional groups present in kraft lignin make its utilization quite challenging (Jiang et al., 2016; Vishtal and Kraslawski, 2011).

Thus, fractionation has been introduced as a convenient method to obtain specific lignin samples with definite molecular weight. Several techniques have been proposed to fractionate various lignins. For instance, selective precipitation based on pH has been conducted by adding acidic agents such as sulfuric and hydrochloric acids (Helander et al., 2013; Lourençon et al., 2015; Sun et al., 1999; Zhu and Theliander, 2015). Ultrafiltration is one technique for separating lignin compounds based on

molecular cut-off. It has sparked great interest in several fields such as chemical, biological, and pharmaceutical industries (Humpert et al., 2016; Toledano et al., 2010). However, each method has its own disadvantages, such as low purity, and high cost associated with filtration.

Organic solvent extraction or fractionation has been considered a promising method and efficient and economical process for lignin fractionation; it has powerful advantages for separating lignin compounds according to definite molecular weight and functionality. Based on these advantages, many studies have already been conducted using this technique over the last few decades (Helander et al., 2013; Jiang et al., 2016; Kim et al., 2017; Li et al., 2012; Park et al., 2018; Thring et al., 1996; Wang et al., 2010). Lignin fractionation with organic solvents is typically performed by liquid-liquid phase separation using a solid lignin sample as a starting material. Although the technique has garnered much attention, it has limited applicability in bulk processing.

In this study, open-column fractionation was used to improve existing techniques, and fractionate lignin compounds according to their molecular weight and functionality. Open-column chromatography is a simple method that is widely used to isolate certain chemicals as value-added products. This technique has certain advantages; it can easily eliminate contaminants from the sample, and low-cost silica-gel is used as a typical stationary. Thus, it can improve process efficiency in terms of price competitiveness.

Lignin liquefaction using peracetic acid (PAA) was conducted before fractionation. In previous chapter, it demonstrated the effect on lignin degradation and changing solubility; in particular, ethyl acetate extraction was performed before open-column chromatography to divide the lignin into organic and water-soluble fractions for definite target application. The ethyl acetate soluble compounds were then used for further column fractionation. Thereafter, the fractionated lignin samples were fully characterized using

various analytical methods to compare each fraction's structural properties.

2. Materials and methods

2.1. Materials

Kraft lignin (KL) was provided by Moorim P&P Co. and it used for liquefaction and fractionation experiments. The chemical composition of the KL is described in Section 2.1 in Chapter 2.

2.2. Peracetic acid liquefaction of lignin

KL was liquefied by modifying the methods described in Section 2.2.3 in Chapter 2. A sufficient quantity of the lignin sample was prepared by putting, 1 g of KL into into a glass bomb (19.5 cm × 4.5 cm, length × diameter) and dissolving it in 30 mL of a solution of acetic acid (AA) and hydrogen peroxide (HP) (4:1, v/v) for the reaction. The solution was titrated using the procedure described in Section 2.2.2 in Chapter 2, and it contained 1.5% of PAA and 5.8% of HP, respectively. The PAA treatment was conducted at 80°C for 1 h using a dry block (MG-2200, EYELA, Japan). After the reaction, the completely liquefied sample (Figure 4-1) was obtained and diluted with 10-fold with distilled water to adjust the pH to 2. The diluted liquid was extracted twice with 300 mL of ethyl acetate (EA). For further fractionation processes, solvent was removed using a rotary evaporator at 40°C, and the remaining solute was freeze-dried. The residual aqueous fraction, which was not extracted by EA, was also freeze-dried.



Figure 4-1. The change from kraft lignin to liquefied lignin after PAA treatment.

2.3. Fractionation of liquefied lignin

The EA-soluble products in KL were fractionated using open-column chromatography system to separate the lignin fractions; 20 g of silica gel was used as a stationary phase and chloroform (CHCl_3) was poured into a glass tube (2.8-cm inner-diameter). Then, the EA-soluble compounds were added to the column. The solvent fractionation was conducted using 200 mL of CHCl_3 -EA (2:1, v/v), methanol (MeOH), and distilled water (H_2O), successively. After fractionation, each fraction was evaporated to remove the solvent and freeze-dried for further analysis. The overall process and nomenclature for each fraction is as shown in Figure 4-2.

- EA: Ethyl acetate fraction
- EA-F1: EA fractionated with chloroform:ethyl acetate (2:1, v/v)
- EA-F2: EA fractionated with methanol
- EA-F3: EA fractionated with water
- Residual: Residual aqueous fraction

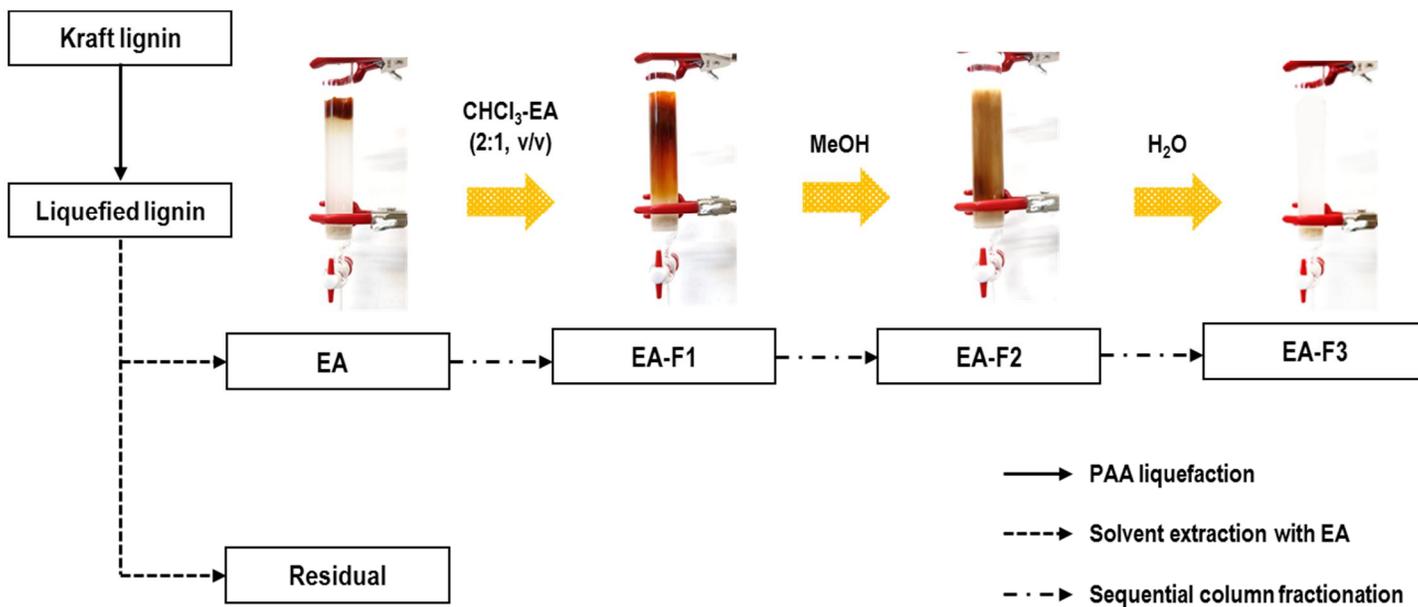


Figure 4-2. Scheme of the overall PAA liquefaction and column fractionation procedures.

2.4. Chemical properties of the lignin fractions

2.4.1. Gel permeation chromatography (GPC)

GPC was performed to analyze the molecular weight of the lignin. Unlike our previous results, some samples obtained through fractionation with several solvents were not dissolved in tetrahydrofuran (THF). Acetylation was conducted before GPC analysis to improve the solubility of the solid samples. The solid samples were acetylated with pyridine and acetic anhydride (1:1, v/v) at 105 for 2 h. The molecular weight distribution of the acetylated samples from each fraction was determined according to the previous methods described in Section 2.3.3 in Chapter 2.

2.4.2. Phosphorus-31 nuclear magnetic resonance (³¹P-NMR)

The hydroxyl group content in each fraction was determined by conducting ³¹P-NMR analysis according to the previous methods described in Section 2.3.2 in Chapter 2.

2.5. Structural analysis

2.5.1. Carbon-13 nuclear magnetic resonance (¹³C-NMR)

The types of carbon linkages of each lignin fraction were investigated by conducting ¹³C-NMR analysis. About 60-80 mg of each solid sample were dissolved in 0.6 mL of dimethyl sulfoxide-*d*₆ (DMSO, Sigma Aldrich Co.). Deuterium oxide (D₂O) was used as a solvent samples, which did not dissolve in DMSO. NMR data was recorded at 60°C for 8 h using a spectrometer set to 600 MHz (ADVANCE 600, Bruker, Germany).

2.5.2. Fourier-transform infrared spectroscopy (FT-IR)

The structural differences between fractions were compared by performing FT-IR analysis according to previous methods described in Section 2.3.3.1 in Chapter 3.

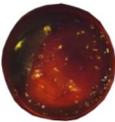
3. Results and discussion

3.1. Yield of each lignin fraction

KL was liquefied using PAA solution and was extracted by EA to divide into organic and water-soluble fractions. As shown in Table 4-1, the yield of the EA fraction was 57.9%, and this fraction was sequentially fractionated followed by CHCl_3 -EA (EA-F1), MeOH (EA-F2), and H_2O (EA-F3). Meanwhile, significant amounts, 41.1% yield, of residual aqueous fraction (Residual) were obtained after PAA treatment.

The yields of the three fractions obtained from EA-soluble compounds were shown in Table 4-1, and EA-F2 showed the highest yields (62.0%) among them. Interestingly, EA-F2 and EA-F3 obtained from EA-soluble fractions were not dissolved in EA after column fractionation due to structural changes in the two fractions after PAA treatment.

Table 4-1. The yields of each fraction obtained by solvent extraction and open-column fractionation

Fraction	Yield (%)	Fraction	Yield (%)
		EA-F1 ¹	
			9.2 (±0.22)
EA	57.6 (±1.49)	EA-F2	62.0 (±0.75)
			
		EA-F3	27.7 (±0.92)
			
Residual	41.1 (±1.80)	-	-
			

¹EA-F1, F2, F3: Calculated based on the yield (g/g) of EA-soluble fraction

3.2. Molecular weight distribution

Solvent extraction and column fractionation were performed in this study to fractionate the PAA-treated lignin with different molecular weight and characteristics. All fractions exhibit bimodal molecular weight curves, as shown in Figure 4-3, which meant that lignin-derived molecules were present with specific molecular weight ranges collectively.

Table 4-2 shows the molecular weight values of all fractions. The M_w of all fractions was significantly decreased compare to that of untreated KL (4,068 Da). EA and Residual fractions were 2,075 and 1,221 Da, respectively. Meanwhile, the EA fraction was sequentially fractionated through the column, which was divided into three fractions. The molecular weight and polydispersity index (PDI) of the three fractions increased in proportion to solvent polarity, as shown in Table 4-2. These tendencies were similar to those of previous studies, and it has been reported that when polar solvents, such as methanol, and acetone, were used for fractionation, the molecular weight and PDI decreased (Li et al., 2012; Park et al., 2018; Thring et al., 1996). The molecular weight of the remaining Residual fraction after EA solvent extraction was the lowest at 1221 Da.

Meanwhile, bimodal curves were observed in the GPC diagram shown in Figure 4-3, and the molecular weight and PDI of all fractions were divided into Group 1 and 2 based on the median value of 8.6 mL. In addition, the relative quantitative percentages were calculated by dividing the entire area by the median value. As can be seen from the results at the bottom of Table 4-2, it was expected that Group 2 in the diagram would consist of low-molecular weight compounds decomposed by PAA in the range of 261~275 Da. However, Group 1 was observed as relatively high-molecular weight lignins, while PDI was significantly decreased compared to untreated KL. In

particular, Group 1 of the EA-F2 fraction was mainly comprised of high weight molecules, which accounted for 87% of the entire area.

It is important to have a uniform structure for lignin application, and thus PDI is considered an important criterion. Among the fractions, the Residual fraction showed the lowest PDI in Group 1. However, since the portion of Group 1 was the lowest at 66%, the separation of the each group according to molecular weight may be necessary to use those that have narrow molecular weight distribution.

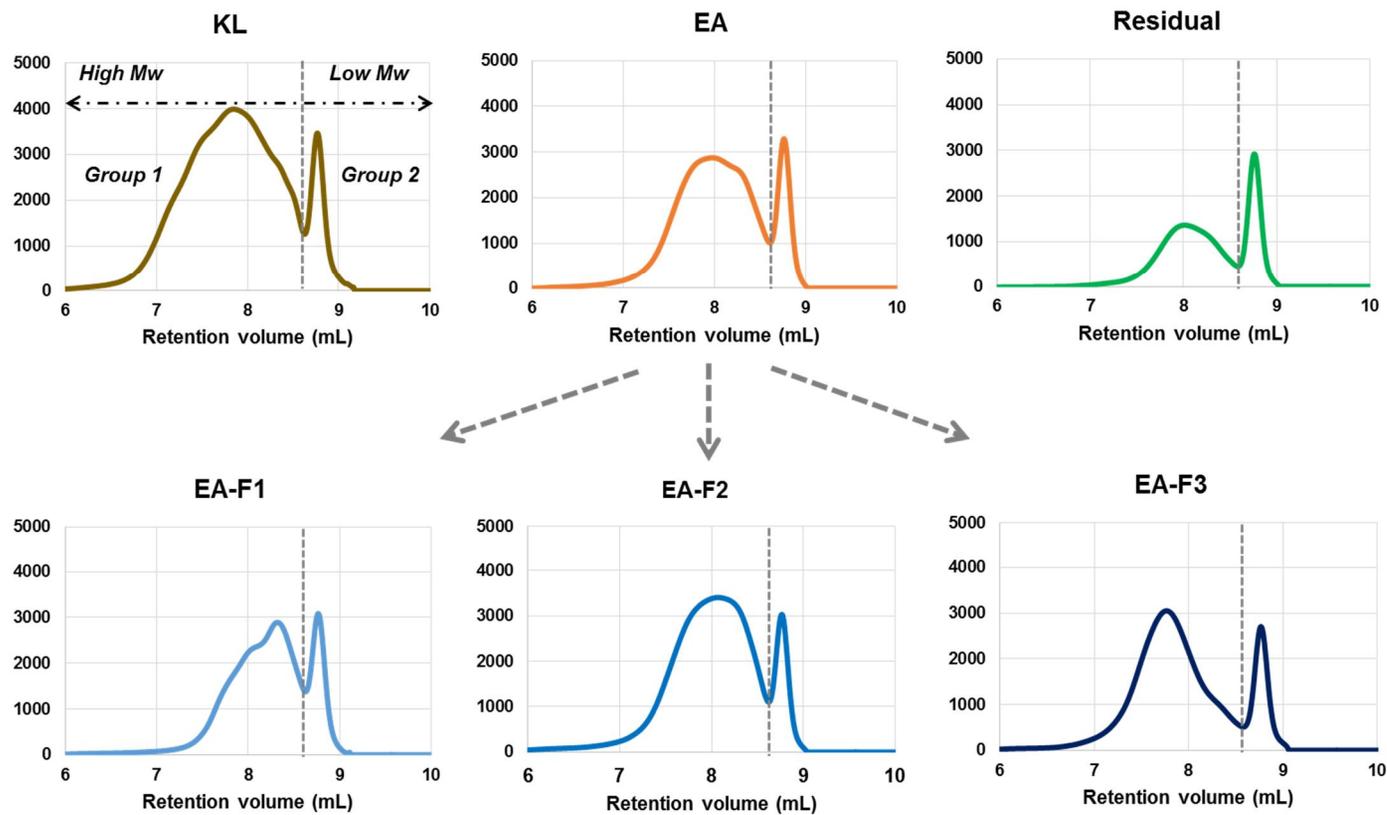


Figure 4-3. Chromatograms of molecular weight for each fraction.

Table 4-2. The molecular weight (Dalton) distribution of each fraction

Broad												
	KL		EA		EA-F1		EA-F2		EA-F3		Residual	
M _w	4068		2075		1366		2075		3031		1221	
M _n	1072		803		635		868		976		543	
PDI	3.79		2.58		2.15		2.39		3.11		2.25	
Separated												
	KL		EA		EA-F1		EA-F2		EA-F3		Residual	
	1	2	1	2	1	2	1	2	1	2	1	2
M _w	4427	266	2425	270	1570	261	2307	275	3552	274	1702	268
M _n	1749	255	1356	261	987	252	1329	267	2078	265	1201	261
PDI	2.53	1.04	1.79	1.03	1.59	1.04	1.74	1.03	1.71	1.03	1.42	1.03
Area (%) ¹	88.5	11.5	82.5	17.5	79.4	20.6	86.6	13.4	83.3	16.7	65.7	34.3

¹Relative area (%) calculated by dividing Groups1 and 2 based on the retention volume at 8.6 mL

3.3. Hydroxyl group contents

The hydroxyl group is the most important functional group in lignin applications, which is related to its reactivity with other polymers. In this study, hydroxyl groups comprised aliphatic-, phenolic-, and carboxylic acid moieties calculated by ^{31}P -NMR analysis. As shown in Figure 4-4, the total content of the hydroxyl groups in untreated KL decreased at both EA and the Residual fraction after PAA liquefaction. PAA degraded lignin by the cleavage of β -O-4 linkages in lignin structures and converted the lignin into large amounts of carboxylic-containing structures, as mentioned in a previous chapter. Therefore, the phenolic hydroxyl content decreased drastically, while those of the carboxylic OH group increased.

Meanwhile, EA-F1, F2, and F3 fractionated by open-column from EA-soluble compounds did not show the proportional values of the total contents. Previous research has shown that hydroxyl contents generally have lower values when lignin structures were changed to more condensed structures with high molecular weights as the solvent polarity increased in the fractionation process (Park et al., 2018; Thring et al., 1996; Mörck et al., 1986). The solvents used in this fractionation process; CHCl_3 , EA, MeOH, and water; have different solubility parameters. The solubility parameter (δ), from Hildebrand theory, and hydrogen-bonding parameter (δ_{h}) from Hansen theory for each solvent and water are listed in Table 4-3 (Sameni et al., 2017). However, although the molecular weight proportionally increased as the solvent polarity increased (F1 \rightarrow F3), the total hydroxyl contents did not show a similar trend.

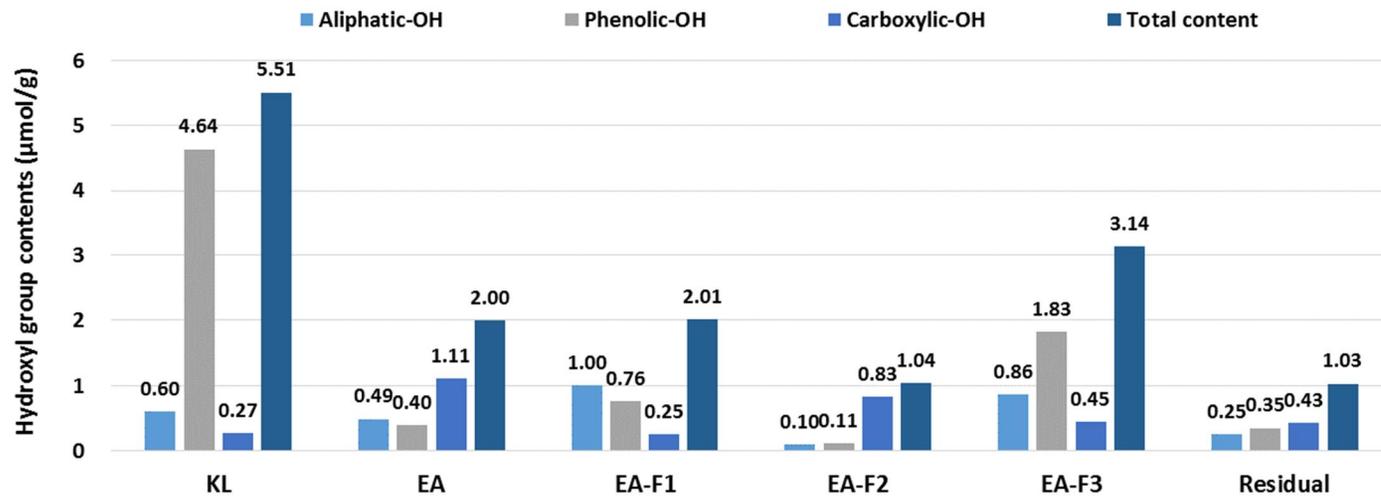


Figure 4-4. The hydroxyl group contents (µmol/g) of each fraction as measured by ³¹P-NMR analysis.

Table 4-3. The solubility parameter (δ - from Hildebrand theory) and hydrogen bonding parameter (δ_h - from Hansen Theory) of the solvents and water used in this study (Sameni et. al., 2017)

No.	Solvent	δ (cal/cm ³)	δ_h (cal/cm ³) ^{1/2}
1	Chloroform	9.2	2.8
2	Ethyl acetate	9.1	3.5
3	Methanol	14.3	10.9
4	Water	23.5	20.6

3.4. Structural analysis

3.4.1. ^{13}C -NMR

The carbon bond types of KL and their fractions, obtained by solvent extraction and column fractionation, were investigated by performing ^{13}C -NMR analysis. At 100-150 ppm as shown in Figure 4-5, carbon signals originating from aromatic structure; which included symmetric carbons in syringyl units, etherified carbons in guaiacyl units and non-etherified carbons; were typically observed. Meanwhile, C- α , C- β , C- γ carbons in β -O-4 were observed around at around 58-100 ppm. In addition, a strong peak at 55 ppm indicated a typical carbon signal from the methoxy group (-OCH₃) in the lignin structure. At 151-200 ppm, the C=O signals of carboxylic and carbonyl groups in aromatic and aliphatic structures were also observed (Capanema et al., 2004; Chen and Robert, 1988; Terashima et al., 2002; Wen et al., 2013). Based on these results, the carbon signal patterns of each fraction were compared.

Untreated KL showed diverse carbon signals in all spectral regions. In particular, carbons were clearly observed in aromatic structures of lignin. In addition, aryl-ether-linked carbon signals at 55-100 ppm and aliphatic carbons were also commonly observed. KL was generated by the kraft pulping process, which made lignin structures more condensed compared to natural lignin in wood because the process needs a comparatively high temperature. However, aryl-ether-linked carbons were maintained at 55-100 ppm. Meanwhile, EA and Residual fractions were obtained after PAA liquefaction, and these fractions showed simple peaks compared to KL. In particular, the number of carbons related to the aromatic structure and those of aryl-ether-linked carbons were clearly decreased; these can explain how PAA cleaved the linkage of lignin

structures and produced less-complex lignin-derived compounds.

Meanwhile, three fractions obtained by open-column fractionation using EA-soluble compounds showed significant differences between each fraction. In particular, the EA-F3 fraction did not show carbon signals originating from aromatic structures in lignin. Only few signals were observed at 151-200 ppm, which means that compounds in the EA-F3 fraction may not have contained aromatic compounds.

Intensive signals of C=O from carboxylic or carbonyl group were observed in all fractions; these functional groups can be involved in solubility. As the lignin moieties containing methoxyl group conjugate bonds decreased, the compounds became hydrophilic substances. According to previous research, PAA solution can result in demethoxylation of the lignin structure. Therefore, some fractions did not dissolve in organic solvents due to changes in the functional groups.

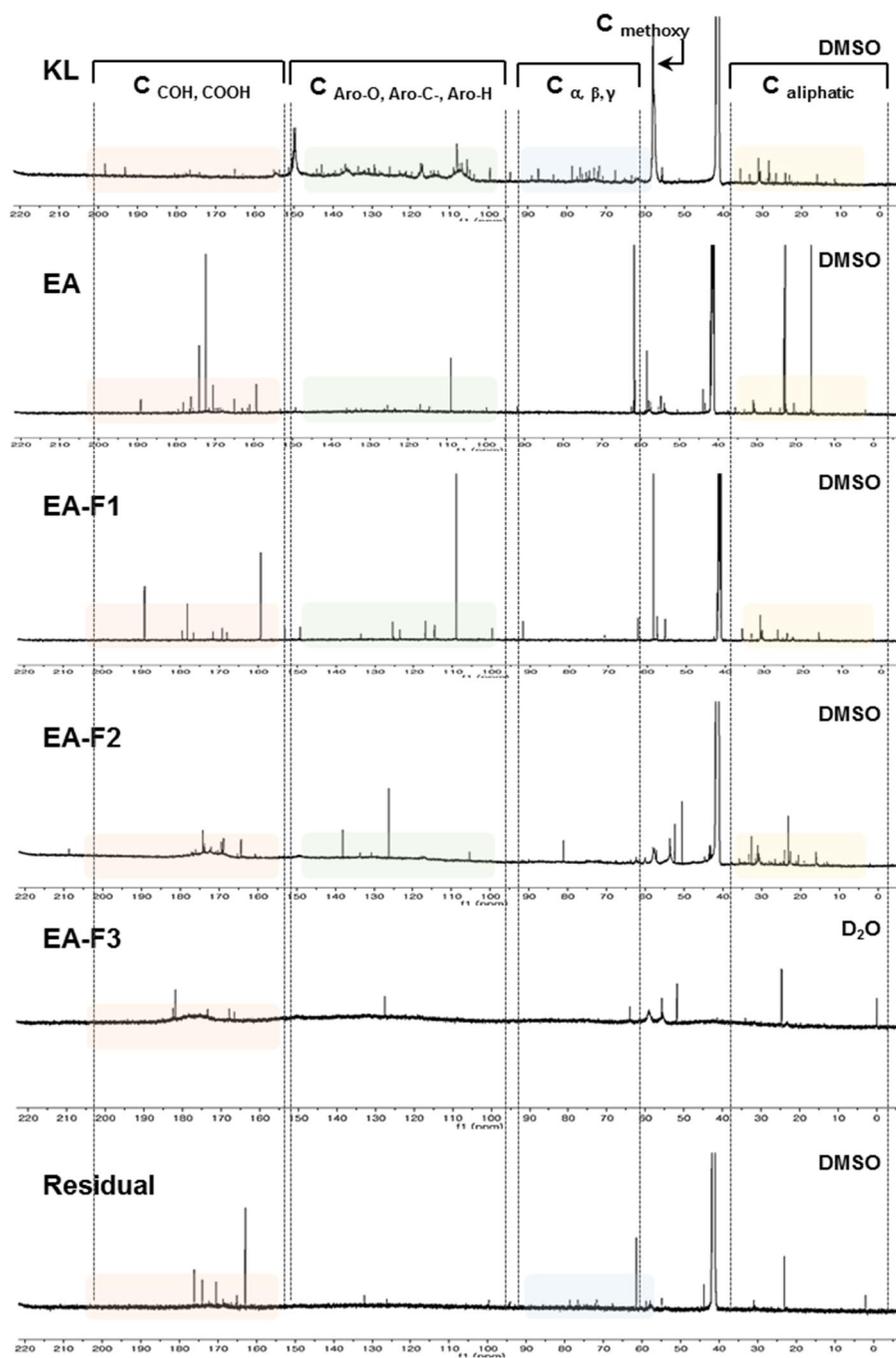


Figure 4-5. The ^{13}C -NMR spectra of KL and its fraction.

3.4.2. FT-IR

Figure 4-6 shows the FT-IR spectuma of each fraction obtained by solvent extraction and fractionation. At the top of the spectra Figure 4-6, KL indicates typical untreated lignin assignment and its spectrum was used as a reference for comparison with other fractions. The bands for aromatic skeleton vibrations were at 1598, 1513, 1455 cm^{-1} , included C=C. In addition, C-O bands related to ether bonds in syringyl and guaiacyl groups were also observed at 1400-1000 cm^{-1} . The band assignments were similar to typical peaks related to lignin structure that are mentioned in previous studies. At around 1660-1800 cm^{-1} , the strong bands indicated that C=O peaks originated from the carboxylic acid carbonyl groups that were present in organic polymers. (Lisperguer et al., 2009; Sills and Gossett, 2012; Zhou et al., 2011).

EA-soluble compounds obtained by solvent extraction after PAA treatment were sequentially fractionated by the column. Interestingly, there was no peak related to the aromatic skeleton from lignin in the EA-F3 fraction. Furthermore, the intensity of the C=O bands was also decreased in the EA-F3 fraction; these corresponded to the ^{13}C -NMR results that also showed that no carbon signal was involved the aromatic structures.

Meanwhile, the Residual fraction showed a simple spectrum pattern compared to other fractions, and this was similar to the patterns of polycarboxylate polymers. The signal at near 1700 cm^{-1} proves the existence of ester bonds; the peaks at around 1400 cm^{-1} represent for $-\text{CH}_2-$, and the signal at 1100 cm^{-1} , proved that it was similar to the polycarboxylate-type molecular structure (S. and Hanaa Rokbaa, 2016; Zhang et al., 2014). This was probably due to PAA oxidation, which converted the carbonyl, hydroxyl, and methoxyl groups in lignin to carboxylic groups.

Among the fractions, EA-F3 and the Residual fractions were well-dissolved in water as mentioned in previous results. In particular, the peaks

originating from CH₂ and CH₃ at 3000-2800 cm⁻¹ were not observed in those fractions, which was evidence of demethoxylation by PAA. In other words, it is expected that the lignin-derived products in the two fractions were dissolved in water due to the change of the functional group.

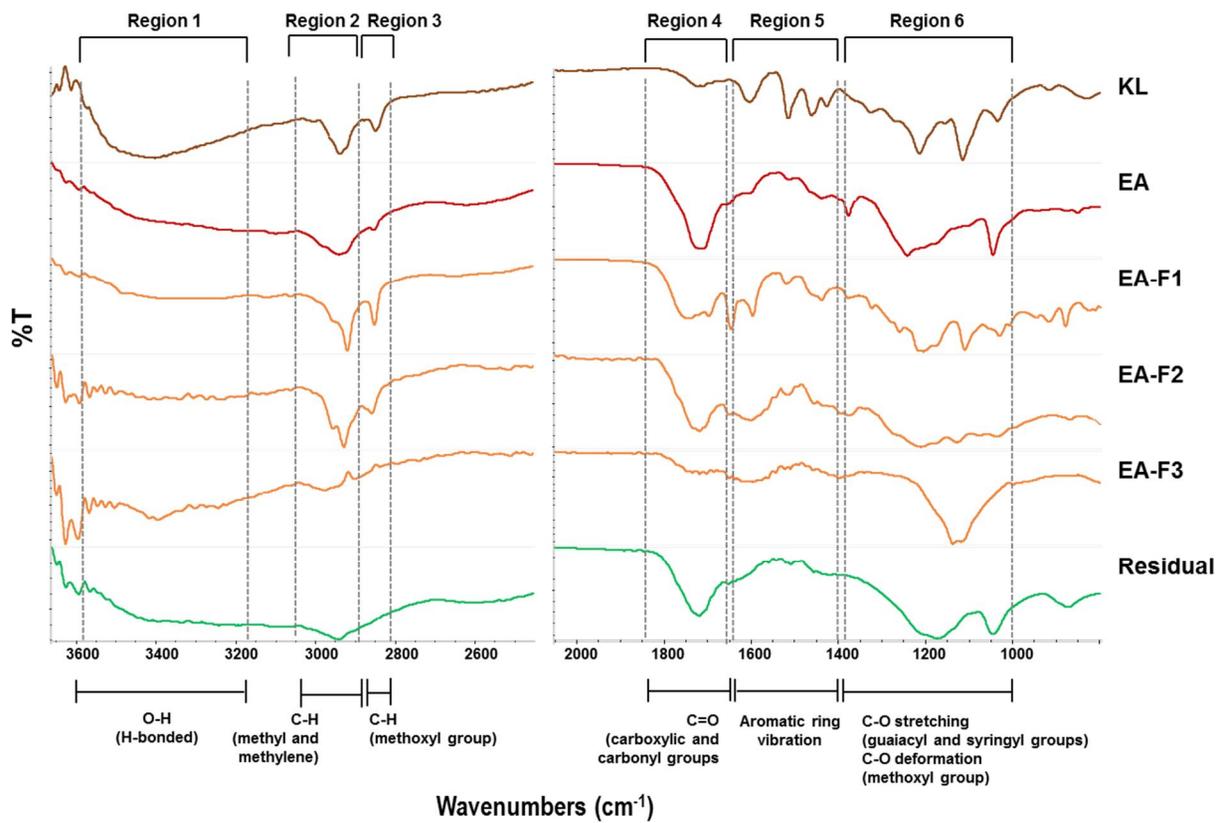


Figure 4-6. The FT-IR spectra of each fraction obtained after solvent extraction and column fractionation.

4. Summary

This chapter performed open-column fractionation using several solvents and water to fractionate kraft lignin-derived compounds according to their molecular weight. Before this process, PAA treatment was first carried out to dissolve and depolymerize the lignin. PAA made the lignin structure more uniform and changed its functionality. After column fractionation, each fraction showed specific molecular weight ranges and structures. In particular, some fractions (EA-F3 and Residual) that were dissolved in water did not maintain the original aromatic skeleton of lignin structure. It seemed that PAA-induced both lignin degradation and structural changes with a large amount of carboxylic acid groups. In summary, combination of PAA treatment and column fractionation could have a simultaneous effect on the low-molecularization of lignin and separation of lignin derived compounds according to their molecular weight range and structure. It was concluded that many problems related to complexity and heterogeneity of lignin could be improved through these techniques.

Chapter 5

Concluding remarks

Lignin is an aromatic biopolymer linked to lignin monolignols, such as *p*-coumaryl, coniferyl and sinapyl alcohols. It has great interest as a promising biodegradable material alternative to petroleum-based chemicals. However, complexity and heterogeneity of lignin has been considered a challenge to be overcome for lignin utilization. It is necessary to develop efficient process to make the lignin value-added chemicals or polymers with more uniform, not only limiting in primary method such as steam and electricity.

In this study, to find the efficient technique for overcoming the problems, peracetic acid (PAA) was used for lignin degradation. In particular, commercial PAA solution shows different properties according to the manufacturers, and thus PAA solution was prepared directly by mixing acetic acid (AA) and hydrogen peroxide (HP) at different ratios (4:1, 1.5:1, 1:1, 1:1.5, and 1:4 (v/v)).

PAA treatment with two types of technical lignins (alkali and kraft lignin) at room temperature was conducted to compare the lignin degradation characteristics. As a result, some part of the lignin became degraded and dissolved in liquid phase. At the 1:1.5 (v/v), the highest degradation rates of AL and KL were 29.0% and 65.3%, respectively. However, degradation rates were differed depending on the concentrations of PAA and HP, which means that not only concentration of PAA and but also that of other chemicals in the solvent may be factors in lignin degradation. In addition, degradation rates were also affected by lignin species, indicating the different reactivity be attributed from monolignol's moiety.

Based on these results, it was confirmed that PAA can degrade and dissolve the lignin at low temperature. However, solid samples still remained despite of the reaction time increase. Therefore, PAA treatment was conducted by heating during the reaction. As a result, two types of technical lignins were completely dissolved in the PAA solutions (mix ratio; 4:1, 1:1, 1:4 (v/v)) at 80°C. In particular, molecular weight and polydispersity of KL in EA fraction

drastically decreased than those of untreated KL. However, the portions of EA-soluble compounds in total liquid accounted for small amounts. It was possible to explain that PAA also converted the lignin structure to other polymers that have large amounts of carboxylic acid groups as the HP concentration in the PAA solution increased; thus solubility of the lignin-derived compounds was changed. FT-IR results of the residual fraction proved the structural changes of the lignins. Therefore, two types of polymers with different structure and solubility were produced.

Open-column fractionation was applied to separate the lignin-derived products with different molecular weight range through a simple method. Even though PAA can lower the molecular weight of the lignin, making the lignin structure more uniform was needed. PAA oxidation at 80°C was first conducted to dissolve and degrade lignin. After that, the liquid samples was divided into EA and water-soluble fraction by solvent extraction. And then, EA-soluble compounds were fractionated using several solvents and water in open-column. As a result, each fraction showed different molecular weight with narrower distribution than untreated KL. In particular, some fractions did not show the lignin aromatic structure, which indicates the possibility of separating the lignin-derived products with different polymer structures. Consequently, the combination of the PAA treatment and open-column fractionation can not only lower the molecular weight of the lignin but also separate the lignin-derived products depending on the molecular weight and structural differences.

In conclusion, this study aimed at complete liquefaction of lignin and separation of the lignin-derived compounds depending on molecular weight. Through various methods applied in this study, new trial for improving the limitation of the lignin uses was carried out. As a result, production of low-molecular lignins and separation of them were possible. By improving the structural problems of lignin, it was possible to suggest that lignin uses will be

more available in a variety of areas.

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초 록

리그닌 유래 유용화합물의 선택적 생산을 위한 과초산 처리

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본 연구에서는 과초산 용액 농도에 따른 리그닌의 구조 전환 특성을 이해하고, 반응 온도 및 시간 조건 설정을 통해 리그닌의 저분자화를 유도하고자 하였다. 또한, 목적하는 유용 화합물을 생성하기 위한 과초산 용액 제조 조건을 탐색하였다. 최종적으로, 여러 용매를 선정하여 컬럼 분획을 통해 과초산 반응 후 저분자화된 리그닌 유래 물질을 분자량별로 분리하고자 하였다.

과초산 용액 제조 시 사용되는 아세트산과 과산화수소를 4:1, 1.5:1, 1:1, 1:1.5 및 1:4 (v/v)의 비율로 혼합하여 상온에서 리그닌 산화 반응을 수행한 결과, 2 종의 리그닌(알칼리 리그닌, 크라프트 리그닌) 모두 1:1.5 (v/v) 혼합용액으로 반응하였을 시 각각 29.0%, 65.3%로 가장 높은 고형분 분해율을 나타내었다. 하지만, 리그닌의 수종에 따른 구조 전환 양상이 상이하였으며, 활엽수종을 원료로 제조된 크라프트 리그닌의 경우 고형 분해가 빠르게 나타났다. 이는 리그닌 수종에 따른 반응성 차이에 기인한 것으로 과초산 반응 시 수종 선택이 중요한 것으로 사료된다. 한편, 초기 리그닌으로부터 분해된 리그닌 유래 화합물이 액상내에서 검출되었으며, 초기 시료보다 분자량 및 다분산성의 감소를 나타내었다.

이와 같은 결과를 바탕으로, 고형 리그닌의 완전 분해와 저분자화를 동시에 유도하기 위하여 80°C 온도를 가하여 과초산 반응을 수행하였다. 과초산 제조를 위한 아세트산-과산화수소 혼합조건은 4:1, 1:1, 1:4 (v/v)로 설정하였다. 과초산 반응 결과, 가열에 의하여 고형 리그닌의 완전 액화가 이루어졌다. 한편, 초기 리그닌으로부터 유래되는 리그닌 물질의 특성을 확인하고자 용매(에틸아세테이트) 추출을 수행한 결과, 과산화수소 농도 비율이

높은 과초산 용액을 사용하였을 때에 용매 추출 분획이 점차 감소하는 것으로 나타났다. 특히, 과산화수소 농도가 높은 용액으로 과초산 반응을 수행한 경우, 분자량이 가장 낮게 나타났으나 리그닌 방향족 골격이 유지되지 않았으며, 카르복실기 구조를 다량 포함하고 있는 구조로 변환 된 것으로 나타났다. 이와 반대로, 아세트산 첨가 비율이 높은 과초산 용액으로 반응을 수행한 결과, 리그닌 구조를 유지하고 있는 저분자 물질의 생성을 관찰 할 수 있었다. 이러한 결과를 통해 리그닌으로부터 목적 화합물의 선택적 생산을 과초산 용액 정보를 통해 조절 할 수 있을 것으로 판단된다.

본 연구는 리그닌의 저분자화와 함께 균일한 구조를 나타내는 리그닌의 선택적 생산을 위한 연구를 목표로 하였다. 따라서, 리그닌 방향족 구조를 유지할 수 있는 과초산 용액(아세트산-과산화수소 4:1, (v/v) 혼합용액)을 선택하여 과초산 분해 반응을 시도한 후, 이를 컬럼 분획을 통해 분자량별 분리 가능성을 평가하고자 하였다. 액화된 리그닌을 유기용성과 수용성 분획물로 나누기 위하여 에틸아세테이트 용매로 선 추출하였으며, 이를 오픈 컬럼을 이용하여 3종의 용매(chloroform-ethyl acetate, methanol, water)로 순차 분획하였다. 그 결과, 분획에 사용된 용매의 극성이 낮은 경우 가장 낮은 분자량 범위를 나타내었다. 한편, 각 분획물에 대한 구조 분석 결과, 특정 분획물에서 리그닌 방향 구조가 분해된, 카르복실기를 다량 가지는 물질을 나타냈다. 이를 통해 컬럼 분획법을 이용하여 분자량 별 분리뿐 아니라, 구조 특성이 다른 리그닌 유래 화합물의 분리 또한 가능한 것으로 판단되었다.

본 연구에서는 리그닌의 저분자화를 위하여 과초산 용액을 직접 제조하여 분해반응에 사용하였으며, 용액 특성에 따라 최종 생성

화합물의 선택적 생산이 가능한 것으로 나타났다. 또한, 컬럼 분획의 간단한 분리법을 적용하여 분자량뿐 아니라 구조 특성이 다른 화합물의 선택적 분리가 가능한 방법을 제시할 수 있었다.

키워드 : 리그닌 탈중합, 과초산 처리, 산업리그닌, 오픈 컬럼 분획,
리그닌 유용화합물 선택적 생산

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