

SAIMAA UNIVERSITY OF APPLIED SCIENCES

Faculty of Technology, Imatra

Paper Technology

Li Jingjing

ISOLATION OF LIGNIN FROM WOOD

Bachelor's Thesis 2011

ABSTRACT

Li Jingjing

Isolation of Lignin from Wood, 57 pages, 3 appendices

Saimaa University of Applied Sciences, Imatra

Unit of Technology, Degree Programme in Paper Technology

Bachelor's Thesis, 2011

Supervisor: Guangyu Yang, D.Sc., Saimaa UAS

The purpose of this bachelor's thesis was to know well the different lignin isolation methods from various wood species and black liquor, and then the lignin obtained was identified by spectrophotometric methods which were UV and FTIR spectroscopy, to analyze and compare the physical and chemical properties of lignin, such as lignin content and color with different pH values, and their chemical structures, etc.

The experimental part of this study was performed in the laboratory of Saimaa University of Applied Sciences, Imatra. Two different methods of lignin isolation were studied: one was isolation according to different pH values from the black liquor which was obtained from four wood species (pine, spruce, birch and aspen chips) in the batch digester by Kraft process, it was called Kraft lignin; another one was isolation from sawdust of four wood species in a flask reactor with reflux condenser, it was called Klason lignin. These lignins were characterized by UV and FTIR spectroscopy.

According to the results, the lignin content and functional group were determined separately. The yields of lignin slightly increased with pH value decreasing, and the yields in softwood were higher than in hardwood. The UV absorption maximum of lignin revealed that lower pH value had a high purity level. In a comparison with Kraft and Klason lignin, they included different functional groups; there was a difference in chemical structure. In addition, the chemical structure was not similar between softwood and hardwood.

Keywords: Kraft lignin, Klason Lignin, Softwood, Hardwood, Black Liquor, Lignin Isolation Methods, UV and FTIR Spectroscopy.

CONTENTS

1 INTRODUCTION	5
2 LIGNIN FROM WOOD.....	6
2.1 Chemical composition of wood.....	6
2.1.1 Cellulose.....	7
2.1.2 Hemicellulose	7
2.1.3 Lignin.....	8
2.1.4 Extractives.....	9
2.2 The chemical structure of lignin	10
2.3 Different methods of lignin isolation.....	14
2.3.1 Isolation of lignin from wood	15
2.3.2 Isolation of lignin from black liquor	17
2.4 Application of lignin.....	20
3 ANALYTICAL METHODS OF LIGNIN	22
3.1 Klason method	22
3.2 UV spectrophotometric method	23
3.3 FTIR spectrophotometric method	24
4 EXPERIMENT PART	26
4.1 Isolation of lignin from black liquor	26
4.2 Extraction of lignin from wood	32
4.3 Identification of lignin	34
5 RESULTS AND DISSCUSSION	36
5.1 Comparisons of the different Kraft lignins.....	36
5.2 Comparisons of the different Klason lignin	39
5.3 Analysis of UV spectra of Kraft lignin.....	41

5.4 Analysis of FTIR spectra of Kraft and Klason lignin.....	44
6 SUMMARY	49
FIGURES.....	51
CHARTS.....	52
TABLES.....	52
REFERENCES	54
APPENDICES	58

1 INTRODUCTION

Lignin is one of the important chemical constituents of lignocellulosic materials in wood and it is one of the most abundant biopolymers in nature. Despite extensive investigation, the complex and irregular structure of lignin is not fully understood. The physical property and the chemical characteristics of lignin vary not only between different wood species, but also according to the method of isolation. Moreover, the molecular structure and function groups differ for the various type of lignin.

In this work, the general knowledge of lignin, e.g. chemical structure of lignin, application of lignin, the different isolation methods for lignin and identification of lignin, are reviewed. Furthermore, two different isolation methods of lignin were implemented to obtain Kraft lignin and Klason lignin in labotoary of Saimaa University of Applied Sciences. The raw materials were four wood species (pine, spruce, birch and aspen). For extraction of Kraft lignin, the black liquor after chemical cooking of woods was precipitated at various pH values by using sulfuric acid. For Klason lignin, the wood was extracted directly in accordance with TAPPI T222 Strandard.

The isolated lignin was characterized with regard to yields of lignin, UV and FTIR spectroscopic analysis. FTIR spectroscopy is a versatile, rapid, and reliable technique for lignin characterization. Using this technique, the p-hydroxyphenyl, guaiacyl, and syringyl units, methoxyl groups, carbonyl groups, and the ratio of phenolic hydroxyl to aliphatic hydroxyl groups can be determined. The UV spectroscopic method was best suited for investigating the topochemistry of lignin in wood and for determining the concentration and purity of lignin. Subsequently the experimental results were discussed from the different viewpoints, such as the effect of isolation method, operating conditions and wood species on the property and structure of lignin.

2 LIGNIN FROM WOOD

2.1 Chemical composition of wood

Wood is one of the most abundant resources in the bio-based industry and yet it is also one of the most complex materials, composed of polymers of lignin and carbohydrates that are physically and chemically bound together. Wood species can be divided into two groups: hardwood and softwood. Softwoods are gymnosperm trees, while hardwoods are angiosperm trees. (Stenius 2000.)

Wood is essentially composed of cellulose, hemicelluloses, lignin, and extractives. In different wood species, however, their relative composition varies greatly, and also the chemical composition of wood varies quantitatively among tree species. Table 2.1 shows some values that are given in the percentages of wood weight for each constituent in the different wood species.

Table 2.1 Chemical comparison of various wood species (Sjöström 1993)

Constituent (%)	Softwood		Hardwood	
	Scots Pine (<i>Pinus sylvestris</i>)	Spruce (<i>Picea glauca</i>)	Silver Birch (<i>Betula verrucosa</i>)	Eucalyptus (<i>Eucalyptus camaldulensis</i>)
Cellulose	40.0	39.5	41.0	45.0
Hemicellulose				
-Glucomannan (%)	16.0	17.2	2.3	3.1
-Glucuronoxylan (%)	8.9	10.4	27.5	14.1
-Other polysaccharides (%)	3.6	3.0	2.6	2.0
Lignin (%)	27.7	27.5	22.0	31.3
Total extractives (%)	3.5	2.1	3.0	2.8

As can be seen from the Table 2.1, the contents of cellulose and hemicelluloses are relatively higher than that of lignin in each wood species. In comparison, the content of extractives is relatively low. According to Table 2.1, which shows the four kinds of wood species, the content of lignin is in softwood slightly higher than in hardwood. (Sjöström 1993.)

2.1.1 Cellulose

Cellulose is the main constituent of wood carbohydrates. It is a polysaccharide consisting of glucose units. The cellulose molecule is linear and it easily forms hydrogen bonds with neighboring molecules (Knowpulp). The structure of cellulose molecule is shown in the Figure 2.1.

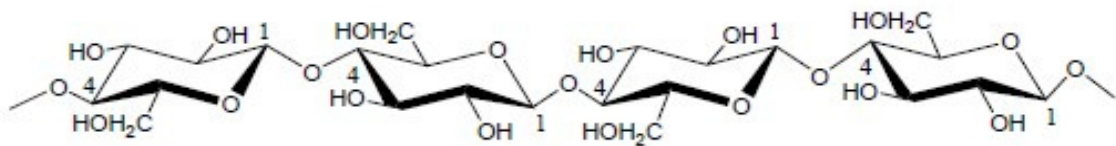


Figure 2.1 The structure of cellulose (Klemn 2005)

It can be seen from above Figure 2.1 that cellulose is a glucan polymer consisting of D-glucose linked by β -1,4-glycosidic bonds (Klemn 2005). As cellulose is an insoluble substance in most solvents including strong alkali, it is hard to separate cellulose from the wood in pure form, because cellulose is closely integrated with lignin and hemicelluloses (Pettersen 1984).

2.1.2 Hemicellulose

Hemicelluloses consist of heteropolysaccharides. The structure and composition of softwood and hardwood hemicelluloses are different. Hemicelluloses play a crucial role in the bonding capacity of fibers, i.e. the ability to form interfiber bonds, which gives the paper fiber network its strength (Knowpulp). In contrast to cellulose that is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. It is easily hydrolyzed by dilute acid or base, but nature provides an arsenal of hemicellulase enzymes for its hydrolysis. (Wise L. 1962.)

2.1.3 Lignin

Lignin is a complex chemical compound and the only aromatic polymer present in wood; it is concentrated mainly in the region of the middle lamella. The amount of lignin in normal wood is 20%-35% depending on the different wood species. (Glennie & McCarthy 1962.) Lignin is bound together to the cellulose and hemicelluloses. The position of lignin within lignocellulosic matrix can be seen in the Figure 2.2.

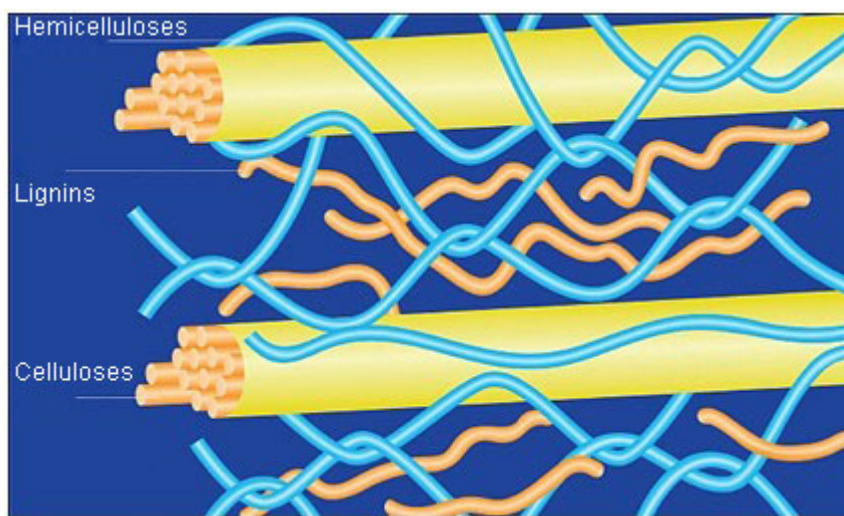


Figure 2.2 The position of lignin within lignocellulosic matrix (Kuhad & Singh 2007)

As it is illustrated in the Figure 2.2, lignocellulosic matrix is a complex structure in which the cellulose is surrounded by a monolayer of hemicellulose and embedded in a matrix of hemicellulose and lignin. Furthermore lignin specifically creates a barrier to enzymatic attack while the highly crystalline structure of cellulose is insoluble in water, then the hemicellulose and lignin create a protective sheath around the cellulose. (Stenius 2000.)

In general, lignins are roughly classified into three major groups: softwood, hardwood, and grass lignins. Besides these native lignins, which are typically separated from the wood in the form of "milled wood lignin" (MWL), "dioxane lignin", or "enzymically liberated lignin", there are several industrially based

technical lignins that are by-products of the chemical pulping. Kraft lignin (or sulfate lignin), alkali lignin (or soda lignin), and lignosulfonates are derived from Kraft, soda-AQ, and sulfite pulping of wood, respectively. (Stenius 2000.)

Most isolated lignins are brown amorphous powders. Depending on the preparation method used and on the fraction represented of the total lignin, there are some changes correspondingly in color and shape. The molecular weight, or average molecular weight, is a particularly important characteristic property of a lignin. Another important property of lignin is its capacity to absorb ultraviolet light. When the intensity of absorption is plotted against a given wavelength of ultraviolet light, an ultraviolet spectrum curve for the lignin is obtained. According to the type of lignin, the lignin solvent, and the pH of the solution and lignin structure, the shape of this curve may change. (Glennie & McCarthy 1962.)

2.1.4 Extractives

Wood contains also other components which are so-called extractives. These substances are usually soluble in one or more of the following solvents: water, ether, the alcohols, acetone, and various simple organic halides. However, the choice of solvent depends on the type of wood being examined. The solvents should be neutral, and in many cases the extractives can be recovered by evaporating the solutions to dryness. Alkaline or acid organic compounds should not be used, because they usually attack the cell wall components. In general, aqueous extraction should also be carried out with cold water, but hot water usually causes some degradation of the cell wall. (Wise L 1962)

2.2 The chemical structure of lignin

Lignin consists of complex and diverse structures. Lignin includes three primary precursors which have different proportion in softwood and hardwood lignin. Lignin precursors are linked together by different functional groups, the frequency of the linkages results in the variation of the structure in lignins.

The distribution of lignin in the cell wall

The cell wall consists of several layers which are depicted in the classical representation in Figure 2.3. The layers of the cell wall from outer to inner are as follows: middle lamella (M), the primary wall, the secondary wall (divided into the S1, S2, and S3 layers), and the hollow inner region called the lumen. The layers of the secondary wall differ based on the thickness of the cell wall layer and the microfibril angle. (Holtman 2003.)

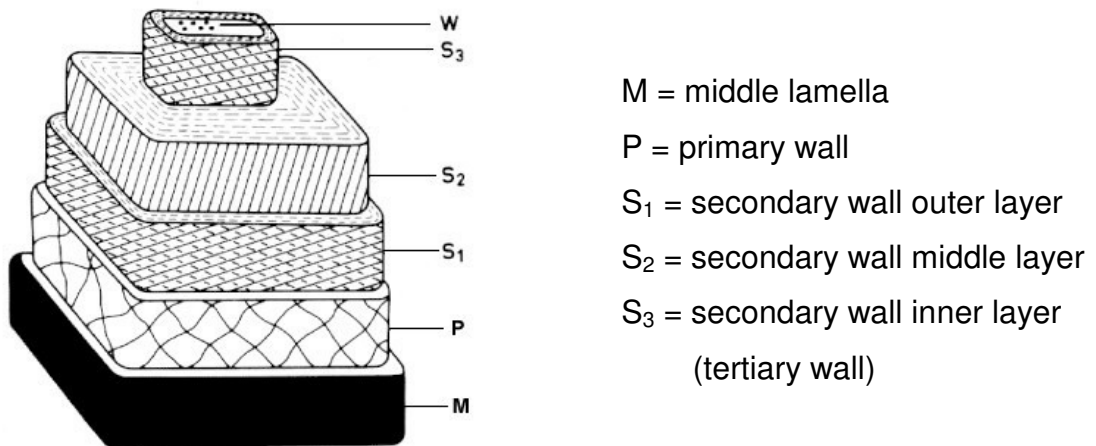


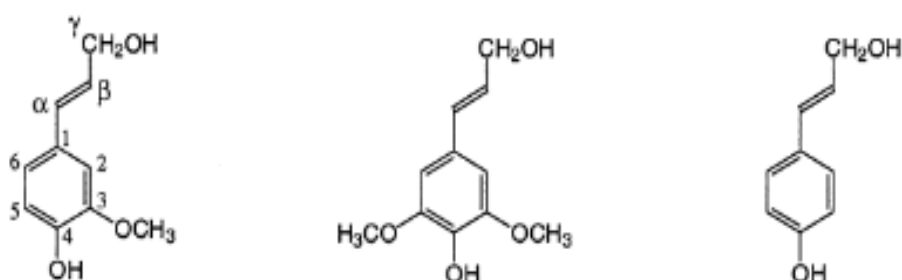
Figure 2.3 The structure of cell wall in wood (Knowpulp)

Lignin is found mainly in the middle lamella and the secondary wall. In the middle lamella the lignin content is high, but, because the layer is thin, only a minor fraction of the total lignin is located in this layer. In hardwood, the lignin concentration in the middle lamella is lower than in that of softwood. In addition, the majority of lignin is contained in the secondary wall although the relative concentration of lignin is low. (Holtman 2003.)

Precursors of lignin

Lignin is an amorphous polymer with a chemical structure that distinctly differs from the other macromolecular constituents of wood. The lignin polymer molecule is made up of a number of structural units. These units are similar in configuration and can be regarded as a common skeleton which is a phenylpropane or C₆-C₃ or C₉ type.

Lignin can be defined as a polyphenolic material arising primarily from enzymic dehydrogenative polymerization of three phenylpropanoid units, which are coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol, respectively (Stenius 2000). Chemical structures of the precursors are presented in Figure 2.4.



trans-Coniferyl alcohol *trans*-Sinapyl alcohol *trans*-*p*-Coumaryl alcohol

Figure 2.4 The structural units of lignin (Stenius 2000)

Lignin classification is traditionally done according to the precursors of the polymer. Guaiacyl lignin (G) is typical of softwood species and it is formed mostly of *trans*-coniferyl alcohol precursors, with the remainder consisting mainly of *trans*-*p*-coumaryl alcohol which contains *p*-hydroxyphenyl (H) units. In contrast, generally guaiacyl-syringyl (GS) lignins found in hardwood species, are mainly composed of *trans*-coniferyl alcohol and *trans*-sinapyl alcohol type units in varying ratios. Grass lignins are also classified as guaiacyl-syringyl lignins, although they contain some structural units derived from *trans*-*p*-coumaryl alcohol and some aromatic acid residues. (Glennie & McCarthy 1962.)

Polymerization of lignin precursors

In the polymerization process of lignin, precursors are turned into resonance-stabilized phenoxy radicals by enzymatic oxidation. Figure 2.5 shows an example of a phenoxy radical formed from coniferyl alcohol by a one – electron transfer and its resonance forms. Delocalization of the singlet electron allows covalent bonds to be formed on three different sites of the molecule. Similar radicalization occurs to lignin polymer when new precursors are added. (Freudentberg & Neish 1968.)

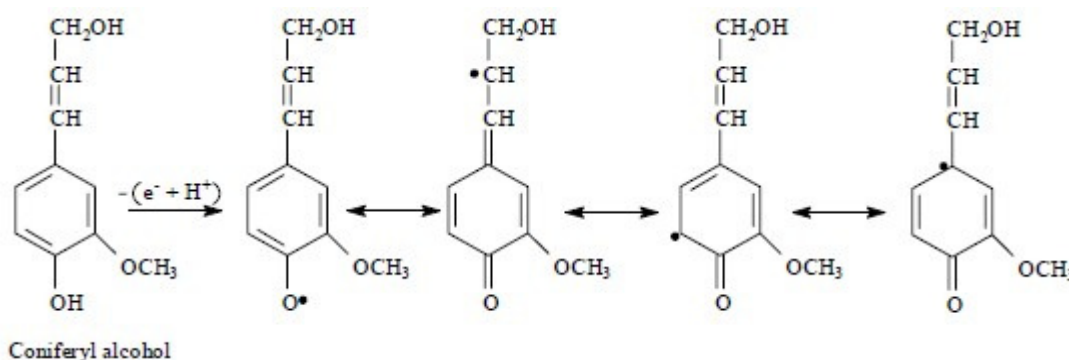


Figure 2.5 Resonance forms of the coniferyl alcohol radical (Freudentberg & Neish 1968)

Lignin precursors are linked together with ether linkages (C-O-C) and with carbon-carbon (C-C) linkages. Carbon-carbon linkages are considered as condensed linkages, whereas ether linkages are non-condensed. Ether-type of linkages is most common and approximately two thirds of bonds between precursors are of ether-type, the rest are of the carbon-to-carbon type. (Harkin 1969.)

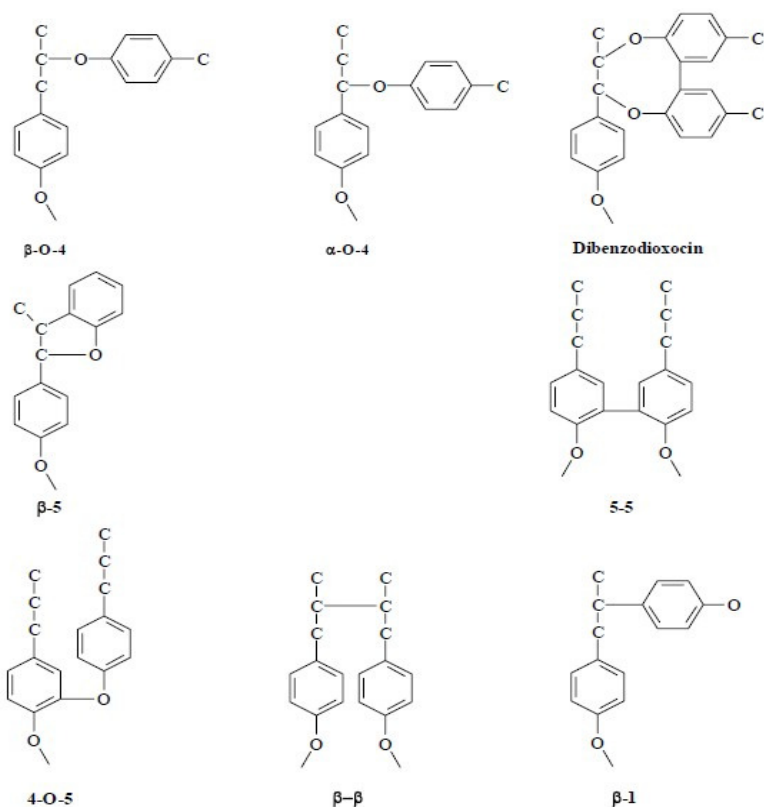


Figure 2.6 The common phenylpropane linkages in lignin (Froass 1996)

Figure 2.6 shows the common linkages between phenylpropane in lignin. The dominant linkage is the β -O-4 linkage, and then, some new linkages were discovered in lignin. The percent of linkages in lignin has been determined and is shown in Table 2.2.

Table 2.2 Proportions of most common linkages in lignin (Froass 1996)

Linkage Type	Dimer Structure	Percent of Total Linkages (%)
β -O-4	Phenylpropane β -aryl ether	45-85
5-5	Biphenyl and Dibenzodioxocin	4-25
β -5	Phenylcoumaran	9-12
β -1	1,2-Diaryl propane	7-10
α -O-4	Phenylpropane α -aryl ether	6-8
4-O-5	Diaryl ether	4-8
β - β	β - β -linked structures	3

The variation of the structure in lignins also comes from the frequency of the linkages in lignin macromolecules. These three units which are *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) in lignin tremendously change the frequency

of the linkages. Hardwood lignin contains relatively more β -O-4 and less 5-5 and β -5 linkages than softwood lignin, though generally the most abundant linkage in lignin is β -O-4. The frequency of a β -O-4 linkage is approximately 45-50% of the phenylpropane units in softwood lignin, while approximately 60-85% phenylpropane units in hardwood lignin. (Chen 1991.)

Lignin polymer contains methoxyl groups, phenolic hydroxyl groups, benzyl alcohol groups and carbonyl groups and some terminal aldehyde groups in the side chain. The same functional groups that are present in the lignin polymer are also present in the lignin precursors (Pearl 1967). There is considerable variation in the distribution of functional groups among different wood species. Therefore only approximate values for the frequencies of different functional groups can be given (Table 2.3).

Table 2.3 Functional groups of softwood and hardwood lignin (Stenius 2000)

Functional group	Softwood lignin	Hardwood lignin
Phenolic hydroxyl	20-30	10-20
Aliphatic hydroxyl	115-120	110-115
Methoxyl	90-95	140-160
Carbonyl	20	15

2.3 Different methods of lignin isolation

Lignin can be isolated from various raw materials, i.e. wood and black liquor. There are several methods for lignin isolation from wood, generally, where lignin is isolated either by removing non-lignin or lignin components. Moreover, carbon dioxide or sulfuric acid is used to isolate lignin from black liquor.

2.3.1 Isolation of lignin from wood

Lignins probably exist in wood as branched-chain polymer molecules which may comprise an almost infinite network, and this network may be integrated and chemically combined with hemicelluloses or other nonlignin components of wood. In this state, lignin will here be called protolignin. Broadly speaking, lignin may be separated from associated wood components either by preferentially dissolving lignin or by preferentially dissolving nonlignin components. Therefore, there are some methods for isolation of lignin from wood. (Glennie & McCarthy 1962.)

Removing non-lignin components

Klason Lignin (sulfuric acid lignin): Wood meal is extracted with alcohol-benzene which is employed to remove materials, such as waxes, fats, some resins, and possibly some portions of wood gums, then stirred at room temperature and hydrolysis with 64 to 75% sulfuric acid. The Klason lignin is obtained after removing the polysaccharides, and refluxed with dilute acid; then the Klason lignin or sulfuric acid lignin is filtered, dried, and weighed. This procedure serves as a method for determination of lignin in wood and other plant materials. But this method is able to change the structure of lignin during the hydrolysis. (López et al. 2010)

Willstätter Lignin: wood meal is extracted and hydrolyzed with concentrated hydrochloric acid, and produces an insoluble lignin residue, this is so-called Willstätter lignin.

Periodate Lignin: mild oxidation of extracted wood meal with periodic acid (HIO_3) dissolved nonlignin components by hot-water hydrolysis, there is less alteration in lignin structure. Degraded carbohydrates are dissolved, finally obtain an

insoluble Periodate or Purves lignin.

Cuproxam Lignin: substantially all carbohydrate components in extracted wood meal may be dissolved with cuprammonium hydroxide with alternate dilute acid hydrolysis, and this is the basis for preparation of Freudenberg or Cuproxam lignin (Glennie & McCarthy 1962).

Removing lignin components

Brauns or Native Lignin (BNL): Fresh wood meal is extracted with cold water, then with ether for 48 hours, and finally with ethanol at room temperature for 8 to 10 days. The solution of lignin in ethanol is then purified by solvent precipitation until the methoxyl content is constant, resulting in a lignin which in yield is only a few per cent based on lignin content of the wood. This method causes the structural changes as minimally as possible. Without removing extractives from wood meal before ethanol extraction, Brauns lignin consists of some impurities, such as carbohydrates and extractive components. Compared to other preparations, such as Milled Wood lignin, Brauns lignin from conifers is characterized by similar elemental compositions, low molecular weight lignin, large amounts of ester groups and higher phenolic hydroxyl content. (Lai & Sarkanen 1971.)

Milled Wood Lignin (MWL): Björkman developed an isolation procedure to extract a larger proportion of lignin from wood. According to Björkman, when extractive-free wood meal of a woody species is ground for 48 hr or more in a vibratory ball mill under nitrogen atmosphere. Wood-meal particles are reduced in size in a vibrational ball mill in the presence of an organic solvent with a non-swelling agent, such as aqueous dioxane. During ball mill the cell structure of the wood is destroyed. The dissolved lignin is purified by solvent precipitation, and as much as 50% of the total lignin can be obtained as an almost white powder. This lignin preparation is known as milled wood lignin (MWL) or Bjorkman lignin. At the same time, MWL preparation always contains some carbohydrate material.

Moreover, Ball milling affects the yield and chemical structure of MWL. (Hu 2006.)

Cellulolytic Enzyme Lignin (CEL): In order to improve the yield of lignin isolated from ball-milled wood, the cellulolytic enzymes are used. Cellulolytic enzymes are used to remove carbohydrates prior to aqueous dioxane extraction of ball-milled wood meal. Cellulolytic enzyme lignin (CEL) was found and it is structurally similar to MWL. This method results in original structure essentially unchanged of cellulolytic enzyme lignin. (Glennie & McCarthy 1962.)

2.3.2 Isolation of lignin from black liquor

Black liquor is generated in the cooking process as the white liquor dissolves the lignin and other organic compounds in the wood. Kraft or Sulfate cooking is the most commonly used pulp production method. Kraft process uses white liquor containing mainly the active chemicals, a mixture of sodium hydroxide (NaOH) and sodium sulfide (Na₂S) as the main cooking chemicals. The sulfite process is characterized by its high flexibility compared to the Kraft process, which is a very uniform method, which can be carried out only with highly alkaline cooking liquor. The sulfite cooking process is based on the use of aqueous sulphur dioxide (SO₂) and a base - calcium, sodium, magnesium or ammonium. (Knowpulp.) Therefore, two kinds of black liquor from Kraft and sulfite process are acidified to generate lignin, which are called Kraft lignin and Lignosulfonate respectively.

Composition of black liquor

Black liquor contains water, organic residue from pulping, and inorganic cooking chemicals. The primary organic compounds are lignin, polysaccharides, carboxylic acids, and extractives, the main inorganic substances in black liquor are Na₂CO₃, Na₂SO₄, Na₂S, Na₂S₂O₃, NaOH and NaCl. The organic material dissolved from wood is approximately 60% of the total black liquor dry solids

(Pettersen 1984, Sjöström 1993). Typical content of spent liquor from various cooking conditions are listed in Table 2.4.

Table 2.4 Typical composition of the black liquor from various cooking condition (Sjöström 1993)

Components	Content (% of dry solid)	
	Kraft liquor	Sulfite liquor
Lignin	39-54	55
Degraded carbohydrates	25-35	28
Extractives	3-5	4
Inorganic components	18-25	13

Black color comes from lignin compounds colored by alkali and dissolved to liquor. There are often large differences between industrial and laboratory liquors, for example industrial black liquor contains minor amounts of sulphate and carbonate as residues from the recovery and more degraded lignin due to several reuses of the black liquors in impregnations of chips to recover heat. There are also differences between different industrial black liquors due to different cooking strategies and variations in equipment as well as raw material. (Sjöström 1993.)

Isolation of Kraft lignin

Normally, isolation of lignin is a by-product of the pulp and paper industry. Lignosulfonate derived from sulfite pulping of wood, and Kraft lignin derived from Kraft pulping of wood are the principal commercially available lignin types.

Kraft lignins, also called sulfate or alkali lignins, are obtained from black liquor by precipitation with acid. Generally, acidification is conducted in two steps. In the first step, carbon dioxide from the waste gases of boiler fires or from lime kilns is used to reduce the pH of the liquor. About three quarters of the lignin is precipitated in this step as a sodium salt. After isolation, the material obtained is refined by washing. By suspending the salt in water and minimizing the pH with

sulfuric acid, refined lignin is obtained. The procedure of lignin precipitation is shown in the Figure 2.7. (Tamminen 1995.)

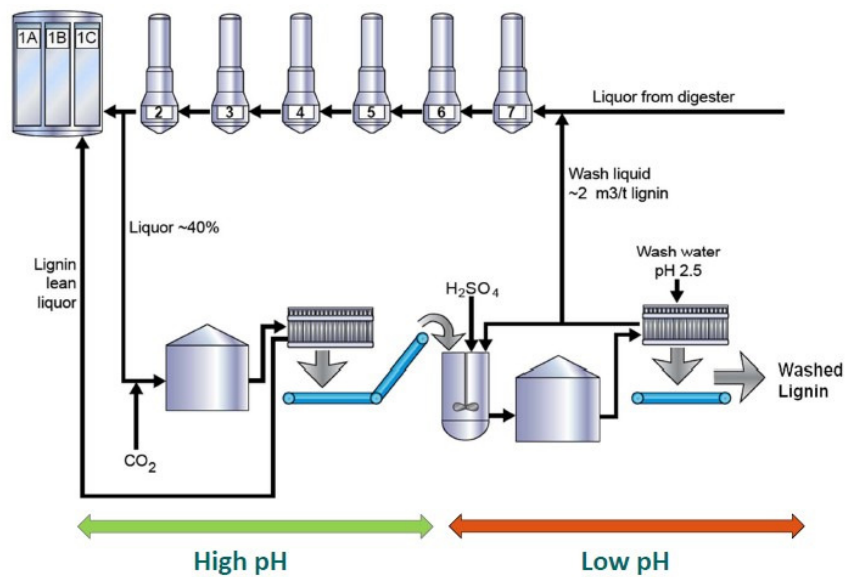
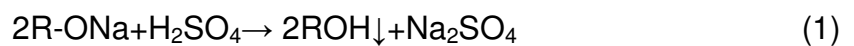


Figure 2.7 Lignin isolation process (TAPPI 2010)

As it is illustrated in the Figure 2.7, in the cooking process, ether bonds break due to the function of caustic soda, and then lignin macromolecules degrade gradually in the form of alkali lignin or lignin sodium salt R-OH. When lignin totally dissolves in the black liquor, it presents hydrophilic gel. Afterwards, electrophilic substitution reaction happens when the black liquor is neutralized by acid; it means that hydrogen ion instead of sodium ion in alkali lignin and hydrophilic gel of alkali lignin is destroyed. Finally, the lignin is precipitated from the black liquor, namely it is called Kraft lignin which is difficult to dissolve in water.



At the same time, the recovery is also an essential part. Water is removed from black liquor in evaporation plant and then black liquor go to the recovery boiler to burned, through a series of chemical reaction, form a circulation process, the cooking chemicals can be recycled, regenerated as well as reused in the cooking process. (Knowpulp.)

Isolation of lignosulfonate

Lignosulfonates, also called lignin sulfonates and sulfite lignins, are derived from the sulfite pulping of wood. Typical compositions for hardwood and softwood spent sulfite liquors are given in Table 2.5.

Table 2.5 Compositions of Spent Sulfite Liquors (John Wiley & Sons, Inc)

Component	Percentage of total solids	
	Softwood	Hardwood
Lignosulfonate	55	42
Hexose sugars	14	5
Pentose sugars	6	20
Noncellulosic carbohydrates	8	11
Acetic and formic acids	4	9
Resin and extractives	2	1
Ash	10	10

There are various methods for isolating and purifying lignosulfonates from spent pulping liquors. For instance, the Howard process is one of most widely used industrial processes, where calcium lignosulfonates are precipitated from spent pulping liquor by addition of excess lime. In addition, other methods used industrially include ultrafiltration and ion exclusion, which uses ion-exchange resins to separate lignin from sugars. Laboratory methods for isolating lignosulfonates include dialysis, electrodialysis, ion exclusion, precipitation in alcohol, and extraction with amines. (John Wiley & Sons, Inc)

2.4 Application of lignin

Natural lignin is a colorless or pale yellow. But when it met acid, alkali treatment, the color changes the brown or dark brown. From the lignin structure, it has a non-polar aromatic ring side chain and polar sulfonic acid group, etc., therefore, it is lipophilic and hydrophilic. Lignin is used as a cement water reducer, cement

grinding aids, bitumen emulsion, drilling mud regulator, plugging agent, viscosity breaking agent, surfactant and dye dispersant. Lignin is a kind of natural polymer, it has bonding itself, and then through the phenol, aldehyde or other method of modification, the bonding will be better. Therefore, it can be used as rubber intensifier, polyolefin and rubber packing. New applications of lignin are in composite materials. In the unsaturated polyester and vinyl ester, it is for filler and comonomer. Lignin has natural affinity for cellulose, it can deal with natural hemp fiber surface. At the same time, the bond strength between resin and fiber is increasing. In addition, lignin molecular structure contains a variety of active groups, so it can apply in the agroforestry. After degradation slowly by microorganisms in the soil, it can be converted into humus; it has certain inhibition for urease activity, promoting the growth of plants, improving soil conditions. (Chen 1991.)

Moreover, the Kraft lignin and lignosulfonate can be as the industrial application. Kraft lignins are used in some foam fire extinguishers to stabilize the foam and in printing inks for high speed rotary presses (John Wiley & Sons, Inc). Kraft lignin products are generally used in high value applications. In many applications, the base lignin must be modified prior to use. Once modified, Kraft lignins can be used in most of the same applications in which lignosulfonates are used. These include usage as emulsifying agents/emulsion stabilizers, as sequestering agents as pesticide dispersants, as dye dispersants, as additives in alkaline cleaning formulations, as complexing agents in micronutrient formulations, as flocculants, and as extenders for phenolic adhesives. In addition, Kraft lignins can also be used as an extender/modifier, and as a reinforcement pigment in rubber compounding. (John Wiley & Sons, Inc.)

One major application of lignosulfonates is for mud viscosity control during deep oil well drilling. There are also some applications in metallurgy. Specific mineral dispersing and depressant effects plus sequestering power make lignosulfonates effective in slime control and improving separation during tabling or flotation of ores. Lignosulfonates are also included in some adhesives. They act as extenders for the phenolic resins used in manufacturing particleboard, nonwoven fiber padding, and molding powders. (John Wiley & Sons, Inc.)

3 ANALYTICAL METHODS OF LIGNIN

There are several standard methods for determination of the total amount of lignin in wood and pulp samples. Lignin content in wood is determined by direct or indirect methods. The direct method includes measurement of acid-insoluble (ie, Klason) lignin, such as Klason method. In contrast to the direct determination of lignin content, indirect methods do not involve the isolation of a lignin residue, these include spectrophotometric methods. (John Wiley & Sons.) Furthermore, UV and infrared spectroscopy are useful techniques for the identification, determination and characterization of analytical and technical lignins and lignin derivatives.

3.1 Klason method

Klason method is based on hydrolysis and solubilization of cellulose and hemicelluloses from the extracted wood or pulp samples with 72% sulfuric acid, final hydrolysis is made with 3% sulfuric acid, and then acid-insoluble (ie, Klason) lignin is washed, dried, and measured (Stenius 2000). For instance, the Klason lignin contents of representative lignified materials are shown in Table 3.1 (John Wiley & Sons).

Table 3.1 The Klason lignin contents of lignified materials (John Wiley & Sons)

Material	Klason lignin, %
Softwood	26-28.8
Hardwood	22-30

From the above table, the figures show clearly that softwoods contain about 26-28.8% lignins, and the lignin present in hardwoods is about 22-30%. Compared with Table 2.1, the contents of lignin are relatively similar, while the contents of lignin are higher in softwood than in hardwood too. Therefore, results indicate

that this method is feasible.

3.2 UV spectrophotometric method

Ultraviolet (uv) spectrophotometric method is best suited for determining the concentration and chemical structure of lignin in wood or black liquor. Instrument is used for quantitative analysis and it is not used for identification of compounds, because many compounds absorb at same wavelengths. In the qualitative and quantitative UV spectroscopic determination of lignin the typical maximum at a wavelength of 280nm is mostly used. Because the lignin molecule contains no large portion of unsaturated aliphatic units in addition to its aromatic structure, it is concluded that there are the two characteristic bands in the lignin spectrum at 200-230 and 260-280 nm. (Jahan & Mun 2007) However, due to common acidic degradation products of carbohydrates which have absorbance maximums near 280nm, the measurement wavelength at 205nm is the better choice. The absorbance of UV spectra is directly proportional to the purity level of lignin. A lower absorbance is due to the co-precipitation of non-lignin material such as polysaccharides degradation product, wax, and lipids. A number of spectral methods for determining lignin content are based on totally dissolving the sample in a suitable solvent and measuring the UV absorbance of the solution. (Stenius 2000)

The essential parts of a spectrophotometer include light source, monochromator, sample and detector. Light source can be one lamp or two lamps in a spectrophotometer. When a single lamp is used, it covers the whole wavelength range. If there are two lamps, one is for UV wavelength range and another one is for visible light range. The sample background can be subtracted with two different methods, single beam instruments and double beam instruments. There are a spectrometer and a photometer in a spectrophotometer. With a spectrometer the measuring wavelength of light is chosen. This is done with a monochromator. A photometer measures the intensity of light. This is done with

a detector.

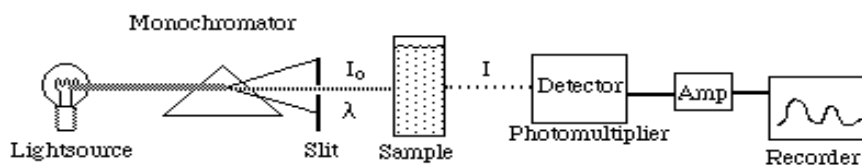


Figure 3.1 Parts of single beam spectrophotometer (Skoog et al. 2007)

Figure 3.1 gives a clear picture of operation UV spectrophotometer. The light source emits light spectrum. A measurement wavelength which is led to the sample is chosen by a monochromator. The light passes through the sample and the sample absorbs the light. A detector measures the intensity of absorbance and converts light into electricity, a photomultiplier is often used. In photomultiplier, a light photon removes photoelectrons from photocathode. Every photoelectron removes photoelectrons from next dynode and an electric current is formed. Finally the current is transformed to a voltage with an anode. The current is amplified before the measurement. (Skoog et al. 2007.)

3.3 FTIR spectrophotometric method

Fourier Transform Infrared is a versatile and rapid technique for identification and determining lignin content. Typical bands are found at about 1500 and 1600 cm^{-1} and between 1470 and 1460 cm^{-1} (Wegener et al. 1983). FTIR can give information on the lignin type, methoxyl groups, carbonyl groups, and hydroxyl groups. FTIR spectra can be obtained directly on solid samples such as wood, pulp, and paper by attenuated total reflectance (ATR), diffuse reflectance (DRIFT), and photoacoustic (PAS) techniques (Stenius, P 2000).

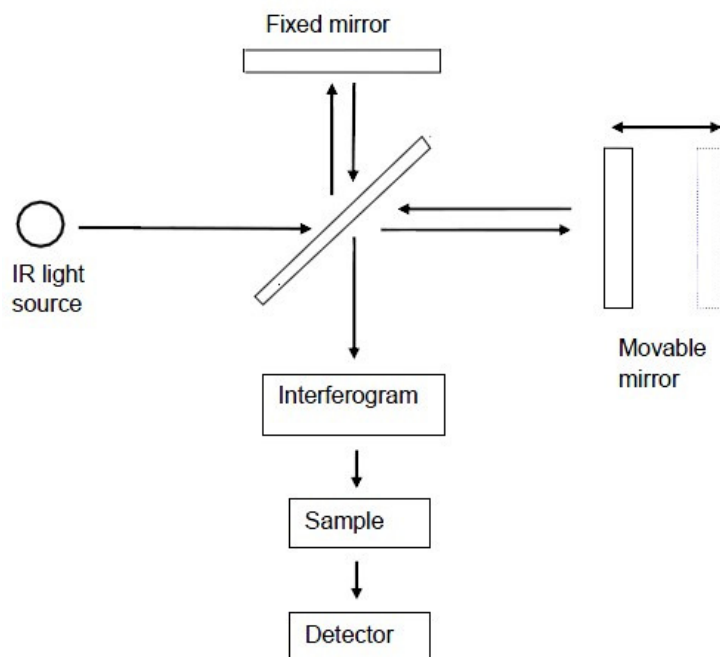


Figure 3.2 Operating principle of FTIR spectrometer (Griffiths & de Hasseth 2007)

The basic components of an FTIR are shown schematically in Figure 3.2. The FTIR is a method of obtaining infrared spectra by first collecting an interferogram of a sample signal using an interferometer. The interferometer consists of a beam splitter, a fixed mirror, and a mirror that translates back and forth; radiation from the source strikes the beam splitter and separates into two beams. One beam is transmitted through the beam splitter to the fixed mirror and the second is reflected off the beam splitter to the moving mirror. The fixed and moving mirrors reflect the radiation back to the beam splitter. Again, half of this reflected radiation is transmitted and half is reflected at the beam splitter, resulting in one beam passing to the detector and the second back to the source. Then a Fourier Transform (FT) is performed on the interferogram to obtain the spectrum. An FTIR Spectrometer collects and digitizes the interferogram, performs the FT function, and displays the spectrum. (Griffiths & de Hasseth 2007.)

4 EXPERIMENT PART

In my experiment, two kinds of lignin were isolated in Saimaa University of Applied Sciences' laboratory, i.e. isolation of Kraft lignin from black liquor and isolation of Klason lignin from wood. Softwood and hardwood were prepared as raw materials. The isolation procedures were performed based on TAPPI Standard.

4.1 Isolation of lignin from black liquor

Equipment

Batch cooking and extraction of Kraft lignin were carried out in a laboratory at Saimaa University of Applied Sciences, Imatra. Batch digester was used as equipment of batch cooking. The equipment is shown in the Figure 4.1.



Figure 4.1 Batch digester for experiment of batch cooking

The batch digester is a pressure vessel, the size of digester is 0.010 m³ (10 litres). In addition, in order to control the cooking temperature and cooking time, the values were adjusted on the control panel. Liquid was circulated between heat exchanger and digester tank continuously. The system can reach maximum temperature of up to 170 °C and stand up to 20 bars pressure.

Raw material

Raw materials for the experiments were chips of birch, pine, spruce and aspen, which were available from Saimaa University of Applied Sciences' laboratory. The dry content of each wood type was measured, which was 67%, 63%, 57% and 93% for birch, pine, spruce and aspen respectively. Chips were screened in Gyratory screen which is shown in the Figure 4.2.



Figure 4.2 Gyratory screen

The purpose of Gyratory screen was to obtain the chips with length of 19mm-25mm, and no barks or knots. The even distribution of chip size can improve the quality of cooking, and also improve defibration speed. The white liquor used in the cooking process came from Stora Enso, Kaukopää mill, Imatra. Moreover, White liquor was used for the cooking; it consisted of a mixture of sodium hydroxide (NaOH) and sodium sulfide (Na₂S).

Batch cooking method and conditions

The cooking was done in alkali conditions as white liquor was used in cooking process. The amount of white liquor required was calculated. The active alkali was 136 [gNaOH /l] of white liquor used for the cooking process and sulfidity was 35%.

For each cooking, about 900 g of oven-dry screened chips were used. First of all, the chips were fed into the digester tank, and then white liquor with a measured volume was poured carefully in to digester tank. In addition to these, the certain amount of water was added according to liquid-to-wood ratio of 4:1. One had to make sure that sealing surfaces were clean and dry without damage, and that the deckle had been closed. Afterwards, the most significant thing was that the valves of cold cooling water had to be opened for the circulation of pump and the circulation of liquor in the pipe.

Then heat exchanger of cooking system was turned on. In the first stage, the temperature inside the cooker fast increased from about 20°C to 80°C. And then it took 90 minutes to raise the temperature of cooking liquor from 80°C to 170 °C. In the secondary stage, the temperature inside cooker increased quite slowly, which was about 1°C /min. Then the chips were homogeneously cooked, cooking time was calculated according to H-factor for various wood species, and thus it took around 70-90 minutes to reach the temperature of 170 °C. Batch cooking conditions are shown in Figure 4.3 and Table 4.1.

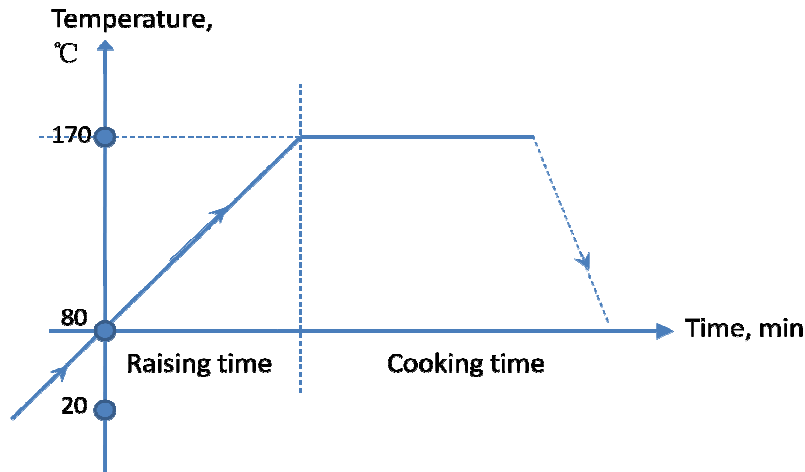


Figure 4.3 Batch cooking process

Table 4.1 The raising time and cooking time for various wood species.

	Raising time (min)	Cooking time (min)	H-factor
Birch	90	67	1200
Pine	90	87	1500
Spruce	90	87	1500
Aspen	90	87	1500

When the cooking was finished, the heating was turned off. The heat exchanger was cooled by opening the valve of cooling water. When the pressure was one bar or lower, the temperature decreased to about 80°C, warm water was injected to digester tank for wash. After washing the digester for 15 minutes, the cooked chips were taken out, and the digester was further washed finally. (Knowpulp)

Treatment of black liquor

After each cook, about 1 liter of black liquor was obtained. The pH value of black liquor was measured by indicator paper, and the value was about 12-13. Black liquor of various wood species is shown in Figure 4.4.

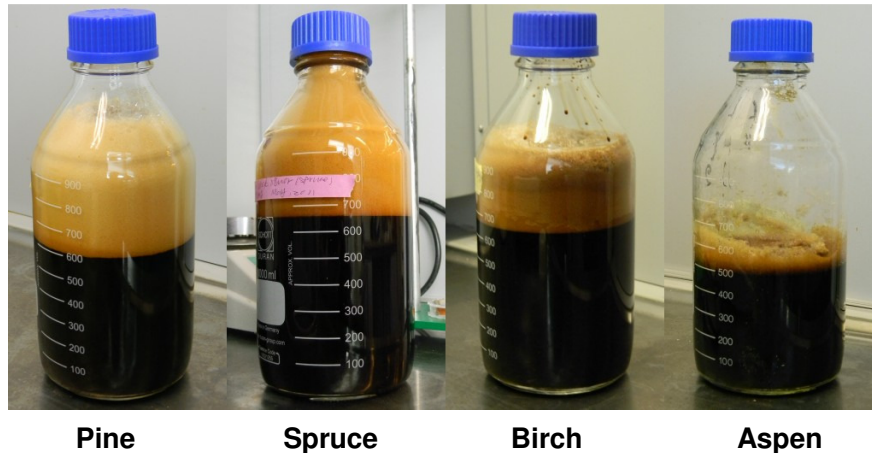


Figure 4.4 The black liquor of various wood species

It can be seen from Figure 4.4 that the black liquors were black and had a strong odour. There was a number of tall oil soap on the surface of the black liquor. Tall oil soap was a mixture of the sodium salts of rosin acids, fatty acids and neutrals that separated from Kraft black liquor. The tall oil soap can be recovered to improve evaporator operation, reduce effluent toxicity, and improve recovery boiler operation, etc. (Drew & Propst 1981.)

Properties of black liquor

Black liquor is generated in the cooking process as the white liquor dissolves the lignin and other organic compounds in the wood. The most important physical properties of black liquor are density, viscosity, thermal conductivity, specific heat, and surface tension. Some waste liquor properties, such as dry solids content and temperature, give a sufficiently exact basis for the determination of the physical properties of black liquor. Black liquor density decreases with increasing temperature. The interdependence between dry solids content and evaporation temperature is almost linear.

The kinematic viscosity of black liquor depends on its dry solids content, temperature, and composition. Viscosity increases sharply at over 30% dry solids content. If black liquor is stored at a high temperature, its viscosity decreases due to the cracking of polymers. Heat conductivity decreases with an increasing dry solids content and increases with an increasing temperature.

Apart from viscosity and density, surface tension is the most important factor in the assessment of drop formation in black liquor firing.

The specific heat of black liquor increases with temperature. At increasing dry solids content, there is a drastic decrease in specific heat. The specific heat calculated per volumetric unit is almost constant, the dry solid content being 10-60% and the temperature 50-120°C. (Gullichsen & Fogelholm 2000)

When the black liquor was acidified by adding strong mineral acid, such as 20% (v/v) sulfuric acid, Kraft lignin was precipitated from black liquor. For each black liquor, Kraft lignin was precipitated by acidifying it to pH 2-3, 4 and 7 respectively and titration process is shown in Figure 4.5. The reaction inside black liquor was much stronger and the yield of Kraft lignin increased with the pH value decreased. During the precipitation process, the odour emitted from the black liquor. Then Kraft lignin precipitates were separated from the liquor by filtration. The filtration of the lignin precipitate was improved at elevated temperatures (about 40°C), because of aggregation to a tighter and a less hydrated form. At the same time, lignins were washed by water for several times. Finally, lignins were dried in the vacuum oven, the temperature was not allowed to exceed 40°C.

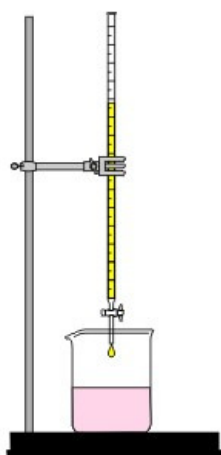


Figure 4.5 Titration

4.2 Extraction of lignin from wood

Lignin isolation from wood was divided into two steps according to TAPPI Standard, which was preparation of extractive-free wood and procedure of isolating lignin. Finally, the Klason lignin was obtained from wood.

Preparation of extractive-free wood

Raw materials for the experiments were fresh sawdusts of birch, pine, spruce and aspen. In these sawdusts, the pine came from UPM paper mill, the rest of them were obtained from logs by the equipment at the laboratory of Saimaa University of Applied Sciences. The four kinds of sawdusts and equipment are shown in Figure 4.6.



Figure 4.6 Raw material

Afterwards, the sawdusts were placed to be extracted in a flask reactor with a reflux condenser (Figure 4.7). Fresh sawdusts of about 15g (40 meshes) were

extracted with 200 ml of ethanol-benzene solvent (1:2 by volume) for 6 hours, keeping the liquid stably boiling. Benzene was highly flammable liquid and toxic, so we had to use plastic gloves and avoided any contact of benzene with skin.

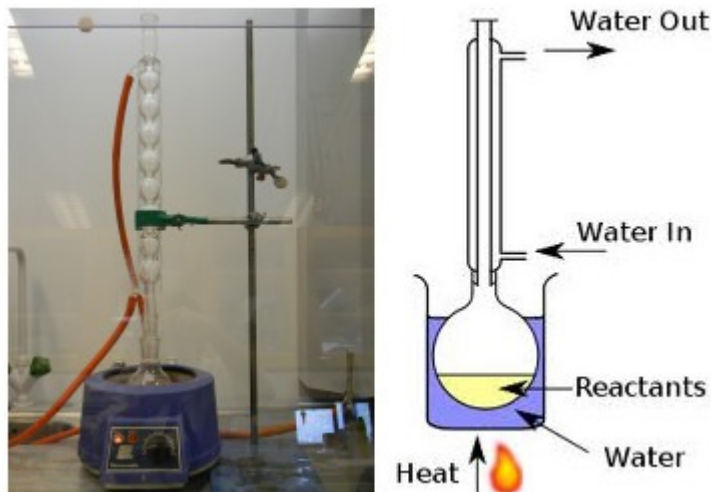


Figure 4.7 Flask reactor with Reflux condenser

After extraction with ethanol-benzene, sawdusts were transferred to a Büchner funnel, the excess solvent was removed with suction and sawdusts were washed with ethanol to remove the benzene. Then sawdusts were returned to the extraction flask and extracted with 95% ethanol for 4 hours. After that, the samples were transferred to a Büchner funnel again, the excess solvent was removed with suction, and sawdust samples were washed with distilled water to remove the ethanol. Finally, the samples were transferred to a 1000-ml Erlenmeyer flask and 500 ml of boiling distilled water was added. The flask was heated for 1 hour in the water bath at boiling temperature. After extraction, the sawdust samples were filtered on a Büchner funnel, while washed with 500 ml of boiling distilled water. Then the sawdusts were allowed to air-dry thoroughly at room temperature. (TAPPI T264)

Procedure of isolating lignin

Two parallel samples were prepared from the extractive-free sawdust, and each

sample had 1 g dry weight. The samples were placed in 100-ml beakers, and also 15ml of cold (10 to 15°C) 72% sulfuric acid was added. Sulfuric acid was added gradually in small increments while the material was stirred and macerated with a glass rod. Each beaker was kept in a bath at $20\pm 1^\circ\text{C}$ during dispersion of the material. After the samples were dispersed, each beaker was covered with a watch glass and they were kept in a bath at $20\pm 1^\circ\text{C}$ for 2 hours. The materials were stirred frequently during this time to ensure complete dissolution.

Afterwards, the solid solutions were transferred from the beakers to the two flasks separately, and about 300 ml of water was added to each flask. Then more water was added to dilute the solution to a 3% concentration of sulfuric acid. Then the solution was boiled for 4 hours by using a flask reactor with a reflux condenser. After that, the lignin was transferred to the filter and hot water was used to wash, and lignin was dried at room temperature. (TAPPI T222)

4.3 Identification of lignin

The analyse of lignin samples were carried out at Saimaa University of Applied Sciences. Ultraviolet spectroscopy (UV) and Fourier transform infrared spectroscopy (FTIR) were used for lignin identification.

For the UV analysis, a Shimadzu spectrophotometer model U-2000 (Figure 4.8) was used. Prior to the analysis, the Kraft lignin was prepared; 5 mg of oven-dry sample was dissolved into 10 ml of 90% (v/v) dioxane-water (aliquot). 1 ml of aliquot was further diluted into 25 ml by using 50% (v/v) dioxane-water.



Figure 4.8 Shimadzu UV-spectrophotometer

For the analysis of FTIR, both Kraft lignin and Klason lignin were identified and the IR spectra were recorded by using a Bruker TENSOR Series FTIR spectrometer (Figure 4.9). The sample can be measured directly with ATR technique (attenuated total reflection technique), no sample preparation was needed. The sample was kept in contact with the ATR crystal using the micrometer pressure clamp. Then acquisition time was only one minute, and IR spectra were obtained. (IBRAHIM et al. 2006.)

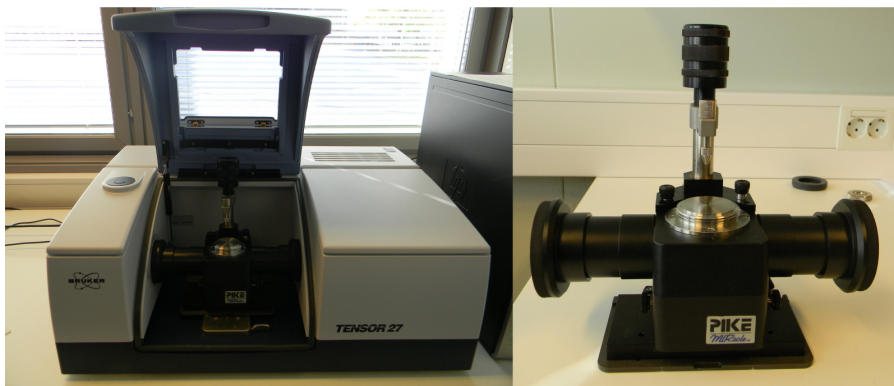


Figure 4.9 Bruker TENSOR Series FTIR spectrometer and pressure clamp

5 RESULTS AND DISCUSSION

The lignins of various wood species were compared in different isolation conditions, the results received from the experiments were illustrated in charts. Furthermore, several analytic techniques and instruments were used, such as ultraviolet-visible (UV-Vis) spectroscopy and Fourier transform infrared spectroscopy (FTIR), in order to identify and determine the lignin content, such as the yield of lignin in various pH values from black liquor, the functional group and bond linkage contained in lignin. A lot of results can be analyzed from the chart and in addition to that detail values could be found in appendices.

5.1 Comparisons of the different Kraft lignins

In general, the precipitation yield of Kraft lignin depended on several factors, for instance, final pH value of liquor and different wood species, e.g. softwood or hardwood. The comparisons of Kraft lignins from various wood species according to different pH values are shown in Figures 5.1-5.4 respectively.

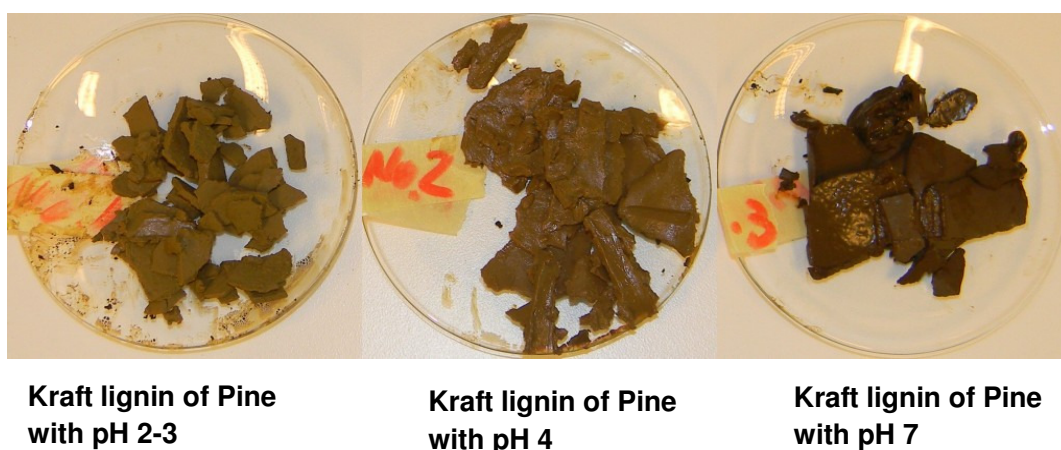


Figure 5.1 The isolation of Kraft lignin from black liquor of pine



**Kraft lignin of Spruce
with pH 2-3**

**Kraft lignin of Spruce
with pH 4**

**Kraft lignin of Spruce
with pH 7**

Figure 5.2 The isolation of Kraft lignin from black liquor of spruce



**Kraft lignin of Birch
with pH 2-3**

**Kraft lignin of Birch
with pH 4**

**Kraft lignin of Birch
with pH 7**

Figure 5.3 The isolation of Kraft lignin from black liquor of birch



**Kraft lignin of Aspen
with pH 2-3**

**Kraft lignin of Aspen
with pH 4**

**Kraft lignin of Aspen
with pH 7**

Figure 5.4 The isolation of Kraft lignin from black liquor of aspen

As can be seen from Figures 5.1-5.4, for a certain type of wood, e.g. pine, due

to different pH value, the color of Kraft lignin changed from light brown to dark brown with pH value increasing. In addition, there were obvious differences in the shape and size of Kraft lignin for softwood and hardwood. Kraft lignin of softwood formed large pieces much easier than that of hardwood, and also filtered relatively faster than that of hardwood during the precipitation process.

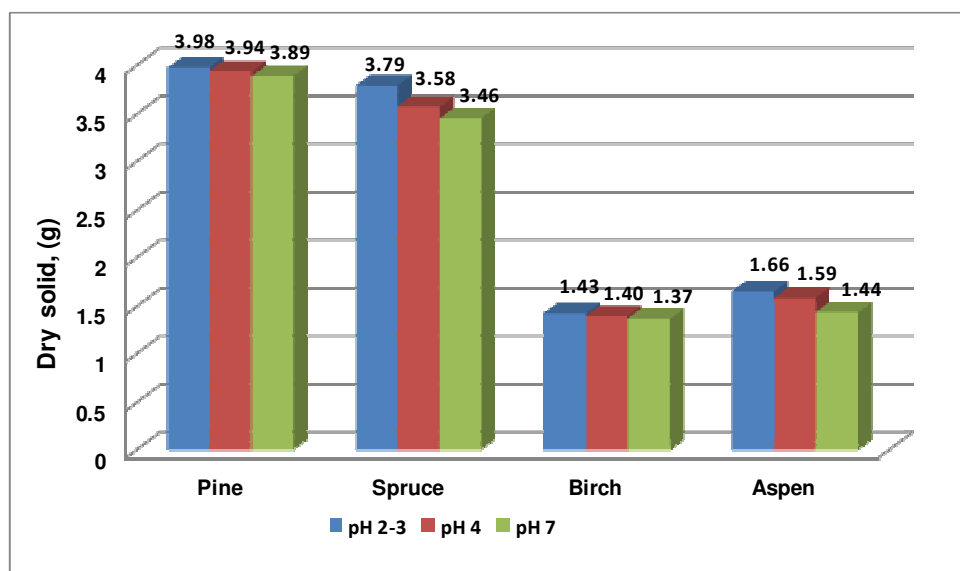


Chart 5.1 The Kraft lignin precipitation from black liquor at different pH values

The yields of Kraft lignin precipitated from black liquor of softwood and hardwood are given in Chart 5.1. The volume of black liquor used for each trial was 50 ml. According to different pH value that was 2-3, 4 and 7, the precipitation yields of Kraft lignin were relatively different. It can be seen that for all wood species, the precipitation yield of Kraft lignin increased with decreasing pH value. This is because the lignin was more soluble at high pH than low pH, and therefore more low-molecular fragments of lignin were dissolved at pH 7 than at pH 2-3 (Gellerstedt et al. 1994). On the other hand, the precipitation yield in Kraft lignin from hardwood was lower than that of Kraft lignin from softwood at the same pH value, because in softwood lignin was typically predominantly composed of guaiacyl units with a minor proportion of unmethoxylated p-hydroxyphenyl units. The typical lignin of hardwood was guaiacyl-syringyl lignin, formed from co-polymerization of coniferyl and sinapyl alcohols. (Higuchi 1985.) Lignin from softwood was more difficult to hydrolyze

because it contained a higher proportion of p-hydroxyphenyl units. The presence of methoxylated syringyl units made hardwood lignin more easily hydrolyzed during isolation process (Chiang and Funaoka 1990). Furthermore, because softwood lignin contained a lower portion of low-molecular-mass compounds as compared to hardwood lignin, these made it more difficult to release or soften the softwood lignin (Gellerstedt et al. 1994). Therefore, based on these two reasons, the yields of Kraft lignin from softwood were relatively higher than that from hardwood.

5.2 Comparisons of the different Klason lignin

To determine acid-insoluble Klason lignin content of the extractive-free sawdust, the procedure described in TAPPI T222 standard was followed. My experiment's Klason lignins extractions from the four kinds of wood species are shown in the Figure 5.5.

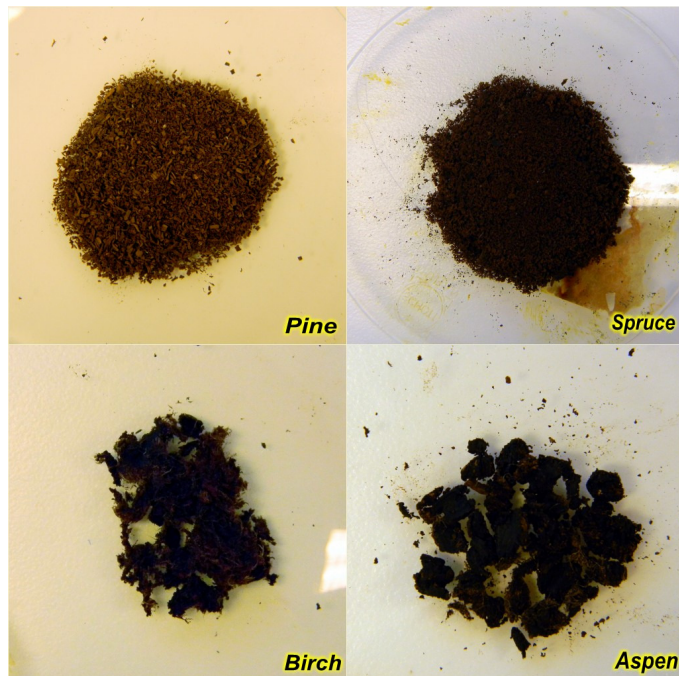


Figure 5.5 The Klason lignins of the four kinds of wood species.

As can be seen from the Figure 5.5, there were some differences in a number of aspects, such as shape, color and lignin content. By contrast between

softwood and hardwood, the color of Klason lignin in pine and spruce was yellow, whereas the color in birch and aspen was dark brown; in another aspect, the isolation of Klason lignin from softwood was much easier than hardwood during isolation process. The shape of Klason lignin had obvious differences between softwood and hardwood. Moreover, the Klason lignin was isolated from about 1g of dry weight of sawdust, then the acid-insoluble Klason lignin contents were calculated in the samples by using the following equation (TAPPI T222 om-06).

$$\text{Lignin, \%} = A \times 100 / W \quad (2)$$

Where: A = weight of lignin, g

W = oven-dry weight of extractive-free sawdust, g

According to the equation 5.1, the Klason lignin contents were calculated and shown in the Chart 5.2.

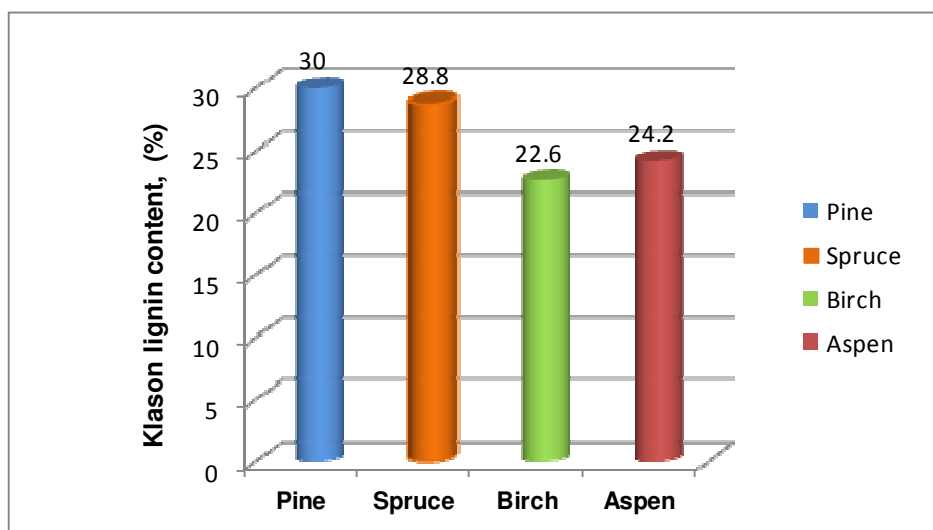


Chart 5.2 The Klason lignin contents of four kinds of wood species.

The Chart 5.2 illustrated the comparison of lignin content in the softwood and hardwood. The Klason lignin content of softwood was relatively higher than that of hardwood.

There were some similarities for isolation process between Kraft lignin and Klason lignin. Isolation of both lignins from softwood was much easier than that

from hardwood. And also the content of softwood lignins was higher than that of hardwood. This could show that the results correspond with data from literature. In addition, comparison with both lignins, the yield of Kraft lignin was obviously higher than that of Klason lignin.

5.3 Analysis of UV spectra of Kraft lignin

The UV spectra of Kraft lignin extracted from black liquor are shown in Figure 5.6. The different spectra were compared for the four wood species. For each wood species, the spectra of lignin obtained at different pH values are also shown. The figure shows that, generally, each Kraft lignin had similar peak regardless of softwood or hardwood. Two peaks were obtained in every UV spectrum of Kraft lignin, which had the absorption maximum at wavelength of 200 -210 nm and at 270-280 nm. For the softwood, e.g. pine and spruce, the spectra of lignin have certain differences at the different pH values.

For Kraft lignin obtained from pine, Fig. 5.6 shows that UV spectrum had an absorption maximum at wavelength of 205-210 nm, and the second maximum was at 279.5 nm. The appearance of two characteristic peaks in the lignin spectrum originated from non-condensed phenolic groups (aromatic ring) in lignin. (IBRAHIM et al. 2006.) Small portion of unsaturated aliphatic units contained in lignin molecule also results in peak (Jahan & Mun 2007). On the other hand, the absorbance maximum value at short wavelength was higher than that at long wavelength. Moreover, absorbance of Kraft lignin of pine has a slight variation at different pH values. The lower absorbance at high pH value (pH 7) was due to the co-precipitated of non-lignin material. The absorbance of UV spectra is directly proportional to the purity level of lignin. (IBRAHIM et al. 2006.) The UV absorbance of Kraft lignin of four wood species is shown in Table 5.1.

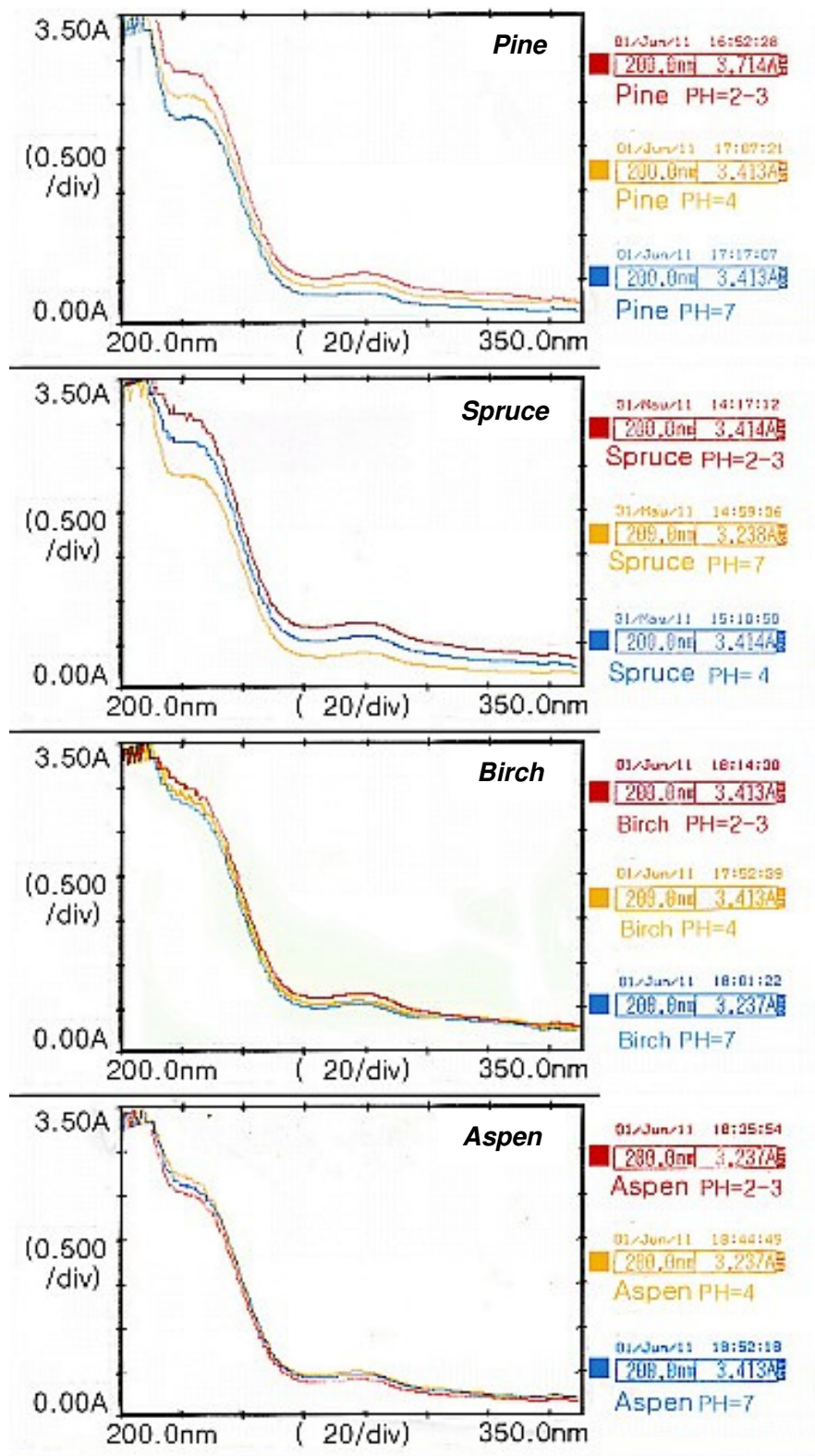


Figure 5.6 The UV spectra of Kraft lignin from black liquor of four wood species

Table 5.1 The UV absorbance of Kraft lignin of four species

Kraft Lignin	Wave length (nm)			Absorbance (A)		
	pH 2-3	pH 4	pH 7	pH 2-3	pH 4	pH 7
Pine	279.5	279.5	279.5	0.586	0.462	0.362
	209.0	208.5	206.5	3.812	3.508	3.492
Spruce	279.5	279.5	279.5	0.762	0.593	0.405
	208.5	208.5	208.0	3.809	3.538	3.504
Birch	278.5	278.5	277.5	0.657	0.585	0.538
	212.0	210.5	209.5	3.350	3.519	3.514
Aspen	278.5	278.5	276.5	0.429	0.504	0.462
	206.5	207.5	207.5	3.492	3.500	3.500

Comparison between softwood, such as pine and spruce, the Kraft lignin samples had similar UV spectra at three different pH values, however, the absorbance of Kraft lignin from spruce was a little bit higher than that from pine at each pH value as shown in Table 5.1.

Figure 5.6 shows that Kraft lignin of birch had well defined maxima at the different pH values, and the values are around 278 nm and 210 nm. The absorbance of Kraft lignin of birch is shown in Table 5.1. Considering another type of hardwood, i.e. aspen, the UV spectrum is similar compared with that of birch, however, the absorbance of Kraft lignin of birch was slightly higher than that of aspen for each pH value.

It can be summarized based on Figure 5.6 and Table 5.1, that both softwood and hardwood had similar UV spectra at different pH values. The two peaks were included in every UV spectrum, and every wavelength value was quite similar corresponding to its maximum absorbance; moreover, absorbance values were also almost the same in Kraft lignin samples. In both softwood and hardwood, there was a significant decrease in absorbance when the wavelength became gradually long. For the softwood, the spectrum has a certain difference at the different pH values, and this difference shows the purity of lignin.

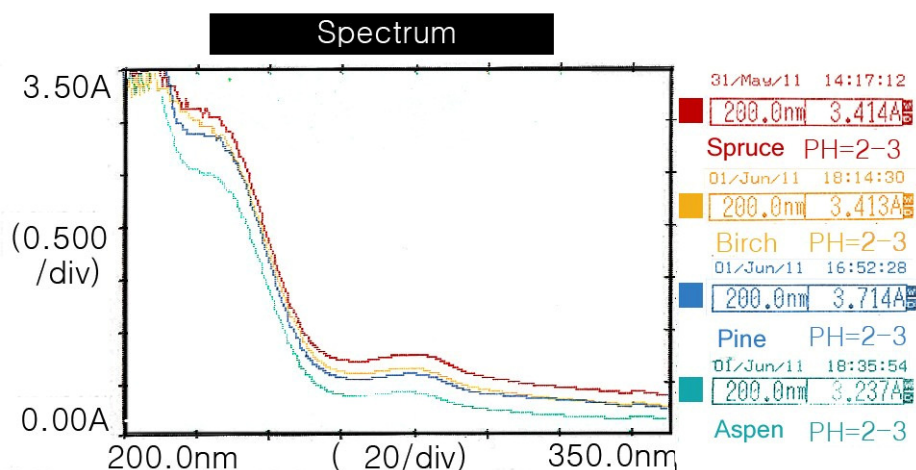


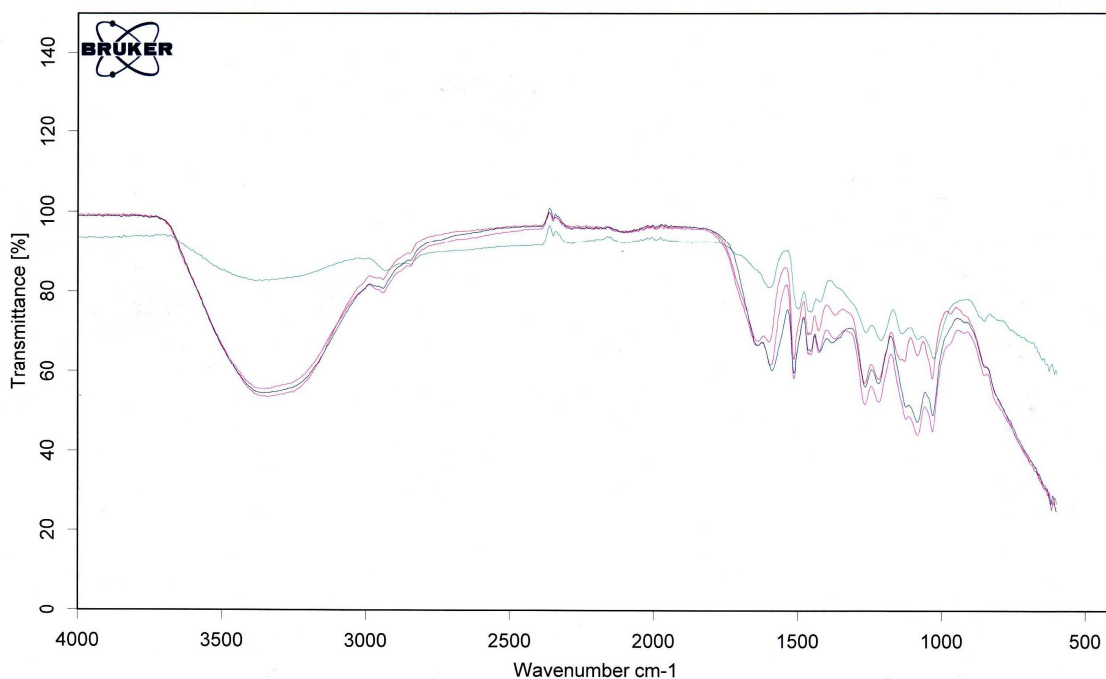
Figure 5.7 The UV spectra of Kraft lignin at pH 2-3

The UV spectra of Kraft lignin were compared at the same pH value of 2-3 for different wood species as shown in Figure 5.7. It can be seen there were significant differences for the spectra of the lignin considering the different wood species. The fluctuation of absorbance values of softwood or hardwood is quite small.

When the pH values were 4 and 7, there were similar results and phenomenon on absorbance values changing for the four species as when the pH value was 2-3. In addition, the absorbance of softwood was the same with hardwood at the same pH value. And also the absorbance values were obviously increasing with the wavelength decreasing at the same pH value for four wood species.

5.4 Analysis of FTIR spectra of Kraft and Klason lignin

In order to elucidate the structure of lignin and also to investigate the differences in the structure of the lignin, Kraft lignin and Klason lignin isolated from the four wood species were analyzed by FTIR. FTIR spectra were recorded and contained most of the characteristic absorption bands for the different chemical structures. For example, the comparison of different FTIR spectra of lignin from pine is shown in Figure 5.8.



C:\Program Files\OPUS\meas\Pine lignin (pH7).0	Pine lignin (pH7)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Pine lignin (pH4).0	Pine lignin (pH4)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Pine lignin (pH2-3).0	Pine lignin (pH2-3)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Pine Klason lignin.0	Pine Klason lignin	Instrument type and / or accessory	23/09/2011

Page 1 of 1

Figure 5.8 The IR spectra of Kraft and Klason lignin of pine

As can be seen from Figure 5.8, Kraft and Klason lignins showed almost the same FTIR spectra for pine at the different pH values. The two kinds of lignin samples indicated the presence of major peaks with corresponding functional groups, which were shown in Table 5.2.

Table 5.2 Distribution of functional group for Kraft and Klason lignin from pine (IBRAHIM et al. 2006, Ghatak 2008)

Band from FTIR	Molecular structure	Lignin
3365-3350 cm^{-1}	OH stretching vibration	Kraft lignin Klason lignin
2932-2930 cm^{-1}	C-H stretching vibration	Kraft lignin Klason lignin
1690 cm^{-1}	Conjugated carbonyl stretching	Kraft lignin

1603 and 1504 cm ⁻¹	aromatic rings	Kraft lignin Klason lignin
1460 cm ⁻¹	C-H deformation and aromatic ring vibration	Kraft lignin Klason lignin
1370 cm ⁻¹	Bending vibrations of OH bonds	Kraft lignin
1280 & 1270 cm ⁻¹	guaiacyl ring breathing with C-O stretching	Kraft lignin Klason lignin
1116 cm ⁻¹	ether stretching	Kraft lignin
1030 cm ⁻¹	C-O deformation	Kraft lignin Klason lignin
840-830 cm ⁻¹	C-H deformation and ring vibration	Kraft lignin Klason lignin

As can be seen from Table 5.2, the absorption bands with distribution of functional groups of Pine Klason lignin were similar to that of Kraft lignin. However, there were still slight differences between Kraft and Klason lignin. The absorption band of O-H stretching shifted to higher bands in Klason lignin, compared with Kraft lignin. In the intermediate and low band regions, the conjugated carbonyl stretching and bending vibrations of OH bonds were absent in Klason lignin, and 1280 cm⁻¹ can be assigned to ring breathing with C-O stretching. (Ghatak 2008.)

In the same way, the IR spectra of other wood species such as spruce, birch and aspen were similar with pine, and they are shown in Appendix 1-2. For hardwood, the absorbance near 1330 cm⁻¹ (syringyl) and 1270 cm⁻¹ (guaiacyl) was typical for hardwood lignin (Faix 1991). Further evidence of the syringyl content in birch and aspen lignin was afforded by it having a band near 835 cm⁻¹ but no band at 855 or 815 cm⁻¹, later two guaiacyl bands were typical for softwood lignin not exhibited by hardwoods (Obst J.R. 1982). The Kraft and Klason lignin for birch indicated the presence of major peaks with corresponding functional groups, which were shown in Table 5.3.

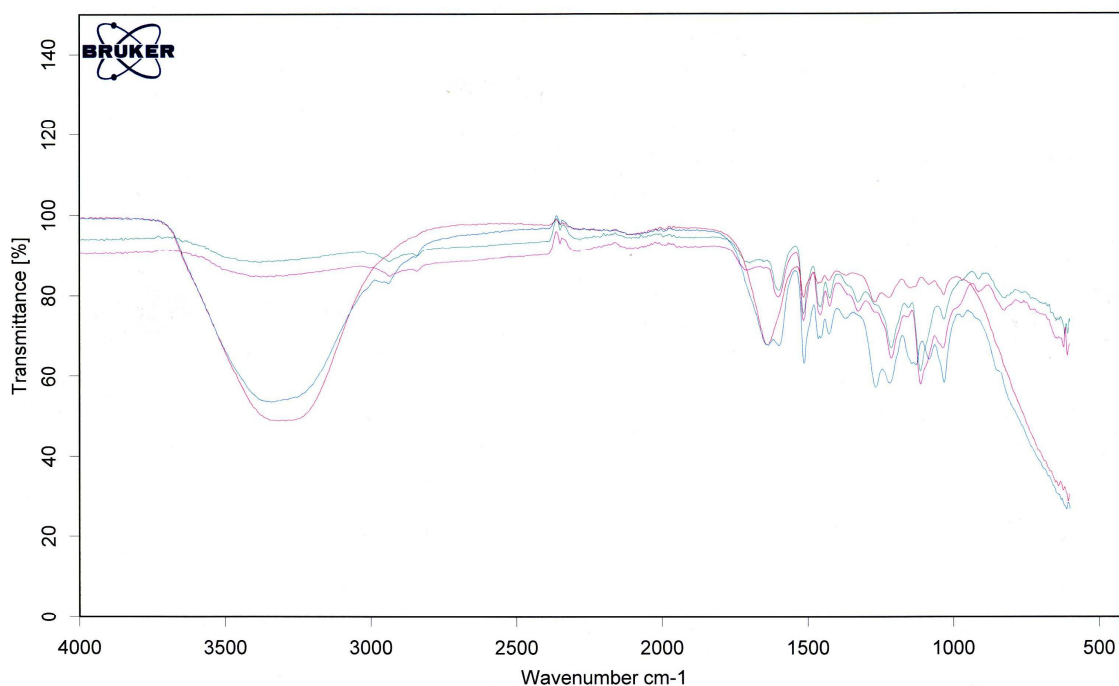
Table 5.3 Distribution of functional group for Kraft and Klason lignin from birch (Lora and Wayman 1980, Jahan & Mun 2007)

Band from FTIR	Molecular structure	Lignin
1710 cm^{-1}	Unconjugated C=O	Kraft lignin
1330 & 1111 cm^{-1}	syringyl structure	Kraft & Klason lignin
1215 & 1028 cm^{-1}	guaiacyl structure	Kraft & Klason lignin

It can be seen from Table 5.3 that some different absorbance bands with functional groups of Kraft and Klason lignin for birch at various pH values were revealed, compared with pine. There were also obvious similarities between Kraft and Klason lignin for birch. The band at 1330 and 1111 cm^{-1} were associated with syringyl structure in the lignin molecules; the band at 1215 and 1028 cm^{-1} were associated with guaiacyl structure in lignin molecules, which indicated the simultaneous presence of both guaiacyl and syringyl unit in the Kraft and Klason lignin molecule. However, presence of some functional groups was different in both Kraft and Klason lignins. For instance, the C=O in unconjugated ketone (β -carbonyl) was absent in Klason lignin. (Jahan & Mun 2007.)

Comparison of the IR spectra of Kraft lignin and Klason lignin for the same wood species, e.g. pine, is shown in Figure 5.8 and other IR spectra are shown in Appendix 1-2. It can be seen from the figures, although the IR spectra of both these two types of lignin were not different, that they still had a little distinction. There was a shift in the high absorption band region, and band at 800-900 cm^{-1} was an entirely diverse absorption pattern. Moreover, the absorbance of Klason lignin in high bond region was obvious lower than that of Kraft lignin. Thus, the different lignin isolation methods were a different regarding the chemical structures.

The FTIR spectra obtained for Kraft lignin from the four wood species are shown in Figure 5.9. Since a general similarity of IR spectra was evident for all the samples, only those bands which varied markedly were discussed.



C:\Program Files\OPUS\meas\Pine lignin (pH2-3).0	Pine lignin (pH2-3)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Spruce lignin (pH2-3).0	Spruce lignin (pH2-3)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Birch lignin (pH2-3).0	Birch lignin (pH2-3)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Aspen lignin (pH2-3).0	Aspen lignin (pH2-3)	Instrument type and / or accessory	23/09/2011

Page 1 of 1

Figure 5.9 The IR spectra of Kraft lignin of four wood species at pH 2-3

It can be seen from Figure 5.9, that the broad band at 3310-3340 cm^{-1} was due to O-H stretching vibration, and the band at 2930 cm^{-1} was characteristic of various types of C-H bonds, generally, which were present in the Kraft lignin of softwood, but they did not exhibit for hardwoods. The spectra of softwood and hardwood exhibited a completely different absorption pattern at 700-900 cm^{-1} region. The band indicated a shift from typical guaiacyl-propane aromatic substitution to a more complicated pattern (Hu 2006). Therefore, the analysis results clearly indicated that Kraft lignins of softwood and hardwood were different in their chemical structures. The common points for the softwood and hardwood show in the medium and weak absorption band regions. The IR spectra exhibited a high intensity condition for all Kraft lignin samples, the C=O stretching vibration, aromatic skeletal vibration, C-H deformation and aromatic ring vibration and C-O stretching vibration would occur.

Similarly, the IR spectra of Klason lignin of the four wood species were analyzed in the same way as in the above method and are shown in Appendix 3. As can

be seen, each wood species had a similar absorption profile for the different types of wood, i.e. softwood and hardwood. However, the adsorption values depend on the various wood species.

6 SUMMARY

In this work two different isolation methods, i.e. Kraft lignin and Klason lignin, from two kinds of wood species, i.e. softwood and hardwood, were investigated. The Kraft lignin and Klason lignin were identified by using the spectroscopic instruments, UV and FTIR spectrometer.

The Kraft lignin was obtained from black liquor from wood. The precipitation of black liquor with sulfate acid was successful to obtain the lignin as solid particles. The results showed that the color, shape and size of lignin particles had a certain difference depending on the pH values and wood species. Klason lignin was isolated from wood continuously with several steps, such as, isolation of extractives with alcohol-benzene, acid treatment of extractive-free wood etc. The results also showed that solid particles of Klason lignin depending on the wood species. For a certain amount of raw materials, the results showed that the content of lignin Kraft and Klason lignin in softwood was higher than that in hardwood, which was supported by the literature study of wood. Furthermore, the Kraft lignin yield was higher than Klason lignin. For the Kraft lignin, the lower the pH value, the higher yield isolated from black liquor.

UV spectrum recorded the lignin purity based on value of absorbance. The UV spectra showed absorption maxima at approximate 208 and 280 nm for Kraft lignins. For the softwood, the absorption profile had a certain difference at the various pH values. The theory explaining the UV analysis was given based on the literature study. The UV results showed that the lignins obtained in this

experimental work were the relative pure product.

IR spectrum recorded the functional groups included in lignin. The different band values in IR spectrum of lignin were explained based on the literature study. The analysis of IR spectra revealed that the chemical structure of lignin varies depending on its wood species and the isolation method employed. Therefore, according to the analysis results, lignin can be quantitative and qualitative identified by Ultraviolet (UV) and Fourier Transform Infrared (FTIR) spectroscopy.

FIGURES

Figure 2.1 The structure of cellulose, p.7

Figure 2.2 The position of lignin within lignocellulosic matrix, p.8

Figure 2.3 The structure of cell wall in wood, p.10

Figure 2.4 The structural units of lignin, p.11

Figure 2.5 Resonance forms of the coniferyl alcohol radical, p.12

Figure 2.6 The common phenylpropane linkages in lignin, p.13

Figure 2.7 Lignin isolation process, p.19

Figure 3.1 Parts of single beam spectrophotometer, p.24

Figure 3.2 Operating principle of FTIR spectrometer, p.25

Figure 4.1 Batch digester for experiment of batch cooking, p.26

Figure 4.2 Gyrotory screen used for experiment, p.27

Figure 4.3 Batch cooking process, p.29

Figure 4.4 The black liquor of various wood species, p.30

Figure 4.5 Titration, p.31

Figure 4.6 Raw material, p.32

Figure 4.7 Flask reactor with reflux condenser, p.33

Figure 4.8 Shimadzu UV-spectrophotometer, p.35

Figure 4.9 Bruker TENSOR Series FTIR spectrometer and pressure clamp, p.35

Figure 5.1 The extraction of Kraft lignin from black liquor of pine, p.36

Figure 5.2 The extraction of Kraft lignin from black liquor of spruce, p.37

Figure 5.3 The extraction of Kraft lignin from black liquor of birch, p.37

Figure 5.4 The extraction of Kraft lignin from black liquor of aspen, p.37

Figure 5.5 The Klason lignins of the four kinds of wood species, p.39

Figure 5.6 The UV spectra of Kraft lignin from black liquor of four wood species, p.42

Figure 5.7 The UV spectra of Kraft lignin at pH 2-3, p.44

Figure 5.8 The IR spectra of Kraft and Klason lignin of pine, p.45

Figure 5.9 The IR spectra of Kraft lignin of four wood species at pH 2-3, p.48

CHARTS

Chart 5.1 The Kraft lignin precipitation from black liquor at different pH values, p.38

Chart 5.2 The Klason lignin contents of four kinds of wood species, p.40

TABLES

Table 2.1 Chemical comparison of various wood species, p.6

Table 2.2 Proportions of most common linkages in lignin, p.13

Table 2.3 Functional groups of softwood and hardwood lignin, p.14

Table 2.4 Typical composition of the black liquor from various cooking condition, p.18

Table 2.5 Compositions of Spent Sulfite Liquors, p.20

Table 3.1 The Klason lignin contents of lignified materials, p.22

Table 4.1 The raising time and cooking time arrangement for various wood species, p.29

Table 5.1 The UV absorbance of Kraft lignin of four species, p.43

Table 5.2 Distribution of functional group for Kraft and Klason lignin from pine, p.45

Table 5.3 Distribution of functional group for Kraft and Klason lignin from birch, p.47

REFERENCES

Chen, C.L. (1991) Lignins: Occurrence in Woody Tissues, Isolation, Reactions, and Structure. In Wood Structure and Composition. Lewin, M., Goldstein, I.S. New York, Marcel Dekker, Inc., pp 183-261.

Chiang & Funaoka (1990) The difference between guaiacyl and guaiacyl-syringyl lignins in their responses to kraft delignification. *Holzforschung* 44: 309-313.

Drew, J & Propst, M. 1981. Tall Oil, Pulp Chemicals Association, New York, p.22-34.

Faix, O. 1991. "Classification of lignin from different botanical origins by FT=IR spectroscopy," *Holzforschung* 45, 21-27.

Freudenberg, K. and Neish, A.C. 1968. Constitution and Biosynthesis of Lignin. ed. Springer, G.F. and Kleinzeller, A. Springer-Verlag: New York. 129 pp.

Froass, P. M. 1996. Chemical structure of residual lignin from kraft pulp. *Journal of Wood Chemistry and Technology* 16.

Gellerstedt, G et al. 1994. Structural and molecular properties of residual birch kraft lignins. *J. Wood Chem. Techno.*

Ghatak, Himadri Roy 2008. Electrolysis of black liquor for hydrogen production: some initial findings, *Int. J. Hydrogen Energy*.

Glennie, D. W. & McCarthy, J. L. 1962. Chemistry of Lignin. In Libby, C. E. (ed.) *Pulp and Paper Science and Technology*. New York: McGraw-Hill Book Company, Inc., pp. 82-107.

Griffiths, P.; de Hasseth, J.A. 2007. *Fourier Transform Infrared Spectrometry* (2nd ed.). Wiley-Blackwell. ISBN 0471194042.

Gullichsen, J. & Fogelholm, C. J. 2000. *Chemical Pulping*. Helsinki: Fapet Oy.

Harkin, John M. 1969. Lignin and its uses.
<http://www.fpl.fs.fed.us/documnts/fplrn/fplrn0206.pdf> (Accessed on 06 May 2011)

Higuchi, T (1985) Biosynthesis of lignin. In T Higuchi, ed, Biosynthesis and Biodegradation of Wood Components. Academic Press, New York, pp 141-160.

Holtman K 2003. An Investigation of the Milled Wood Lignin Isolation Procedure by Solution- and Solid-State NMR Spectroscopy
<http://repository.lib.ncsu.edu/ir/bitstream/1840.16/5436/1/etd.pdf> (Accessed on 10 July 2011).

Hu Zhoujian 2006. Elucidation of the structure of cellulolytic enzyme lignin from loblolly pine
<http://repository.lib.ncsu.edu/ir/bitstream/1840.16/26/1/etd.pdf> (Accessed on 12 June 2011).

IBRAHIM, M. N. MOHAMAD, AZIAN, H. & YUSOP M. R. MOHD 2006.
<http://www.penerbit.utm.my/onlinejournal/44/F/JTJun44F7.pdf> (Accessed on 28 May 2011).

John Wiley & Sons, Inc
<http://www.scribd.com/doc/30137763/Lignin> (Accessed on 12 June 2011).

Jahan, M.S. & Mun, S.P. (2007) Characteristics of Dioxane Lignins Isolated at Different Ages of Nalita Wood (*Trema orientalis*). *J. of wood Chemistry and Technology* 27 (2).

Klemn, D. Cellulose: Fascinating biopolymer and sustainable raw material. *Angew. Chem. Int. Ed.*, 2005. 44: p. 3358-3393.

Knowpulp 10.0. (Accessed on 06 May 2011).

Kuhad, R.C. & Singh Ajay. 2007. Lignocellulose biotechnology: future prospects. I. K. International Pvt Ltd.

Lai, Y.Z., Sarkanen, K.V. (1971). Isolation and structural studies. In *Lignins: Occurrence, Formation, Structure and Reactions*. Sarkanen, K.V., Cludwig, C.H. New York, Wiley interscience, pp 165-230.

López M, Huerta-Pujol O, Martínez-Farré FX, Soliva M. (2010). Approaching compost stability from Klason lignin modified method: Chemical Stability Degree for OM and N quality assessment

<http://upcommons.upc.edu/e-prints/bitstream/2117/11181/1/SD%20pre-print.pdf>
(Accessed on 12 June 2011).

Lora J.H. and Wayman M. 1980. Autohydrolysis of aspen lignin milled wood lignin. *Can J. Chem.*

Obst J.R. 1982. Guaiacyl and syringyl lignin composition in hardwood cell components. *Holzforschung.*

Pearl, I.W. 1967. *The Chemistry of Lignin.* Marcel Dekker, Inc.: New York. 339 pp.

Pettersen, R.C. 1984. *The Chemical Composition of Wood.* U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, WI 53705.

Sjöström, E. (1993). *Wood Chemistry Fundamentals and Applications* (2nd edition). San Diego, CA: Academic Press, Inc.

Skoog, Douglas A, Holler, F.James & Crouch, Stanley R. *Principles of Instrumental Analysis.* 6th ed. Thomson Brooks/Cole. 2007, 349-351.

Stenius, P. 2000. *Forest Products Chemistry.* Helsinki: Fapet Oy.

Swartz, J.N & MacDonald, R.C 1962. Alkaline Pulping. In Libby, C. E. (ed.) *Pulp and Paper Science and Technology.* New York: McGraw-Hill Book Company, Inc., pp.162.

Tamminen T., Vuorinen T., Tenkanen M., Hortling B. Analysis of lignin and lignin-carbohydrate complexes isolated from black liquor. 8th International Symposium on Wood and Pulping Chemistry, Helsinki, Finland, June 6–9, 1995, Vol. II, pp. 297–302.

TAPPI 2010. Black Liquor Lignin Recovery as a Means for Pulp Mill Capacity Raise. (Accessed on 28 September 2011).

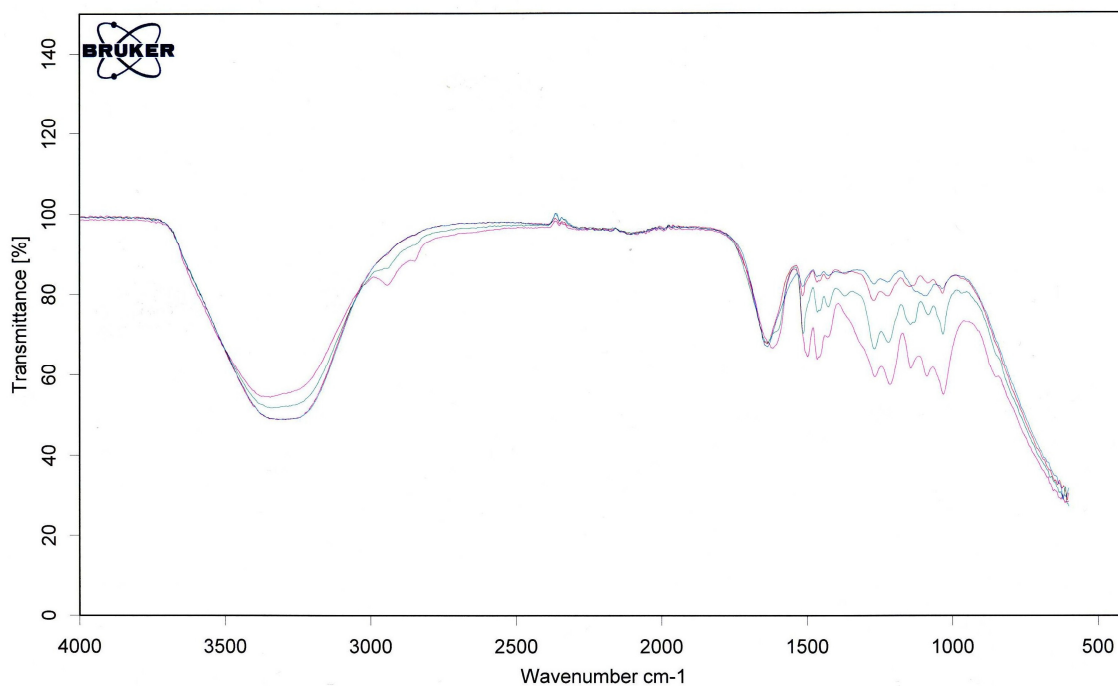
TAPPI T222 om-06, 2011. Acid-insoluble lignin in wood and pulp.

TAPPI T264 om-97, 2011. Preparation of wood for chemical analysis.

Wegener, Gerd; Przyklenk, Margarete & Fengel, Dietrich. 1983. Hexafluoropropanol as Valuable Solvent for Lignin in UV and IR Spectroscopy. *Holzforschung - International Journal of the Biology, Chemistry, Physics and Technology of Wood*. Volume 37, Issue 6, Pages 303–307.

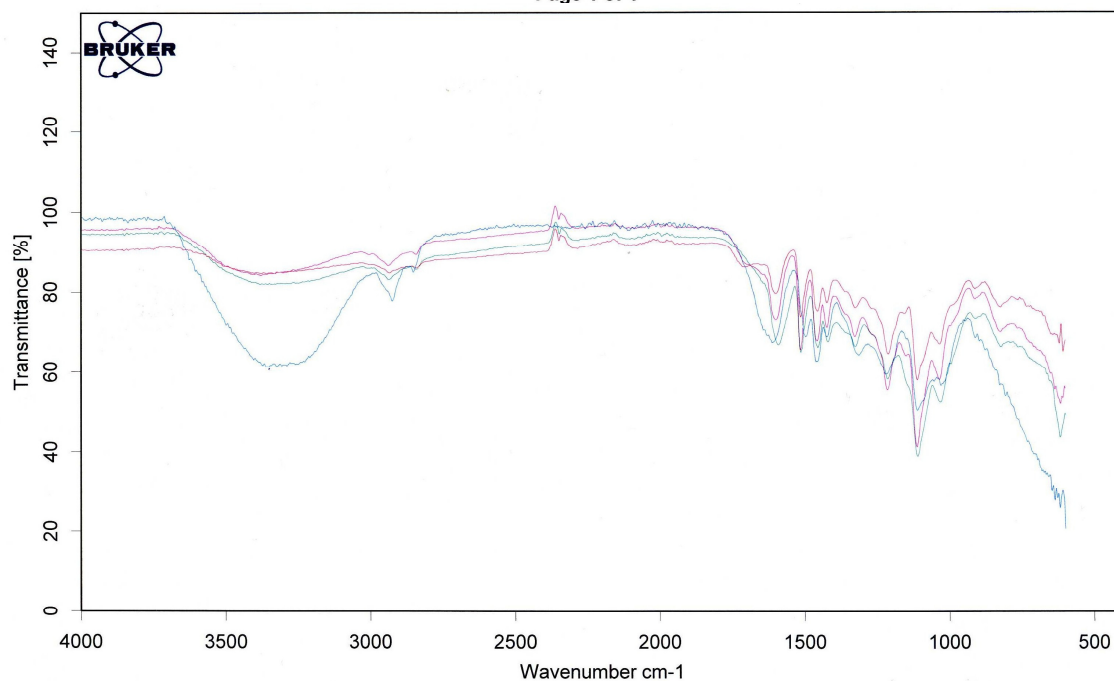
Wise L. 1962. Garcia, Consuelo *Tappi* 34(4).

APPENDIX 1



C:\Program Files\OPUS\meas\Spruce lignin (pH7).0	Spruce lignin (pH7)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Spruce lignin (pH4).0	Spruce lignin (pH4)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Spruce lignin (pH2-3).0	Spruce lignin (pH2-3)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Spruce Klason lignin.0	Spruce Klason lignin	Instrument type and / or accessory	23/09/2011

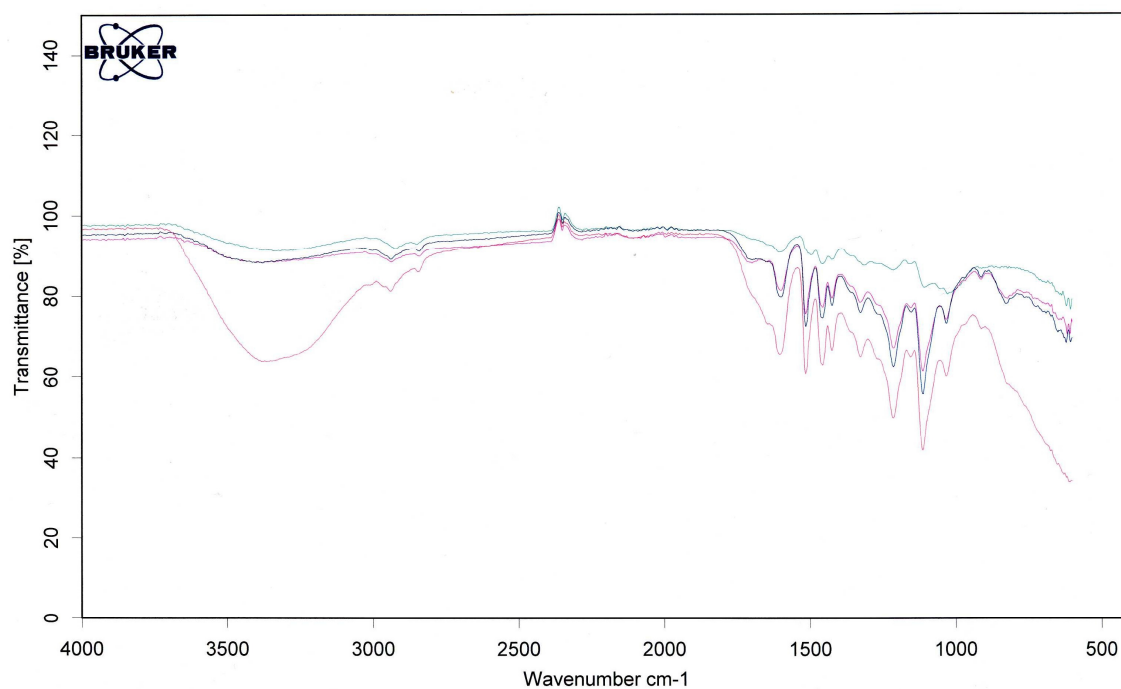
Page 1 of 1



C:\Program Files\OPUS\meas\Birch Klason lignin.0	Birch Klason lignin	Instrument type and / or accessory	06/10/2011
C:\Program Files\OPUS\meas\Birch lignin (pH2-3).0	Birch lignin (pH2-3)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Birch lignin (pH4).0	Birch lignin (pH4)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Birch lignin (pH7).0	Birch lignin (pH7)	Instrument type and / or accessory	23/09/2011

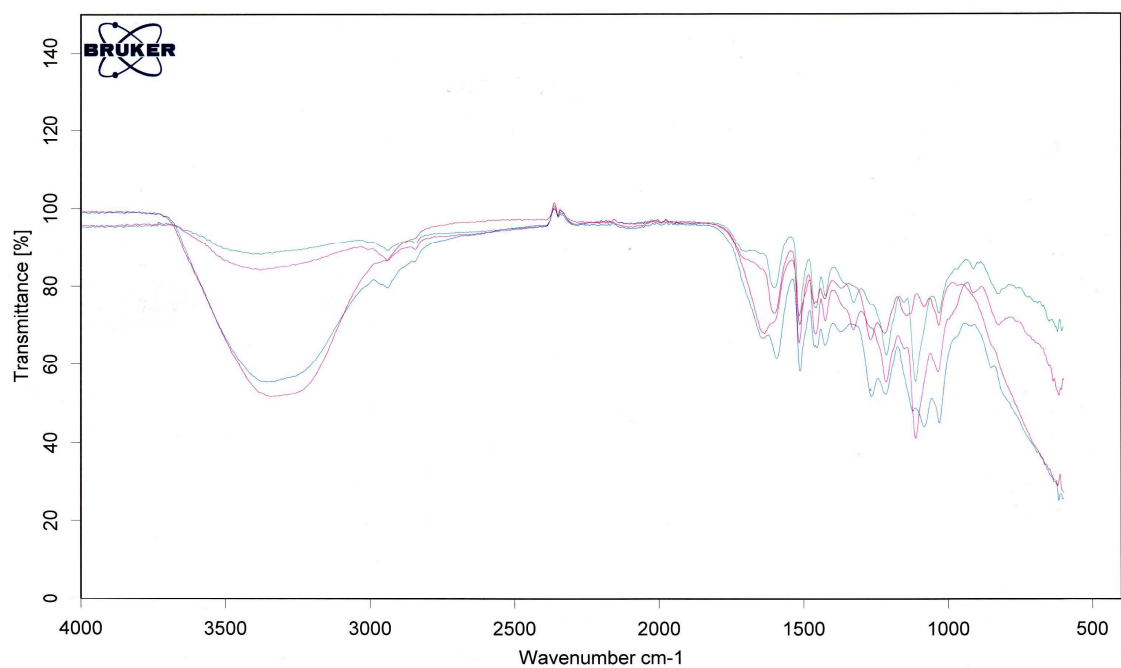
Page 1 of 1

APPENDIX 2



C:\Program Files\OPUS\meas\Aspen lignin (pH7).0	Aspen lignin (pH7)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Aspen lignin (pH4).0	Aspen lignin (pH4)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Aspen lignin (pH2-3).0	Aspen lignin (pH2-3)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Aspen Klason lignin.0	Aspen Klason lignin	Instrument type and / or accessory	23/09/2011

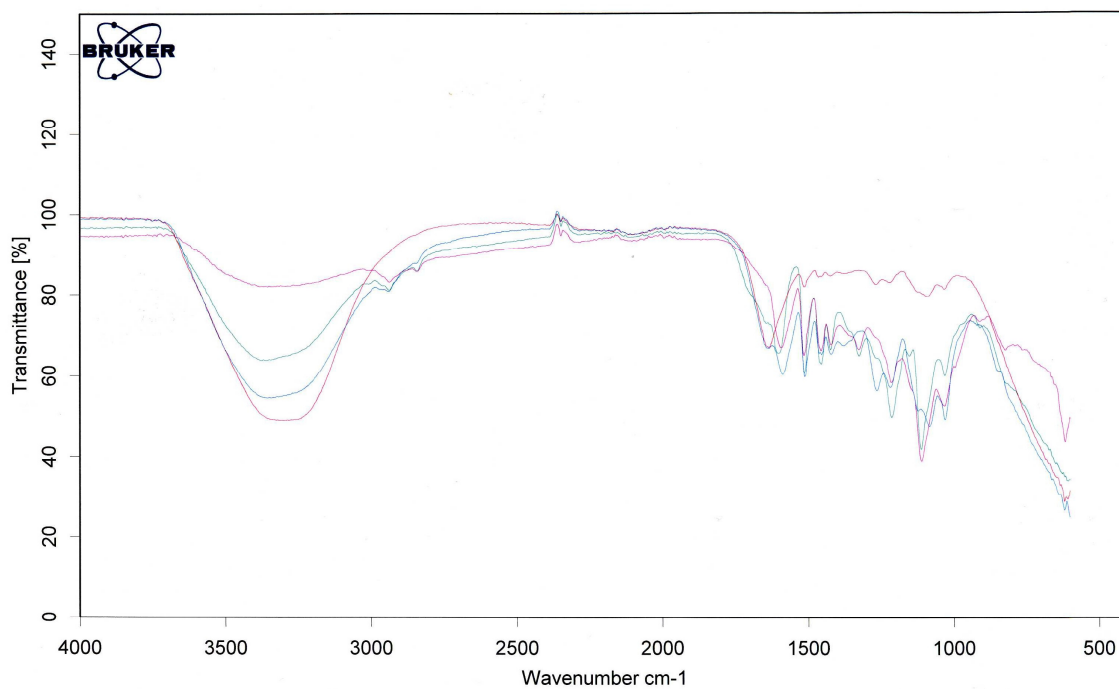
Page 1 of 1



C:\Program Files\OPUS\meas\Pine lignin (pH4).0	Pine lignin (pH4)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Spruce lignin (pH4).0	Spruce lignin (pH4)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Birch lignin (pH4).0	Birch lignin (pH4)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Aspen lignin (pH4).0	Aspen lignin (pH4)	Instrument type and / or accessory	23/09/2011

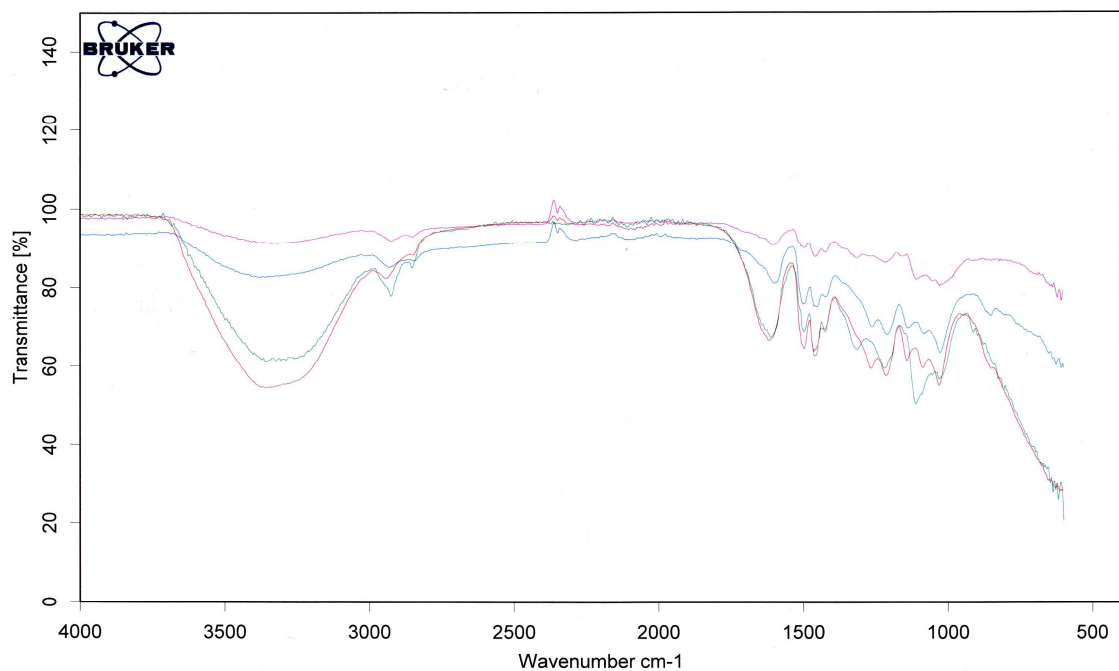
Page 1 of 1

APPENDIX 3



C:\Program Files\OPUS\meas\Pine lignin (pH7).0	Pine lignin (pH7)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Spruce lignin (pH7).0	Spruce lignin (pH7)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Birch lignin (pH7).0	Birch lignin (pH7)	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Aspen lignin (pH7).0	Aspen lignin (pH7)	Instrument type and / or accessory	23/09/2011

Page 1 of 1



C:\Program Files\OPUS\meas\Pine Klason lignin.0	Pine Klason lignin	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Spruce Klason lignin.0	Spruce Klason lignin	Instrument type and / or accessory	23/09/2011
C:\Program Files\OPUS\meas\Birch Klason lignin.0	Birch Klason lignin	Instrument type and / or accessory	06/10/2011
C:\Program Files\OPUS\meas\Aspen Klason lignin.0	Aspen Klason lignin	Instrument type and / or accessory	23/09/2011

Page 1 of 1