

**THE EFFECTS OF HYDROTHERMAL TREATMENT ON
THE PHYSICO-CHEMICAL PROPERTIES OF OIL PALM
FROND (OPF) DERIVED HEMICELLULOSE**

by

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LIST OF SYMBOLS

τ	Shear stress
η	Viscosity / intrinsic viscosity
γ	Shear rate
σ	Yield stress
η/η_s	Relative viscosity
η_{sp}	Specific viscosity
*C	Critical or coil overlap concentration
c	Concentration
CaCl ₂ .2H ₂ O	Calcium chloride solution
CED	Cupriethyldiamine
cm	Centimeter
DMSO	Dimethyl sulfoxide
DP	Degree of polymerization
DSC	Differential Scanning Calorimetry
K	Consistency coefficient
kg	Kilogram
KOH	Potassium hydroxide
M	Molecular weight
ML	Middle lamella
Mn	Number average molecular weight
Mw	Molecular weight average
n	Flow behavior index
P	Primary wall
R _g	Radius of gyration
S	Secondary wall
TGA	Thermogravimetric Analysis
W	Warty layer
μ	Newtonian viscosity
μ_{pl}	Plastic viscosity

KESAN PENGOLAHAN HIDROTERMAL KE ATAS SIFAT-SIFAT FIZIKO-KIMIA HEMISELULOSA DARIPADA PELEPAH KELAPA SAWIT

ABSTRAK

Kajian ini telah dijalankan ke atas hemiselulosa yang diekstrak dari pelepah kelapa sawit (OPF). Hemiselulosa telah diekstrak menggunakan 3 M KOH pada 40°C dengan pengadukan pada 400 rpm selama 4 jam. Bahagian yang pertama dimendakkan apabila ekstrak alkali tersebut ditambahkan dengan 50 % (i/i) asid asetik sehingga pH di dalam julat 4.7 – 5.0 dan dinamakan sebagai Hemiselulosa A (HA). Baki hemiselulosa yang larut dimendakkan pula dengan 95 % etanol dan dinamakan sebagai Hemiselulosa B (HB). Jumlah keseluruhan hemiselulosa yang terekstrak adalah sebanyak 16.57 g per berat kering 50 g OPF. Walau bagaimanapun, hasilan HA (13.12 g) adalah lebih tinggi daripada HB (3.45 g). Satu pengolahan hidrotermal pada 121°C, 15 psi selama 10, 30 dan 50 minit telah dijalankan ke atas HA dan HB. Pencirian dari aspek fiziko-kimia dan sifat-sifat berfungsi seperti kandungan lembapan, kandungan abu, kandungan Klason lignin, kandungan gula penurunan, analisis monosakarida, taburan berat molekul, kapasiti pengikatan air (WBC), kelikatan intrinsik, keterlarutan, kelakuan aliran dan sifat termal telah dijalankan bagi mengkaji kesan proses autohidrolisis ke atas hemiselulosa. Didapati kandungan lembapan HA (5.34 %) adalah lebih rendah secara signifikan berbanding HB (11.25 %). Kandungan klason lignin di dalam HA (17.5 %) adalah lebih tinggi daripada HB (5.67 %). Selepas pengolahan hidrotermal, kandungan klason lignin berkurang. Pengolahan hidrotermal telah meningkatkan keterlarutan hemiselulosa yang mana ditunjukkan dengan kandungan gula penurunnya yang tinggi. Analisis monosakarida menggunakan HPLC menunjukkan komposisi monosakarida di dalam HA dan HB berbeza kerana perbezaan struktur kimia dan konstituennya. Xilosa merupakan monosakarida

utama dalam kedua-dua pecahan dan HA mengandungi kandungan xilosa yang lebih tinggi berbanding HB. HA dicadangkan mempunyai rantaian lurus dengan berat molekul yang tinggi manakala HB adalah polimer yang bercabang dengan berat molekul yang lebih rendah. HA didapati kurang larut di dalam air berbanding HB. Kedua-dua pecahan hemiselulosa menunjukkan kelakuan aliran bukan Newtonian tiksotropik atau penipisan ricih. Walau bagaimanapun, HA adalah lebih likat daripada HB pada kepekatan yang sama. Analisis thermogravimetrik menunjukkan kesemua sampel hemiselulosa mempunyai kestabilan termal di bawah 200°C walaupun sampel yang diolah termal didapati kurang stabil berbanding sampel yang tidak diolah. Kajian ini telah menerangkan kesan signifikan pengolahan hidrotermal ke atas perhubungan-struktur dan sifat-sifat berfungsi kedua-dua sampel HA dan HB.

THE EFFECTS OF HYDROTHERMAL TREATMENT ON THE PHYSICO-CHEMICAL PROPERTIES OF OIL PALM FROND (OPF) DERIVED HEMICELLULOSE

ABSTRACT

This study has been conducted on oil palm frond (OPF) derived hemicellulose. Hemicellulose was extracted using 3M KOH at 40°C with stirring at 400 rpm for 4 hours. The first fraction precipitated when the alkali extract is acidified using 50 % (v/v) acetic acid to pH 4.7 – 5.0 and named as Hemicellulose A (HA). The remaining soluble hemicellulose precipitated by the addition of 95 % ethanol and named as Hemicellulose B (HB). Total hemicellulose isolated was 16.57 g per dry weight of 50g OPF. However, yield of HA (13.12 g) was significantly higher than HB (3.45 g). Both of HA and HB then were subjected to hydrothermal treatment at 121°C, 15 psi for 10, 30 and 50 minutes respectively. Physico-chemical and functional characterization such as moisture content, ash content, Klason lignin content, reducing sugar content, monosaccharides analysis, molecular weight distribution, water binding capacity (WBC), intrinsic viscosity, solubility, flow behavior and thermal behavior were performed in order to study the effect of autohydrolysis process on OPF derived hemicelluloses. It has been found that moisture content of HA (5.34 %) was significantly lower than HB (11.25 %). Klason lignin content in HA (17.5 %) was significantly higher than HB (5.67 %). After hydrothermal treatment, klason lignin content decreased. Hydrothermal treatment enhanced the solubility of hemicelluloses which reflects in its higher reducing sugar content. Monosaccharides analysis using HPLC shows that monosaccharides composition in HA and HB were different due to its different chemical structure and constituents. Xylose was the predominant monosaccharides in both fractions. HA has relatively higher xylose content than HB suggesting that HA is a linear polymer with higher molecular weight while HB is a

branched polymer with lower molecular weight. HA was relatively less soluble in water than HB. Both HA and HB exhibited non Newtonian thixotropic or shear thinning flow behavior. However, HA was more viscous than HB at the same concentration. Thermogravimetric analysis shows that all hemicellulosic samples were fairly stable below 200°C although hydrothermally treated samples were slightly less stable than untreated hemicelluloses. This study has elucidated the significant of hydrothermal treatment on structural–functional relationship of both OPF derived hemicelluloses A and B.

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Agricultural wastes from oil palm tree such as empty fruit bunch (EFB), oil palm trunk (OPT) and oil palm frond (OPF) are rich in cellulose and hemicellulose. Fractionation of these agricultural wastes into their main components could be of interest to obtain separate streams useable for different product applications. Among these enormous amounts of agricultural residues, only a minor quantity is reserved for animal feed or household fuel and a major portion of the oil palm tree is burnt, creating environmental pollution (Suhaimi and Ong, 2001). Recently, an increasing knowledge and fast growing willingness to develop new biopolymer-based materials may lead to an increasing application of hemicelluloses and their derivatives. Thus it is particularly interesting to exploit the hemicelluloses from annual agricultural residues such as oil palm frond and oil palm trunk as environmentally friendly materials for industries.

Applications of hemicellulose have been demonstrated during the past two decades (Methacanon *et al.*, 2003). Hemicellulose showed a potential feedstock for the production of sugars, furfural and used as an additive in paper-making, pharmaceutical, cosmetic and food areas (Fenden *et al.*, 2002; Sun and Tomkinson, 2002). A novel product like hydrogel has been also exploited since hemicellulose are responsible for the interaction with water, in their native form (Gabiellii *et al.*, 2000). In addition, a hydrophobic film from maize bran hemicellulose has been developed as a bioplastic with chemical modification (Fenden *et al.*, 2002). Hemicellulose from olive stones has been used as an energy source and has recently been employed to produce activated carbon (Gonzalez *et al.*, 1994).

Since the main component of the hemicelluloses from oil palm frond is xylan, fractionation of this polysaccharide may result in both xylose, an intermediate for the production of xylitol and a variety of differently substituted xylo-oligosaccharides (XO'S). Because of its anti-cariogenic properties, xylitol has been already used in food applications such as chewing gum and tooth paste. Xylo-oligosaccharides are reported to enhance growth of bifidobacteria and they are frequently defined as prebiotics (Campbell *et al.*, 1997; Fooks *et al.*, 1999; Van Laere *et al.*, 2000).

Classical acid hydrolysis, autohydrolysis at high temperature based on formation of acetic acid from the release of acetyl groups, endoxylanase treatment alone or in combination with steaming and microwave heating was used to prepare xylo-oligosaccharides from various xylan-rich materials (Bobleter, 1998). The hydrothermal treatment is of interest because it enables the fractionation of hemicellulose from cellulose and lignin, and separation of the formed oligosaccharides (Koukios *et al.*, 1999). This has been demonstrated recently on *Eucalyptus* wood and brewery spent grain (Kabel *et al.*, 2002).

An environmental friendly way to fractionate xylan rich materials is to perform a mild hydrothermal treatment. Such a hydrothermal treatment will result in a selective release and degradation of xylan of the used resource, leaving the cellulosic residue available for other purposes (Garrote *et al.*, 1999). Furthermore, since the xylans in the various by-products are expected to possess rather diverse structures, differences in size and structural features of the oligosaccharides released are expected upon the treatment for each material.

In this study, hemicellulose was extracted using alkaline extraction to obtain two different fractions of hemicelluloses that is hemicellulose A (HA) and hemicellulose B (HB). Both of HA and HB were subjected to hydrothermal treatment using an autoclave at 121°C, 15 psi with different treatment times. Low temperature processes was chosen as more advantageous for the protection of oil palm frond against carbohydrate degradation and production of furfural and hydroxymethylfurfural (HMF) under drastic conditions such as steam explosion. Furthermore, this mild treatment also can reduce energy consumption.

Physico-chemical and functional properties of hydrothermally treated HA and HB such as monosaccharides composition, molecular weight distribution, solubility, water binding capacity, thermal behavior and flow behavior were investigated in this study. A better understanding of their physico-chemical properties and chemical composition could allow new opportunities for both food and non-food application of this abundant renewable resources.

1.2 RESEARCH OBJECTIVES

The general objective of the research was to characterize hydrolysates of six hydrothermally treated hemicelluloses derived from OPF. An environmental friendly hydrothermal treatment was performed to obtain monosaccharides particularly xylose from oil palm frond. Since structure and functions are intimately related, knowledge of the chemistry of this material is of crucial importance for its proper utilization. Therefore, the specific objectives of the research were as follows;

- To extract and to quantify the yield of hemicelluloses (HA and HB) from fresh OPF without prior delignification process using 3 M KOH at 40°C with stirring at 400 rpm and 4 hours extraction process.
- To apply the physical pretreatment method or hydrothermal treatment using an autoclave at 121°C, 15 psi and different treatment times (10, 30 and 50 minutes) on HA and HB.
- To characterize the physico-chemical and functional properties of HA, HB and hydrothermally treated hemicelluloses A and B such as monosaccharides composition, solubility, intrinsic viscosity, water binding capacity, flow behavior, thermal behavior and molecular weight distribution.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil Palm

2.1.1 History and Industrial Development

African oil palm *Elaeis guineensis*, is one of the most important plants in Malaysia. It produces palm oil and palm kernel oil, which are widely used in food and other industries such as detergents and cosmetics. The oil palm was introduced as an ornamental in 1871, but it was commercially exploited as an oil crop (Wan Rosli *et al.*, 2007) only from 1911 when the first oil palm estate was established.

The first commercial planting was done in 1911 at Tenammaran Estate, Kuala Selangor but the growth was initially very slow. It was only during the last 50 years that plantation development was accelerated through large scale investments in the cultivation of the oil palm (Yusof, 2007). Table 2.1 shows the area under oil palm plantation (mature and immature) by states in year 2006.

Table 2.1: Area under oil palm plantation (mature and immature) by states in year 2006 (hectares). (Source : Anon, 2006)

State	Mature	Immature	Total
Johor	607,663	63,762	671,425
Kedah	71,705	4,624	76,329
Kelantan	75,825	18,717	94,542
Melaka	49,105	3,127	52,232
N. Sembilan	141,864	19,208	161,072
Pahang	551,713	71,577	623,290
P. Pinang	13,895	224	14,119
Perak	321,656	26,344	348,000
Perlis	258	0	258
Selangor	121,140	7,775	128,915
Terengganu	137,866	26,199	164,065
P.Malaysia	2,092,690	241,557	2,334,247
Sabah	1,139,535	99,962	1,239,497
Sarawak	471,029	120,442	591,471
Sabah/Sarawak	1,610,564	220,404	1,830,968
MALAYSIA	3,703,254	461,961	4,165,215

The rapid increase in plantation area in Malaysia indicates the economics importance of this plantation crop and the growing world demand for palm oil. Malaysia is the world's largest producer and exporter of palm oil and currently producing about 50 % of the world's supply of palm oil. Indonesia is the second largest world producer of palm oil producing approximately 30 % of world palm oil volume. Besides palm oil, the industry also generates massive amounts of lignocellulosic residues such as trunks, fronds and the empty fruit bunches (EFB) with an estimated amount of 30 million tonnes (Anon, 2002).

Figure 2.1 shows the photograph of oil palm tree.



Figure 2.1 : Photograph of Oil palm tree

2.1.2 Availability of Oil Palm Fronds

The average economic life-span of oil palm is 25 years (Hamzah and Wan Mohd, 1991). Generally, oil palm tree start producing fruits for oil at the age of three years after field planting and achieve the maximum yield at the age of 9 – 10. The area needs to be replanted after every 25 years. According to Supranto (1999), there are about 300 oil palm trees per hectare. About 20 pieces of fronds can be obtained from a single tree. The average weight of each frond is about 5 kg. Hence, about 100 kg of fronds can be obtained in a year per tree. Moreover, about 30 000 kg of fronds can be produced in one hectare in a year. Frond length gradually increases over the first few years, and from about year 4, the stem starts to become taller, at about 30 – 60 cm/year (Wood, 1986). The fronds are arranged in two opposing sets of spirals. Usually every 8th frond falls on one spiral and every 13th on the opposing set (Arasu, 1966).

This will be a good opportunity to harness the lignocellulosic biomass or by-products of the oil palm, including fronds. Oil palm fronds (OPF) are available daily throughout the year when the palms are pruned during the harvesting of fresh fruit bunches for the production of oil.

Meanwhile, oil palm plantations, encouraged by oil price, grew in leaps and bounds and presently stand at 3.6 million hectares. 90 % of the total biomass produced by the oil palm plantation is non-oil, which comprises of over 22 million tonnes of fronds (OPF), 8 million tonnes of trunks (OPT), 5 million tonnes of empty fruit bunches (EFB) and others (Mohamad *et al.*, 2002). The changes in area of plantation tree crops in Malaysia are shown in Table 2.2.

Table 2.2: Changes in area of plantation tree crops (10^6 ha) in Malaysia. (Source : Chan, 2002).

Year	Oil palm	Rubber	Cocoa	Coconut	Total
1990	1.984	1.823	0.416	0.315	4.538
2000	3.377	1.430	0.078	0.108	4.993

Oil palm frond (OPF) belongs to the category of fibrous crop residues, which also includes by products such as rice straw. Currently, OPF are left rotting between the rows of palm trees, mainly for soil conservation, erosion control and ultimately the long- term benefit of material recycling. The large quantities of fronds produced by a plantation each year make these a very promising source of valuable lignocellulosic materials. Table 2.3 shows the total of oil palm waste in year 1997 and 2000 (million tones of dry weight).

Table 2.3 : Oil palm waste in year 1997 and 2000 (million tonnes, dry matter basis) in Malaysia (Source : Chan, 1999).

Type of waste	Year	
	1997	2000
Oil palm trunk (*)	5.553	6.363
Oil palm frond (*)	1.065	1.221
Oil palm frond (#)	25.532	29.255

* during field replanting

during harvesting

Based on the above figures, Malaysia therefore has a great potential in turning its abundant supply of oil palm industry by-products such as OPF into value added products.

2.2 Wood Structure

Wood is a complex material, composed of cellulose, hemicellulose and lignin, in addition to extractives (terpenes, tannins, fatty acids, resin, etc.), water and mineral water. Wood composition varies from species to species and within the same species it varies with the habitat, age and location in the tree (trunk, branches, top and roots). Plant tissues are primarily composed of thick-walled cells whose shape and size varies among different wood species. The structural integrity of the plant tissue is attributed to the existence of an intervening layer that binds cells together like a cementing agent. This layer, called 'middle lamella' (ML) is almost entirely composed of lignin and together with two adjacent primary walls, forms the compound middle lamella in lignified heartwood cells.

In general, plant cell walls are subdivided into primary wall (P) and secondary wall (S). The primary wall is mainly composed of polysaccharides and proteins although phenolic substances can occur (Carpita and McCann, 2000). The distribution of cellulose, hemicellulose and lignin varies among these layers (Fengel and Wegener, 1989). The

primary cell wall is a thin layer and can be divided into an outer and an inner surface. It is permeable and flexible in physiologically active tissues (sapwood) but may become highly lignified in heartwood cells. The arrangements of the microfibrils in the primary wall are increasingly disperse from inner to outer surface.

The secondary walls are rigid (Möller *et al.*, 2006b) and thicker (1 – 3 mm) than the primary wall. It provides structural support (Möller *et al.*, 2006b) through their ability to resist tension and compressive forces (Aman and Wasterlund, 1988). The impregnation of the walls with lignin makes them hydrophobic and more resistant against attack from microorganisms. The secondary wall is formed by a sequence of three layers, outer layer (S₁), middle layer (S₂) and inner layer (S₃). The microfibrils are oriented in a cross-helical structure (S helix) in the outer layer of the secondary wall. The orientation of microfibrils in the middle layer of secondary wall (S₂), which is the thickest layer, is relatively consistent. In contrast, the microfibrils of the inner layer of the secondary wall (S₃) may arrange in two or more orientations. In some cases, there is a warty layer (W) on the inner surface of the cell wall. Figure 2.2 illustrated the general structure of cell wall of wood cells.

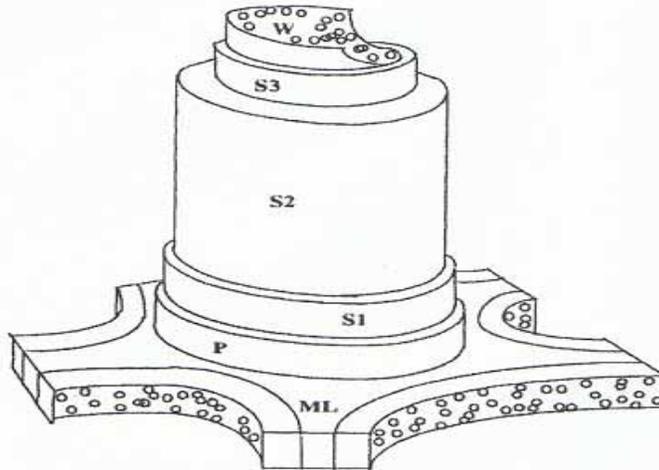


Figure 2.2 : General structure of cell wall of wood cells. ML = middle lamella; P = Primary wall; S1, S2, S3 = outer, middle and inner layers of the secondary wall respectively; W = warty layer (Source : Cote, 1967).

Pure hemicelluloses are also present between the cellulose fibrils and the matrix. Pores in the matrix are small, and permit water, salts and small organic molecules to transfer. Although hemicelluloses are at least partially water soluble, they cannot diffuse out of the cell wall due to their molecular size.

The composition of cell walls varies depending on the plant species, tissue type, cell type, region within the cell wall and development stage of the cell. Cell wall composition can also be influenced by genetic variation within a species, growth conditions and age of the tree. This is important, because cell wall composition and structure will affect the isolation or saccharification process (Möller *et al.*, 2006b). Wan Rosli *et al.*, (2007) reported that OPF are rich in holocellulose and also high in α -cellulose. The lignin content is lower (Wan Rosli *et al.*, 2007) than normally found in common hardwood such as aspen and

eucalyptus. The composition of biomass feedstock based on dry weight is shown in Table 2.4.

Table 2.4: Composition of biomass feedstock based on dry weight. (Source : Möller *et al.*, 2006a).

Feedstock	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)	Ash (%)	Protein (%)
Corn stover	36.4	22.6; Xylose (18), Arabinose (3), Galactose (1), Mannose (0.6)	16.6	7.3	9.7	-
Wheat straw	38.2	24.7; Xylose (21.1), Arabinose (2.5), Galactose (0.7), Mannose (0.3)	23.4	13	10.3	-
Hardwood	43.3	31.8; Xylose (27.8), Mannose (1.4)	24.4	-	0.5	-
Softwood	40.4	31.1; Mannose (22.2), Xylose(8.9)	28	-	0.5	-
Switchgrass (late cut)	44.9	31.4	12	-	4.6	4.5

2.2.1 Cell Wall Structure of OPF

Oil palm frond contains various sizes of vascular bundles. The vascular bundles are widely imbedded in thin-walled parenchymatous ground tissue. Each bundle is made up of a fibrous sheath, vessels, fibers, phloem and parenchymatous tissues. Xylem and phloem tissues are clearly distinguishable (Abdul Khalil *et al.*, 2006). Phloem is divided into two separate areas in each bundle. Some vascular bundles also contain several well-defined protoxylem elements. Protoxylem and metaxylem vessels in the bundle are separated by a layer of parenchyma cells. According to Tomlinson *et al.*, (2001), within the stem and leaves, proto- and metaxylem vessels are separated by at least one layer of live parenchyma cells which form a living barrier to allow gas bubbles to transfer. The

Transmission Electron Microscopy (TEM) views of transverse sections of OPF are shown in Figure 2.3 and 2.4.

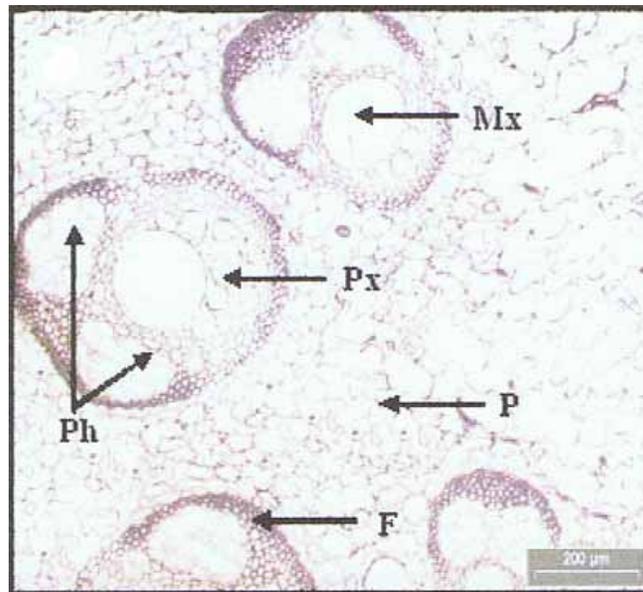


Figure 2.3 : Transverse section of OPF at low magnification (4x). F = Fiber; P = Parenchyma; Mx = Metaxylem; Px = Protoxylem; Ph = Phloem (Source : Abdul Khalil *et al.*, 2006)

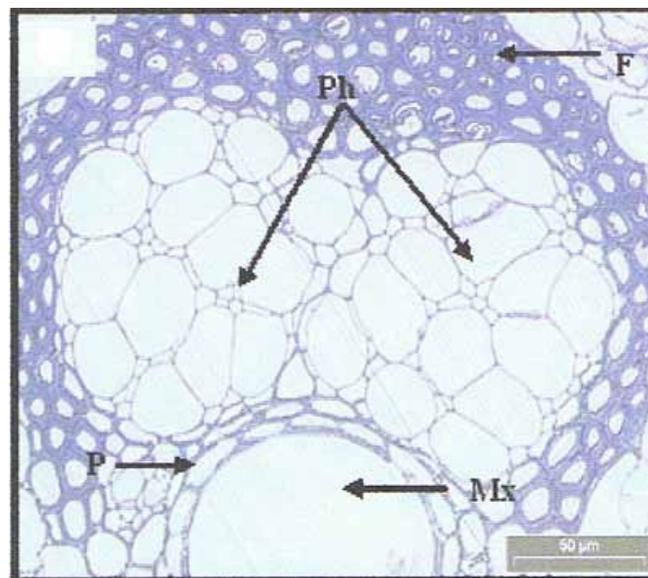


Figure 2.4 : Transverse section of OPF at high magnification (20x). F = Fiber; P = Parenchyma; Mx = Metaxylem; Px = Protoxylem; Ph = Phloem (Source : Abdul Khalil *et al.*, 2006)

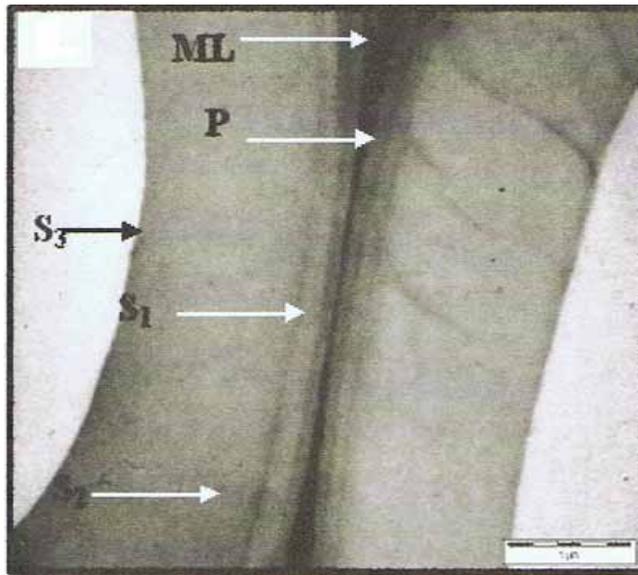


Figure 2.5 : Transverse section of multi layered structure of OPF at high magnification (17 000x). ML = Middle lamella; P = Primary wall; S₁, S₂ & S₃ = Secondary wall sub layers. (Source : Abdul Khalil *et al.*, 2006)

The transverse section of multi layered of OPF at high magnification is shown in Figure 2.5. The figure shows that the layered structure of OPF wall contains primary (P) and secondary (S₁, S₂ and S₃) wall layer. The primary wall appears as a solid boundary of the cell. The middle lamella shows a clear transition to the adjacent primary wall layers. The S₁ layer of OPF is well-defined and could be distinguished from the adjoining S₂ layer, as it is the brightest layer compared to other layers. The presence of distinct S₃ layer in the cell wall of OPF also observed using TEM micrograph. This structure was similar to the wood cell wall structure that has been proposed by Harada and Cote (1985) and other lignocellulosic fibers such as flax, jute, etc.

2.3 Lignocellulosic Materials

Lignocellulose constitutes the major portion of plant cell walls and is mainly composed of lignin, cellulose and hemicelluloses. Xylan which comprises 20 – 30 % by weight of wood and agricultural wastes is the major component of the hemicelluloses portion. Xylan therefore represents a significant resource of renewable biomass which can be utilized as a substrate for the preparation of many useful products such as fuel, solvents and chemicals.

However, the industrial utilization of these materials has been compromised by several factors such as the close association that exists among the three main components of the plant cell wall; cellulose, hemicellulose and lignin, and the low efficiency by which lignocellulosic substrates are converted through biological processes such as hydrolysis and fermentation (Emmel *et al.*, 2003).

2.3.1 Hemicelluloses

The hemicelluloses are composed of a linear as well as branched hetero and homopolymers of pentosans (D-xylose and L-arabinose) and hexosans (D-glucose, D-mannose and D-galactose). Unlike cellulose, hemicelluloses show structural variability (Lachke, 2002). The hemicellulose content of softwoods and hardwoods differ significantly. Hardwood hemicelluloses are mostly composed of highly acetylated heteroxylans, generally classified as 4-O-methylglucuronoxylans. Hexosans are also present but in low amounts as glucomannans.

Hardwood xylans are relatively labile to acid hydrolysis and may undergo autohydrolysis under relatively mild conditions. In contrast, softwoods hemicelluloses consist of glucomannan, galactoglucomannan, arabinan and a small amount arabino-(4-O-

methylglucurono)-xylan. Softwood xylans do not contain acetyl groups, there are separated as a gel from acidic solutions, one of them, in methylated form, being presented as a strong film, indicating the xylan's linear structure (Popa and Spiridon, 2002). As a result, softwood hemicelluloses (mostly hexosans) are more resistant to acid hydrolysis than hardwood hemicelluloses (mostly pentosans) (Ramos, 2003). The average content of hemicelluloses in wood is 25 – 35 %. Hardwood species contain in average 1.5 times more hemicelluloses than softwood species.

As compared to cellulose, hemicelluloses are low molecular weight polymers with a degree of polymerization of around 200 (Cowling, 1975; Sjoström, 1981). Hemicelluloses have more branches and are less crystalline than cellulose. They are readily hydrolysable predominantly to xylose in the case of hardwood, and to mannose in the case of softwood (Popa and Spiridon, 1998).

2.3.1.1 Xylan

Xylan is a kind of heteropolysaccharides. The main chain is composed of D-xylose, and the branches consist of L-arabinofuranose linked to the O-3 position of D-xylose residues and D-glucuronic acid or O-2-methyl-D-glucuronic acid linked to the O-2-position. Some of the D-xylose residues are acetylated (Yang *et al.*, 2004).

Xylan exists in the form of lignin-xylan complex in plant materials (Fengel, 1976). Xylan appears to be a major interface between lignin and other carbohydrate components in plant cell walls. The arabinoxylans are known to bind strongly to cellulosic polysaccharides and also bound with ferulic acid residues forming strong ester and ether linkages (Ford and Hartley, 1989; Hartley and Jones, 1976). Physical access to xylosic linkages in xylan

is restricted by the surrounding lignocellulosic components as well as substituents on its backbone.

Hardwood xylan is slightly branched and the degree of polymerization is 150 to 200. Further studies showed that the reducing end of xylan consists of a combination of xylose, rhamnose and galacturonic acid, the latter two being responsible for the alkali resistance of the xylan molecule (Johansson and Samuelson, 1977). Softwood xylans are arabino-4-O-methylglucuronoxylans. They differ from hardwood xylan in not being acetylated and in having arabinofuranose units linked by α ,1-3-glycosidic bonds. Softwood xylans have higher amounts of 4-O-methylglucuronic acid than hardwood xylans. Softwood xylans have shorter chain lengths (DP 70 to 130) than hardwood xylans and are less branched (Zinbo and Timell, 1967).

In spite of these general characteristics, the source from which the xylan is extracted strongly influenced the specific features with regard to the type, the amount, position and distribution of substituents over the xylan backbones (Huisman *et al.*, 2000). Furthermore, within one plant source, different populations of xylans may occur. For example, in arabinoxylan extracted from wheat flour rather high substituted populations as well as less substituted populations have been described (Gruppen *et al.*, 1993).

The alkali-extracted xylans are partially fractionated on the basis of solubility. Comparatively high molecular weight components, named 'hemicellulose A', precipitate when the alkali extract is acidified to about pH 4. The remaining soluble hemicelluloses, which are normally lower in molecular weight, precipitate by the addition of ethanol and are named 'hemicellulose B'. Interestingly, because of the physico-chemical properties of xylan such as solubility, rank intermediate between those of starch and cellulose, there is

potential for use of xylan as an industrial polymer. The segment of hardwood xylan is shown in Figure 2.6.

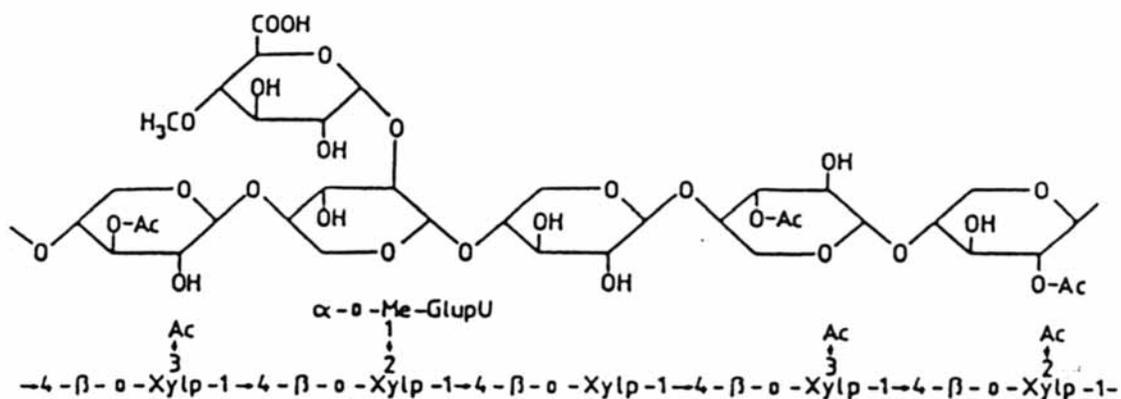


Figure 2.6 : Segment of hardwood xylan (Source : Bobleter, 1998)

2.3.1.2 Mannan

In some plant cell walls, glucomannans and galactomannans may be present as the major non – cellulosic hexosans. The glucomannans are comprised of (1→4)–β–linked glucose and mannose units, whilst the galactomannans are consist of a (1→4)–β–mannan backbone substituted with single units of (1→6)–α–galactose (Choct, 1997). The mannose units occur in combination with glucose or galactose units. The glucomannans are less soluble than the xylans in alkali. They are isolated first by an extraction with dilute alkali and then by an exhaustive extraction with strong alkali. Figure 2.7 shows the segment of softwood glucomannan.

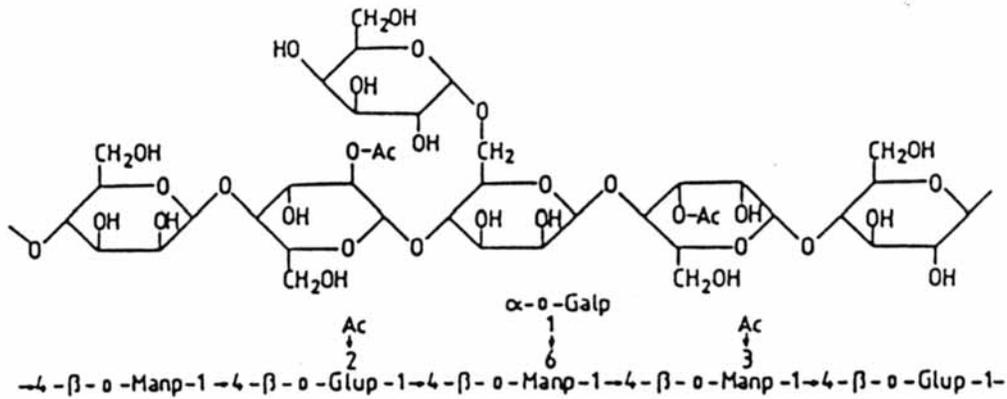


Figure 2.7 : Segment of softwood glucomannan (Source : Bobleter, 1998)

2.3.1.3 Arabinogalactan

The arabinans are polymers of (1→5)-α-L-arabinose residues branched through O2, O3 or both positions, whereas the galactans are polymers of (1→4)-β-D-galactose residues (Choct, 1997). Arabinogalactan is a water-soluble polyose. The distribution of the galactan within the tree is not uniform. It shows great similarity in its physical and chemical properties to natural gums and has therefore been of special interest for industrial application. The segment of larch wood arabinogalactan is shown in Figure 2.8.

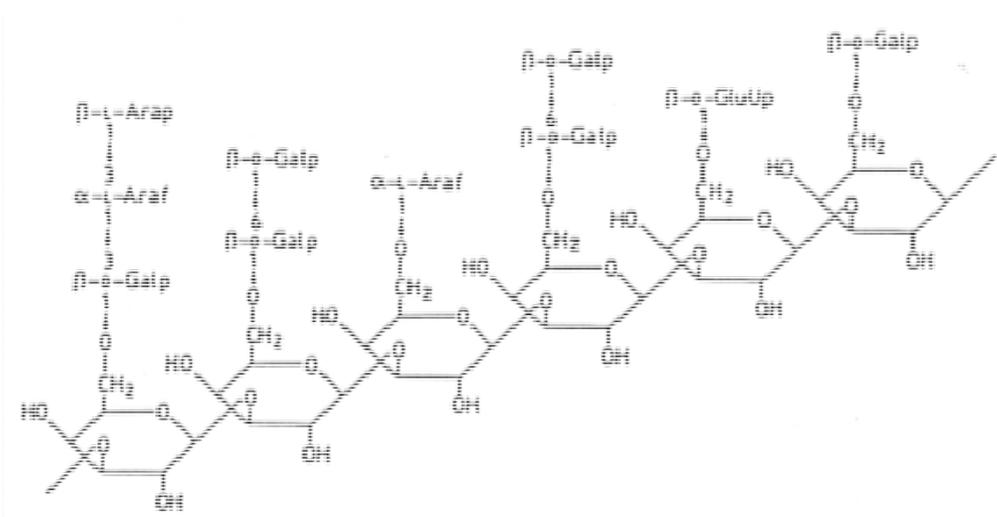


Figure 2.8 : Segment of larch wood arabinogalactan (Source : Bobleter, 1998)

2.3.2 Applications of Hemicelluloses

Pentosans (arabinoxylans) are the major non-starch polysaccharides in wheat and consist of xylan backbone with branches of arabinose residues in various linkages. The pentosans are known to play an important role in the water balance in dough (Courtin and Delcour, 2002), rheological properties of dough (Michniewiez *et al.*, 1991), retrogradation of starch (Gudmundsson *et al.*, 1991) and bread making quality (Delcour *et al.*, 1991; Shrogren *et al.*, 1987).

According to Popa and Spiridon (1998), hemicelluloses may be used as food additives, thickeners, gelling agents, adhesives and adsorbants. Lelli (2003) has used water-soluble hemicellulose as a thickener and stabilizer in confectionary ice-cream. Barnett *et al.*, (1990) has employed hemicellulose A, hemicellulose B and modified hemicellulose A and B as water-soluble bulking agents to replace the functional properties of carbohydrates or fats. Hemicellulose B extracted from wheat bran also has been used to inhibit ice crystal growth (Barnett, 1990). Thus, derivatives of xylans (acetate, butyrate and benzoate) are used as extruders for fatty acids. Arabinoxylans are used as thickeners or stabilizers in food, cosmetic or pharmaceutical industries.

Glucomannans are used in food industry (as carrier substituent), whereas arabinogalactans have applications in the mining (for processing of iron and copper ores) or pharmaceutical industry (as a tablet binder or emulsifier). The gels obtained from different hemicelluloses find applications in cosmetic, pharmaceutical and photographic industries. Hemicelluloses can be successfully used as binders or adhesives in papermaking, and as a retention aids for fibers (Popa and Spiridon, 1998). However,

bioconversion of hemicelluloses to useful chemicals required first hydrolysis of the polysaccharides to their sugar components.

Höije *et al.*, (2005) was studied the method for isolating arabinoxylans from barley husks in order to get a product with high molecular weight that could be used for the preparation of oxygen barrier films or coatings. Haq and Gomes (1977) have isolated a xylan from pulp fruit of date balm and Ishurd *et al.*, (2002) have purified a linear glucan which shows mixing linkages, (1–3)- and (1-4)-. More recently, Ishurd *et al.*, (2004); Ishurd and Kennedy (2005) reported that this glucan was found to exhibit potent antitumor activity.

2.3.3 Cellulose

Cellulose is the most abundant organic compound in nature, comprising over 50 % of all the carbon in vegetation. Cellulose is believed to be identical in chemical composition regardless of the source and it is insoluble in water and aqueous solutions in alkalis (Choct, 1997). Cellulose makes up 40 – 45 % of wood depending on species and growing conditions and is the most important component of wood in papermaking. The term cellulose is also used more loosely in a technological context to mean the residues obtained when materials of plant origin are subjected to certain pulping processes (Myasoedova, 2000).

Cellulose is a linear homopolysaccharide that consists of glucose (D-glucopyranose) units linked together by β -(1-4)glycosidic bonds (β -D-glucan). Normally, the size of the cellulose molecule is given in terms of its degree of polymerization which is the number of anhydroglucose units present in a single chain. Typical degree of polymerization of cellulose in wood is 8 000 – 10 000. The DP depends on the source and history of the

sample (Myasoedova, 2000). Long molecules of cellulose form microfibrils, which in their turn form the structure of a cell wall (fibre wall).

The chains are stiffened by Van der Waals forces and by inter- and intra molecular hydrogen bonding. Single chains never exist under natural conditions but occur in the form of microfibrils which consists of many ordered parallel chains (Myasoedova, 2000). These structures give cellulose a rigid, strong, dense, partially crystalline, chemically and enzymatically resistant nature.

Cellulose can exist in more than one crystalline form with different orientation of parallel chains relative to each other. The most common crystalline form in nature, cellulose I is metastable. Dissolution and reprecipitation lead to the stable form, cellulose II (Myasoedova, 2000). This form is manufactured commercially and sold as rayon. However, some cellulose (roughly 10 %) can also exist in an amorphous state. The cellulose fibrillous structure is shown in Figure 2.9.

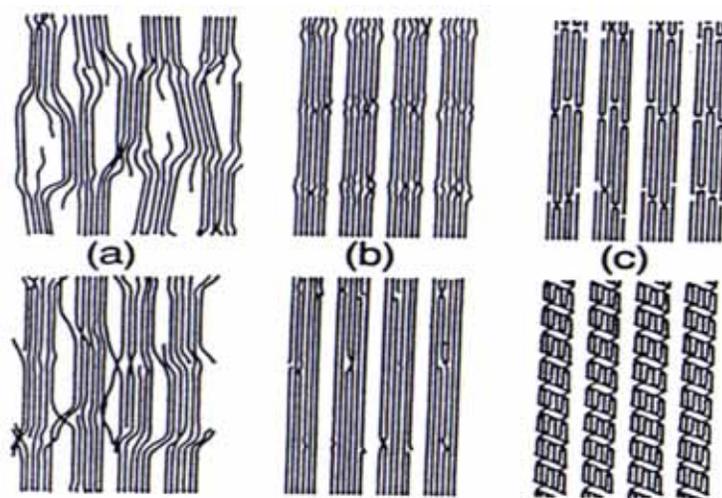


Figure 2.9 : Cellulose fibrillous structures : (a) low crystallinity; (b) high crystallinity; (c) folded models (Source : Fengel & Wegener, 1989)

To degrade cellulose, water temperatures of $\sim 250^{\circ}\text{C}$ or strong acid are needed. The enzymatic attack requires specific pretreatment methods, otherwise the saccharification yields are dramatically low (Bobleter, 1998). Figure 2.10 shows the stereo chemical formula of cellobiose and cellulose.

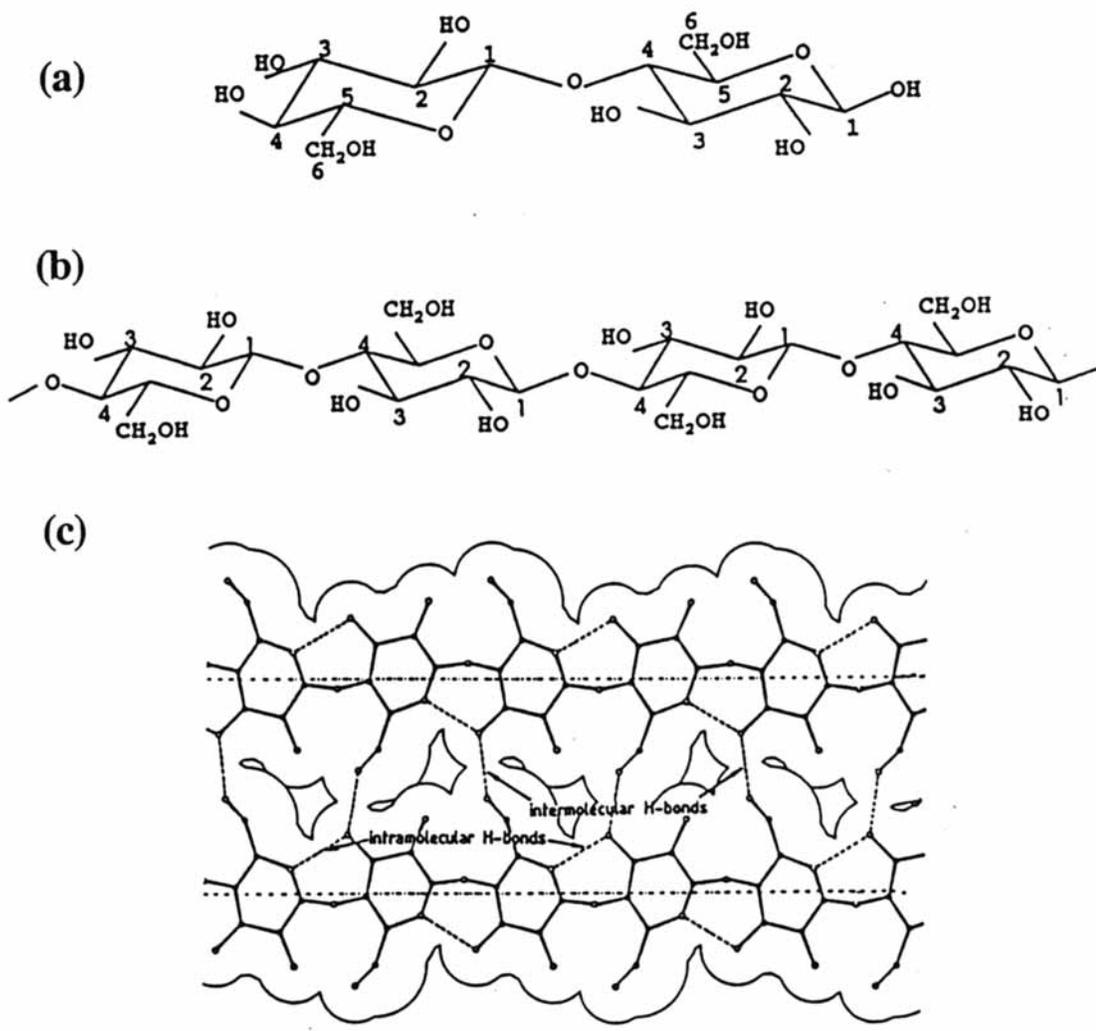


Figure 2.10 : Stereo chemical formula of cellobiose and cellulose. (a) Cellobiose; (b) Segment of cellulose; (c) Two sections of cellulose chains and their intermolecular and intramolecular bonds (Source : Bobleter, 1998).

2.3.4 Lignin

Lignin is an aromatic biopolymer, which is highly insoluble in water. It is responsible for the structural adhesion of the plant cell wall components because it excludes water when it forms networks by cross-linking with other saccharide-type molecules in plants (Nelson, 2001). Lignin is very resistant to chemical, enzymatic and bacterial breakdown. It is a very rigid component and is most associated with the structural integrity of wood. Lignin content in wood can vary from 20 to 35 % (Bobleter, 1998).

The structural units of lignin are various derivatives of phenyl propane with various bonding combinations (Erdtman, 1957). Lignin is built up by oxidative coupling of three major C₆ – C₃ (phenylpropanoid) units, namely syringyl alcohol, guaiacyl alcohol, and *p*-coumaryl alcohol, which forms a randomized structure in a tri-dimensional network inside the cell walls (Xioa *et al.*, 2001). Two main types of lignin units are guaiacyl (with one methoxyl group in phenol ring) and syringyl (with two methoxyl groups).

The structure of lignin is believed to vary between cell wall layers and between separate morphological regions of the tree. According to Christiernin *et al.*, (2005); generally middle lamella and outer cell wall layers contain more guaiacyl units than the secondary cell wall of hardwoods. The lignin of softwoods is mainly (almost exceptionally) guaiacyl units while hardwoods contain both guaiacyl and syringyl. Besides the 20 different types of bonds present within the lignin itself, lignin seems to be particularly associated with the hemicellulosic polysaccharides. Figure 2.11 shows the lignin precursors ; (I) = *p*-coumaryl; (II) = coniferyl and (III) = sinapyl alcohol.