CHARACTERIZATION OF PHYSICO-CHEMICAL AND FILM PROPERTIES OF HEMICELLULOSES B FROM OIL PALM FROND (*ELAEIS GUINEENSIS*)

by

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TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION

CHAPTER 2: LITERATURE REVIEW

2.1. Oil Palm in Malaysia

2.2. Oil Palm Biomass

2.3. Lignocellulosic Material

2.5. Fractionation of Hemicelluloses

2.5. Biological Polymers as Packaging Materials

CHAPTER 3: MATERIALS AND METHODS

3.4. Composition Analysis

3.5. Structure Determination

CHAPTER 4: RESULTS AND DISCUSSION

4.4. Structure Determination

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

KOH	Potassium Hydroxide
EFB	Empty Fruit Bunches
OPT	Oil Palm Trunk
OPF	Oil Palm Frond
HA	Hemicelluloses A
HB	Hemicelluloses B
HC	Hemicelluloses C
DP	Degree of polymerization
DMSO	Dimethyl sulfoxide
SEM	Scanning Electron Microscopy
WVP	Water vapour Permeability
DSC	Differential Scanning Calorimetry
GRAS	Generally Recognized As Safe
ASTM	American Society for Testing and Materials
TEM	Transmission Electron Microscopy
FFB	Fresh Fruit Bunches
D2O	Deuterium Oxide

PENCIRIAN FIZIKOKIMIA DAN SIFAT-SIFAT FILEM HEMISELULOSA B DARI PELEPAH KELAPA SAWIT (*ELAEIS GUINEENSIS*)

ABSTRAK

Hemiselulosa B (HB) diekstrak dari pelepah kelapa sawit (OPF) dengan menggunakan tiga kepekatan alkali yang berbeza (1.0, 2.0 and 3.0M KOH) pada suhu 40 ^{o}C selama 4 jam. HB yang diekstrak kemudian dibuat pencirian melalui analisis komposisi gula, asid uronik, berat molekul, struktur molekul, penghabluran dan kestabilan termal. Keputusan menunjukkan peningkatan kepekatan KOH dari 1.0 ke 2.0 dan 3.0 M menyebabkan peningkatan ekstrak HB dari 5.0 ke 8.7 dan 9.5 %. Walaubagaimanapun, kandungan lignin setiap ekstrak HB menunjukkan tren yang berlawanan. Analisis komposisi monosakarida menunjukkan 1.0M HB mempunyai komposisi arabinosa yang tinggi berbanding xilosa dan glukosa manakala 2.0 dan 3.0M HB mempunyai komposisi xilosa dan glukosa yang lebih tinggi berbanding arabinosa. Nisbah xilosa/arabinosa menunjukkan peningkatan nilai dari 0.8 ke 1.6 bagi ekstrak 1.0 ke 3.0M HB. Semua ekstrak HB di dapati mempunyai peratusan kecil asid uronik. Data FTIR dan 13C-NMR menunjukkan kepekatan alkali yang digunakan tidak mengubah struktur molekul HB dan struktur rantai yang dicadangkan adalah L-arabino-(4-O-methyl-D-glucurono)-D-xylan. Peningkatan kepekatan alkali di dapati tidak mempengaruhi berat molekul HB. Data dari analisis penghabluran dan kestabilaan terma menunjukkan peningkatan kepekatan alkali akan meningkatkan nilai penghabluran dan kestabilan terma HB. Semua ekstrak HB menunjukkan kebolehan untuk membentuk filem. Ketebalan filem HB adalah di dalam julat 0.13 – 0.14 mm. Data yang diperolehi untuk filem HB bagi kekuatan mekanikal adalah di dalam julat 11.22 – 13.18 MPa, 1.5 – 2.2% untuk

pemanjangan, 96.5 – 99.2 % kelarutan di dalam air, 8.3 – 14.0% untuk kandungan air dan 8 x 10^{-11} - 1.15 x 10^{-10} $gm^{-1}s^{-1}Pa^{-1}$ untuk ketelapan wap air. Analisis permukaan filem HB dengan SEM menunjukkan permukaan yang sekata dan licin untuk 1.0M HB manakala permukaan yang agak kasar di dapati untuk 2.0 dan 3.0M HB. Berdasarkan analisis keseluruhan untuk pencirian dan sifat-sifat filem HB yang di ekstrak dengan kepekatan berbeza, dapat disimpulkan kepekatan alkali yang berbeza memberikan kesan kepada sifat-sifat fiziko-kimia dan sifat filem HB.

CHARACTERIZATION OF PHYSICO-CHEMICAL AND FILM PROPERTIES OF HEMICELLULOSES B FROM OIL PALM FROND (*ELAEIS GUINEENSIS*)

ABSTRACT

Hemicelluloses B (HB) from oil palm frond (OPF) were extracted using three alkaline concentration (1.0, 2.0 and 3.0M KOH) at 40 °C for 4 hours, respectively. HB extracted was then characterized based on monosaccharide composition, uronic acid, molecular structure, molecular weight, crystallinity and thermal stability in order to study the effect of different alkaline extraction on HB from OPF. HB extracted from all alkaline concentrations were casted and their film properties were investigated. The increase in KOH concentration from 1.0 to 2.0 and 3.0 M, were observed to increase the yields of each fraction from 5.0 to 8.7 and 9.5 %, respectively. However, lignin content displayed a reverse trend. Monosaccharide composition analysis showed that 1.0 M HB had a higher composition of arabinose compared to xylose and glucose, while higher composition of xylose and glucose was detected for 2.0 and 3.0 M HB. Ratio of xylose/arabinose also increased from 0.8 to 1.6 for 1.0 M to 3.0M HB, respectively. In addition, all HB extracts also contained a significant percentage of uronic acid. FTIR and 13C-NMR data showed that alkaline concentration used did not alter the integrity of the HB chain and the structure proposed is L-arabino-(4-O-methyl-D-glucurono)-D-xylan. Increased KOH concentration did not result in significant difference in average molecular weight (M_w) of HB extracts. Data from relative crystallinity and thermal stability analysis suggested that increased KOH concentration to extract HB would result in higher crystallinity and thermal stability. All HB extracts were observed to produce

self-supporting films. Film thickness ranged from 0.13 - 0.14 mm. Data measured for the HB films were 11.22 – 13.18 MPa for tensile strength, 1.5 – 2.2 % for elongation, 96.5 - 99.2 % for solubility in water, 8.3 - 14.0% for water content and 8×10^{-11} - 1.15×10^{-10} $gm^{-1}s^{-1}Pa^{-1}$ for water vapor permeability (WVP). SEM micrograph of film surface revealed a uniform and smooth surface for 1.0M HB film, on the contrary with 2.0 and 3.0M HB films which exhibit a rougher surface. Based on the overall analysis of characterization and film properties of HB extracted with different concentration of alkaline, it can be concluded that different alkaline concentration have effect on the physico-chemical and film properties of HB.

CHAPTER ONE

INTRODUCTION

1.1. Research background

Production and utilization of edible, biodegradable films and coatings prepared from various biological polymers such as polysaccharides, proteins, lipids or combinations of those components have received great interest in recent years (Kayserilioglu *et al*., 2003). Up to now, the common polysaccharides of interest for renewable packaging material production have been cellulose, starch, gums, chitosan, xanthan, pullulan and curdlan (Weber, 2001). Hemicelluloses, although they are as abundant as cellulose in many plants; they have not been fully commercially utilized as a source for edible/biodegradable films.

Lately, there is an increasing interest in the potential application of hemicelluloses both in food and non-food areas. Promising results have been obtained in the field of papermaking, baking and food additives (Ebringerova and Heinze, 2000). Film-forming properties of hemicelluloses have also been studied, and the results have been promising (Gabrielii and Gatenholm, 1998; Gabrielii *et al*., 2000; Kayserilioglu *et al*., 2003; Zhang and Whistler, 2004; Hoije *et al*., 2005 and Hartman *et al.*, 2006). Hemicelluloses extracted from Aspen wood (Gabrielii *et al*., 2000) did not form a free standing film, on the contrary with hemicelluloses from barley husks (Hoije *et al*., 2005). Mean while, plasticized hemicelluloses film developed by Zhang and Whistler (2004) from corn hull shows antiplasticization properties. Thus it can be suggested that hemicelluloses extracted from different sources will give different film properties.

1.1.2. Hemicelluloses from oil palm frond

The oil palm industry in Malaysia is over a century old. Introduced as an ornamental in 1871, the oil palm was commercially exploited as an oil crop only from 1917 when the first oil palm estate was established (Yusof *et al.,* 2000). Today it is the largest in agricultural plantation sector. In terms of hectare, the total area under oil palm cultivation is over 2.65 million hectares, producing over 8 million tonnes of oil annually. The oil consists of only 10% of the total biomass produced in the plantation. The remainder consists of huge amount of lignocellulosic materials such as oil palm fronds, trunks and empty fruit bunches.

It is estimated that about 30 million tonnes of fibrous biomass can be generated from empty fruit bunches, palm press fibre, fronds and trunks (Khoo and Chandramohan, 2002). The fibrous biomass is a renewable natural resource that can be exploited. Therefore, a huge potential exists in commercialization of oil palm biomass. The industry can practice zero waste policy by utilizing and converting the biomass into value-added products. This biomass will become a new source of raw material which can be considered as an environmentally friendly material (Abdul Razak and Mohd Nor, 2000). Commitment to zero waste has in fact led to the creation of new products from oil palm, using fronds, trunk, bunch wastes and refinery distillate.

Among the above mentioned residues, oil palm frond (OPF) contributes 70% of the overall oil palm industry waste in Malaysia (Eng *et a*l., 2004). For that reason it has been chosen as the raw material in this study. Almost all pruned fronds are discarded in plantation, mainly for nutrient recycling and soil conservation (Wan Zahari *et al*., 1980).

Chemically, OPF consists of four main components; celluloses, hemicelluloses, lignin and extractives. Hemicelluloses are usually defined as the polysaccharides part of plant tissue, which is accessible to the actions of dilute acids and alkalis. Hemicelluloses can be further isolated by fractionation to Hemicelluloses A (HA), Hemicelluloses B (HB) and Hemicelluloses C (HC). However, according to Doner and Hicks (1997), HB is potentially the most useful fraction to be used in food and industrial applications

Zhang and Whistler (2004) also reported further advantages of HB, are the potential health benefits of improving lipid metabolism and mineral balance, improving colon function, protecting against colon cancer, reducing the risk of heart disease, and improving general body health. Therefore, development of edible films with HB may provide alternative ways for consumers to consider this potential dietary nutrient and hence would be of interest to the food industry.

Presently, no study has been done on the characterization and film forming properties of alkaline extracted hemicelluloses, specifically hemicelluloses B (HB) from oil palm frond. Therefore this study will give a better understanding of physico-chemical properties, structural composition and film properties of HB from OPF which will open up possibilities for new opportunities in food applications.

1.1 Research objectives

The general objective of this study is to develop an edible/biodegradable film of HB from OPF extracted with different concentration of alkaline. In view of the fact that physico-chemical and films properties are related, understanding of this relationship is important in order to fully utilize the material. Specific objectives of this research are:

i. To extract and to quantify the yield of hemicelluloses B from OPF without delignification process using three different concentration of alkaline;

ii. To characterize the physico-chemical properties of HB extracted from different alkaline concentration by monosaccharides composition, uronic acids, molecular weight, molecular structure, crystallinity and thermal stability;

iii. To develop and evaluate film forming properties of HB extracted from different alkaline concentration.

CHAPTER TWO

LITERATURE REVIEW

2.1. Oil Palm in Malaysia

2.1.2. Botanical description

Oil palm is a perennial tree crop, which is cultivated extensively in the humid tropical land. Climatic conditions in Malaysia which include a tropically humid climate throughout the year, ample sunshine and good rainfall patterns are ideally suited for oil palm cultivation. The *Tenera* is the common types of oil palm species grown in Malaysia. It is a cross between a *Dura* and a *Pisifera* species (MPOPC, 2003).

Oil palm can reach 60 - 80 feet in height in nature, but is rarely more than 20 or 30 feet in cultivation. The trunks are stout, straight, and grow about 50 - 70 cm per year. Leaf color is green, and leaf bases are persistent for years. Old or dead leaves snap and remain attached on wild palms, but are pruned off for cultivated palm to facilitate harvest in plantations. Leaves are up to 6-8 m in length, with leaflets numbering 200 - 300 per leaf. The fruits are oval in shape, and it ripens about 5-6 months after pollination (Rieger, 2006).

Oil palm tree annually bears 8 – 12 fruit bunches each weighing between 15 – 25 kg. Each fruit bunch carries 1000 – 3000 fruits. Each palm tree produces about 40 kg of palm oil a year (MPOPC, 2003). Average planting cycle of a palm tree is about 25 years for efficient productivity (Sumiani, 2004).

2.1.1. History and Development

Oil palm is the golden crop that has helped to change the scenario of Malaysia's agriculture and the Malaysian economy (Dahlan, 2000; Sumiani, 2004; Yusof and Chan, 2004; Kamarulzaman *et al.*, 2004). In 1763, Nicholaas Jacquin produced one of the earliest illustrations of the oil palm tree (Figure 2.1) and thus he is remembered in its scientific name '*Elaeis guineensis* Jacq' (Janice and Daphne, 2000).

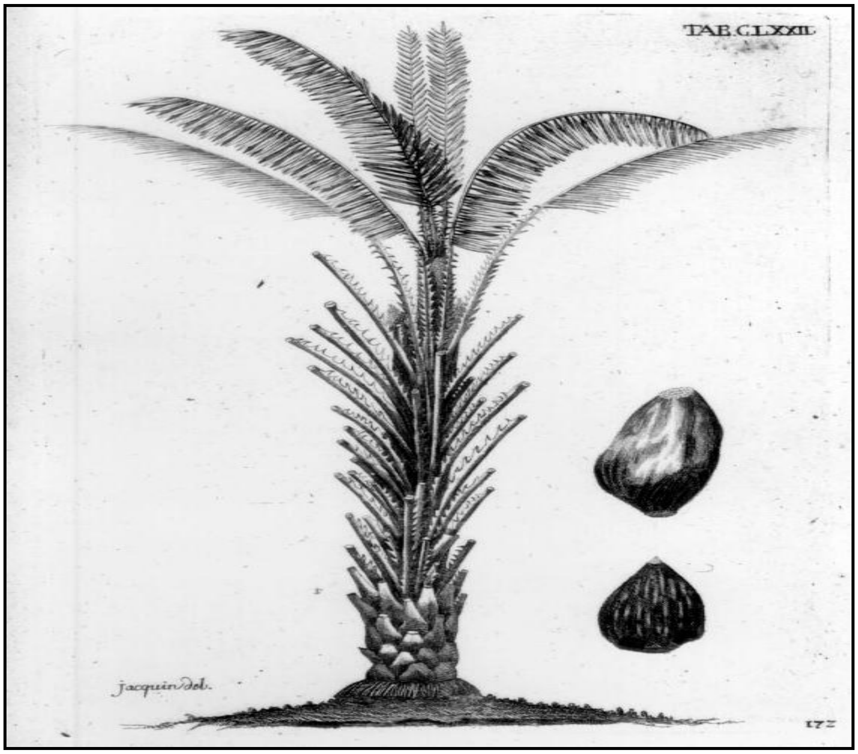


Figure 2.1: The earliest illustration of the oil palm (Janice and Daphne, 2000)

The oil palm industry in Malaysia had a humble beginning. It all started from a mere four original palms introduced from West Africa to the Bogor Botanical Gardens, Indonesia in 1848, their seeds soon arrived on Malaysian shores in 1871 (Yusof and Chan, 2004). In 1911, the first palms (*Deli Dura*), were planted in Malaysia as ornamental plants (MPOPC, 2003). The R&D undertaken soon showed the potential of the new crop. Following this, the first commercial planting was done in 1917 at Tennamaran Estate, Kuala Selangor (Yusof and Chan, 2004).

During the late 1950s, the expansion of the industry started as part of government’s diversified cautious policy from rubber to oil palm and also to raise the socio-economic status of the expanding population in the country (Sumiani, 2004). Oil palm areas expanded from a mere 54,000 hectares in early 1960s to 3.4 million hectares in 2004. In the next 10 years, the present oil palm hectarage in Malaysia is forecast to increase to 4.3 million hectares, and by 2020 Malaysia is expected to have 4.6 million hectares of oil palm (Yusof and Simeh, 2005). With the expansion of oil palm area, the total biomass produced by this industry also increased, and thus providing an abundant renewable natural resource that can be exploited.

Oil palm biomass has great potential to be converted into high value-added and useful income-generating products. Basiron and Simeh (2005), estimated that effective utilization of oil palm biomass has the potential to generate another RM20 billion to Malaysian palm oil industry. Apart from that, utilization of the oil palm biomass effectively could help to achieve zero-waste strategy in the Malaysian palm oil industry and thus preserve a clean environment (Kamarulzaman *et al*., 2004).

2.2. Oil Palm Biomass

Oil palm is a prolific producer of biomass. Palm oil and palm kernel oil constitutes only about 10% of the palm production while the rest 90% is oil palm biomass (Basiron, 2000). Oil palm biomass consists of trunks, fronds, empty fruit bunches, fibre, shell and effluent. The first two biomass, trunks and fronds were obtained from oil palm growing in the plantations and the remaining four from palm oil processing (Chan, 1999). The oil palm industry generates more than 30 million tonnes (dry weight) of empty fruit bunches, oil palm fronds and oil palm trunk (Basiron, 2000; Yusof and Simeh, 2005).

2.2.1. Empty Fruit Bunches (EFB)

Oil palm EFB is one of the main residues of oil palm mills in Malaysia. At palm oil mills, EFB are produced when fruits are removed from bunches prior to the oil extraction process of the fruits. Annual production of the EFB in Malaysia was estimated to be about 8.5 million tons (PORLA 1997). The conventional method for disposal of the material is by leaving it to degrade in oil palm plantations, or termed as mulching. Besides that, it is also incinerated to produce ash as potash fertilizer for oil palm (Sameni, 2000). However, incineration can cause pollution to the environment, and to reduce this practice, studies have been made to utilize EFB. Research have found that EFB can been utilized as a raw material for value added products such as pulp for paper making, and fibre board in the wood industry (Chan, 1999).

2.2.2. Oil Palm Trunk (OPT)

Oil palm trunks are available only when the economic life-span of the palm is reached at the time of replanting, which is the average of 25 years (Chan, 1999). According to Lim *et al*., (1997), most of the felled trees are sectioned and either left to rot on the plantation grounds or piled into stacks for burning. Instead of these practices, OPT can now be converted into various value-added product.

Yusof and Simeh (2005) reported that 60 000 - 70 000 ha of oil palm plantations are replanted annually and this will give yield to 8 - 9.5 million of oil palm trunks. OPT has been utilized as a cellulosic raw material in the production of panel products such as particle board (Chan, 1999). OPT also can be made as plywood. Oil palm plywood has the advantage of being lightweight and environmentally friendly, thus it's readily acceptable to overseas buyers (Basiron and Simeh, 2005).

2.2.3. Oil Palm Frond (OPF)

Oil palm frond is the leaf-like part produced continuously from the oil palm plant (Figure 2.2). OPF grows in tightly clustered bunches or heads. Unlike the trunk, OPF are obtained during felling on replanting and at pruning during harvesting time. Pruning is the process of cutting fronds (usually 2-3 fronds per palm tree), and it was done to facilitate harvesting since fresh fruit bunches (FFB) are compactly packed and hidden in the leaf axils (Dahlan, 2000).

The weight of fronds obtained from pruning during harvesting varies considerably with the age of the palm (Mohamad *et al*., 1986). Chan *et al.*, (1981) reported that 24 fronds are pruned annually, and the average weight from annual pruning is 82.5 kg of fronds/palm/year. On the other hand, during replanting, each palm tree would give approximately 115 kg of dry fronds (Chan *et al.*, 1981). According to Dahlan (1996), the total production of dried OPF is about 8.2 millions t/yr, and this amount is increasing every year.

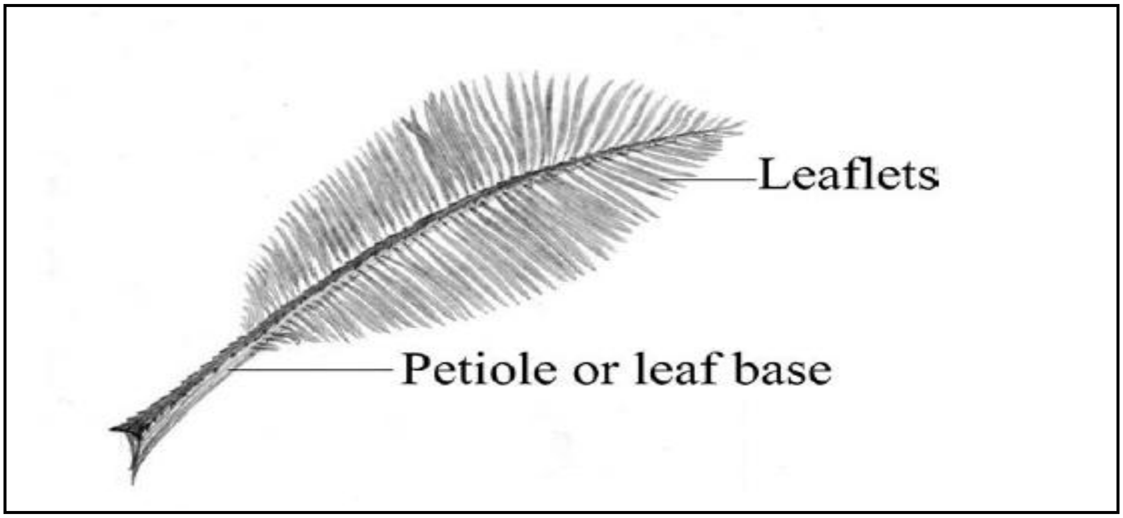


Figure 2.2: Oil palm frond drawing (Daniel, 2000)

The production of felled fronds is only about 20% of the trunks, but unlike pruned fronds, they are concentrated in a replanted area together with the trunks. Thus, felled fronds intensify the problems created by felled trunks (Mohammad *et al*., 1986). In order to avoid this problem, OPF needs to be shredded and pulverized mechanically to enhance the decomposition. However, this will not only add to the cost, but also requires intensive labour (Dahlan, 2000). Therefore, OPF had been studied to be used as various value added-products such as animal feed (Dahlan, 2000), paper (Wan Rosli *et al.*, 2007), and feedstock for production of ethanol and furfural (Yusof and Sameh, 2005).

2.2.3.1. Anatomy

OPF structure can be represented by the basic model of wood cell wall as shown in figure 2.3. The wood cell walls are composed of an intercellular layer, primary and secondary wall. These layers differ from one another with respect to their morphology as well as their chemical composition. Between the cells, middle lamella (ML) acts as a glue to join the cells together. Secondary wall is a thick layer deposited inside the thin layer of primary wall (Abdul Khalil *et al*, 2006). Secondary wall consists of three layers; outer layer (S_1), middle layer (S_2), and inner layer (S_3) (Mazlan, 1998).

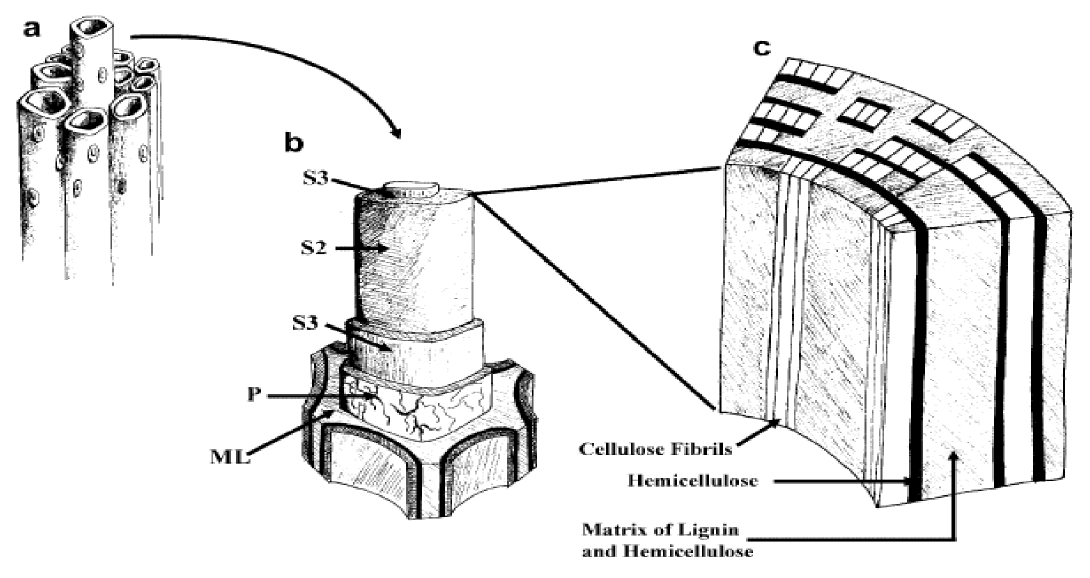


Figure 2.3 a–c: Configuration of wood tissues. a-adjacent cells, b-cell wall layers, S1, S2, S3 - secondary cell wall layers, P - primary wall, ML - middle lamella, c-distribution of lignin, hemicellulose and cellulose in the secondary wall (Perez, 2002).

Abdul Khalil *et al*., (2006) studied the chemical composition, anatomy, lignin distribution and cell wall structure of oil palm frond. In his study, Transmission Electron Microscopy (TEM) was used to view the walls of fibers within the vascular bundle. TEM results showed that OPF fibers contained various sizes of vascular bundles. The vascular bundles were widely imbedded in thin-walled parenchymatous ground tissue. Each bundle was made up of a fibrous sheath, vessels, fibers, phloem, and parenchymatous tissues (Figure 2.4).

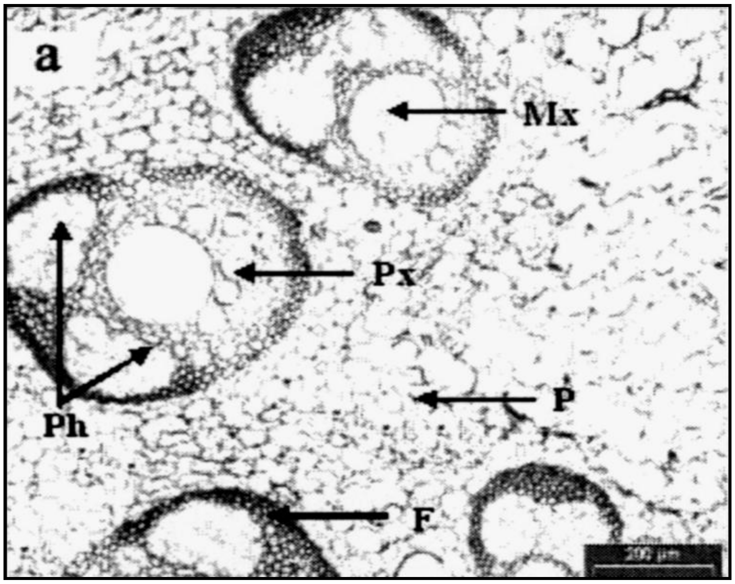


Figure 2.4: Transverse section of OPF fiber at low magnification (4x) (Abdul Khalil *et al*., 2006)

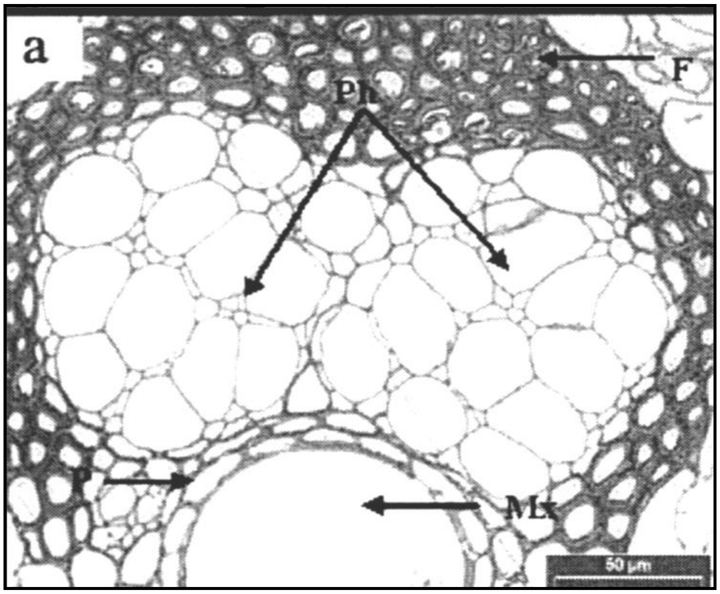


Figure 2.5: Transverse section of OPF at high magnification (20x). F=Fiber, P=Parenchyma, Mx=Metaxylem, Ph=Phloem (Abdul Khalil *et al*., 2006)

Xylem and phloem tissues are clearly distinguishable. Phloem was divided into two separate areas in each bundle. Some vascular bundles also contained several well-defined protoxylem elements. Protoxylem and metaxylem vessels in the bundle were separated by a layer of parenchyma cells (Figure 2.5).

TEM electron micrograph also proved that the layered structure of OPF contained primary (P) and secondary wall layers (S_1,S_2,S_3) (Figure 2.6). The primary wall appeared as the solid boundary of the cell, whereas middle lamella showed a clear transition to the adjacent primary wall layers. The total cell wall thickness is largely controlled by S_2 layer, because it is reinforced with microfibrils (Abdul Khalil *et al*., 2006).

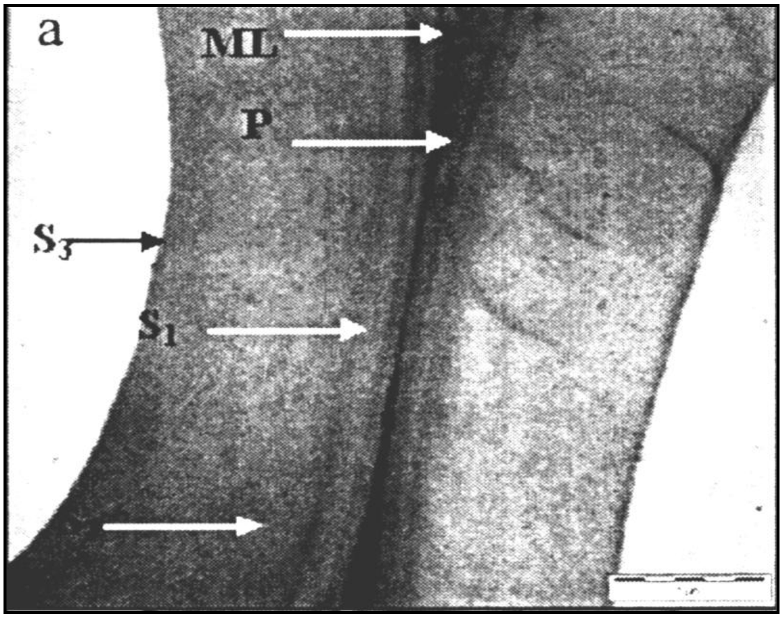


Figure 2.6: Transversed section of a multi-layered structure of OPF at high magnification (17,000x). ML=Middle Lamela, P =Primary wall, S_1, S_2, and S_3 =Secondary wall sub-layers (Abdul Khalil, *et al*., 2006)

Abdul Khalil *et al*. (2006) also observed that thick-walled fiber in OPF was strongly lignified after being stained with toluidine blue. The middle lamella showed a high lignification in OPF fibers. Phloem and parenchyma cells in OPF (Figure 2.7), which consists only of a primary wall, were unlignified, as shown by weak positive reaction with toluidine blue. Therefore, in the OPF vascular bundle, fibers were more lignified than metaxylem vessels.

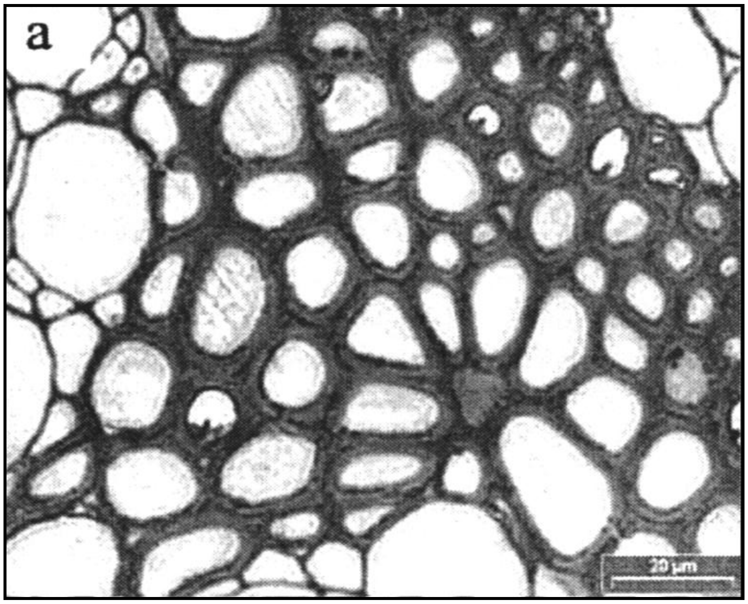


Figure 2.7: Transverse section of OPF after stained with toluidine blue at high magnification (40x) (Abdul Khalil *et al.,* 2006)

2.2.3.2. Chemical Composition

Oil palm frond (OPF) is a lignocellulosic material, which consists of cellulose, hemicellulose, lignin and extractives. The first three components have high molecular weights and contribute much mass, while the latter component is of small molecular size, and it is available in little quantity (Mazlan, 1998). The major carbohydrate portion of wood is composed of cellulose and hemicellulose polymers with minor amounts of other sugar polymers such as starch and pectin (Stamm, 1964). Softwoods (gymnosperms) and hardwoods (angiosperms) have been found to be different with regard to their chemical composition (Roger *et al*., 2005). Based on weight percentage, cellulose and hemicelluloses are higher in hardwoods compared to softwoods and wheat straw (Table 2.0), while softwoods have higher lignin content (Mazlan, 1998).

Table 2.0: Average chemical composition of softwoods, hardwoods and wheat straw (Thomas, 1977)

Chemical component	**Weight, % of dry material**		
	Softwoods	**Hardwoods**	**Wheat straw**
Cellulose	42 ± 2	45 ± 2	36 ± 5
Hemicellulose	27 ± 2	30 ± 5	27 ± 3
Lignin	28 ± 3	20 ± 4	11 ± 3
Extractives	3 ± 2	5 ± 3	26 ± 5

Generally, oil palm frond has low lignin and high holocellulose content (Table 2.1). Holocellulose is the combination of cellulose (40–45%) and the hemicelluloses (15–25%) and usually accounts for 65–70% of the wood dry weight. Lignin constitutes 23% to 33% of the wood substance in softwoods and 16% to 25% in hardwoods (Miller, 1999). Lignin content in OPF is lower than common hardwood; since oil palm trees are non woody, therefore the requirement for structural support is lower. OPF is less resinous, based on the low percentage of extractives soluble in alcohol-benzene. High percentage of ash in OPF was probably contributed by high silica content (Wan Rosli *et al.*, 2007).

Table 2.1: Chemical composition of oil palm frond (Wan Rosli *et al*., 2007)

Chemical Composition	**Dry Weight (%)**
Lignin	15.2
Holocellulose (*Hemicellulose+cellulose*)	82.2
α-Cellulose	47.6
Alcohol-benzene extractives	1.4
Ash	0.7
Polysaccharide composition	
Arabinose	1.5
Mannose	2.2
Galactose	0.9
Glucose	66.6
Xylose	28.9

2.3. Lignocellulosic Materials

2.3.1. Cellulose

Cellulose is the most abundant cell wall component in plants. The links in the cellulose long chain are β-D-glucose. Linking just two of these sugars produces a disaccharide called cellobiose, which is the repeat unit for cellulose chain (Mazlan, 1998) (Figure 2.8). The number of glucose units in a cellulose molecule is referred to as the degree of polymerization (DP). Molecular weight determinations done by light-scattering experiments indicate that wood cellulose has an average DP of at least 9,000–15,000 (Roger *et al*., 2005).

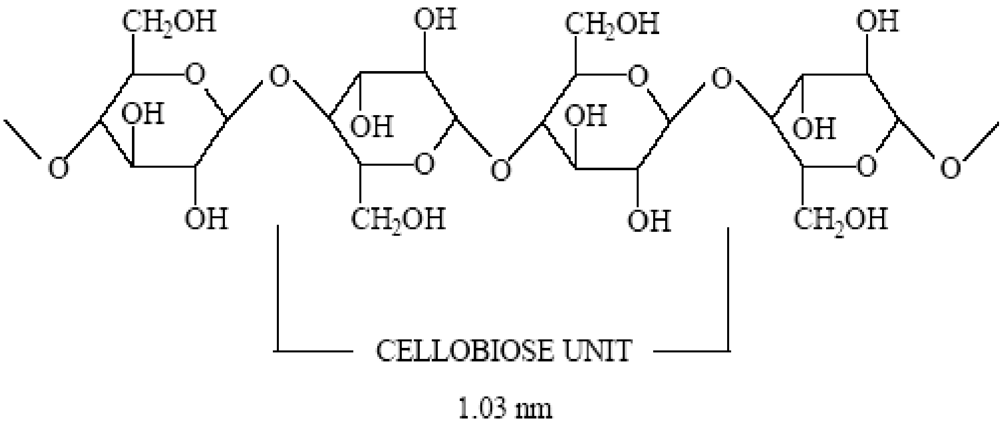


Figure 2.8: Schematic illustration of the cellulose chain (Mazlan, 1998)

Most wood-derived cellulose is highly crystalline and may contain as much as 65% crystalline regions. The remaining portion has a lower packing density and is referred to as amorphous cellulose (Roger *et al.*, 2005). Cellulose has a high tensile strength as a consequence of its fibrous structure and strong hydrogen bonds (Sjostrom, 1981). Example of some cellulose uses are as an anti-cake agent, emulsifier, stabilizer, dispersing agent, thickener, and gelling agent (Martin, 2005).

2.3.2. Lignin

Lignin is the main non-carbohydrate component (phenolic polymer) present in the mature plant cell wall that gives wood its characteristic brown color, density and mass (Armstrong, 2006). Lignin also often called the cementing agent that binds individual cells together (Miller, 1999). It is distributed throughout the secondary cell wall, with the highest concentration in the middle lamella (Roger *et al.*, 2005). Lignin is an amorphous, high molecular weight condensed polymer of phenylpropane units linked by carbon-carbon (C-C) and ether (C-O-C) bonds. The strong covalent bonds in the complex structure of lignin, makes lignin highly resistant to hydrolytic action of acid and alkali (Van Soest, 1982).

The monomeric building units of lignin are shown in Figure 2.9. The guaiacyl unit is dominant in the softwoods. In contrast, syringyl units are dominant in hardwoods (Roger *et al*., 2005). Besides the 20 different types of bonds present within the lignin itself, lignin seems to be particularly associated with the hemicellulosic polysaccharides (Xiao *et al*., 2001). Lignin can be isolated using several methods such as Klason lignin. Klason lignin is obtained after removing the polysaccharides from the extracted wood by hydrolysis with 72% sulfuric acid (Sjostrom, 1981).

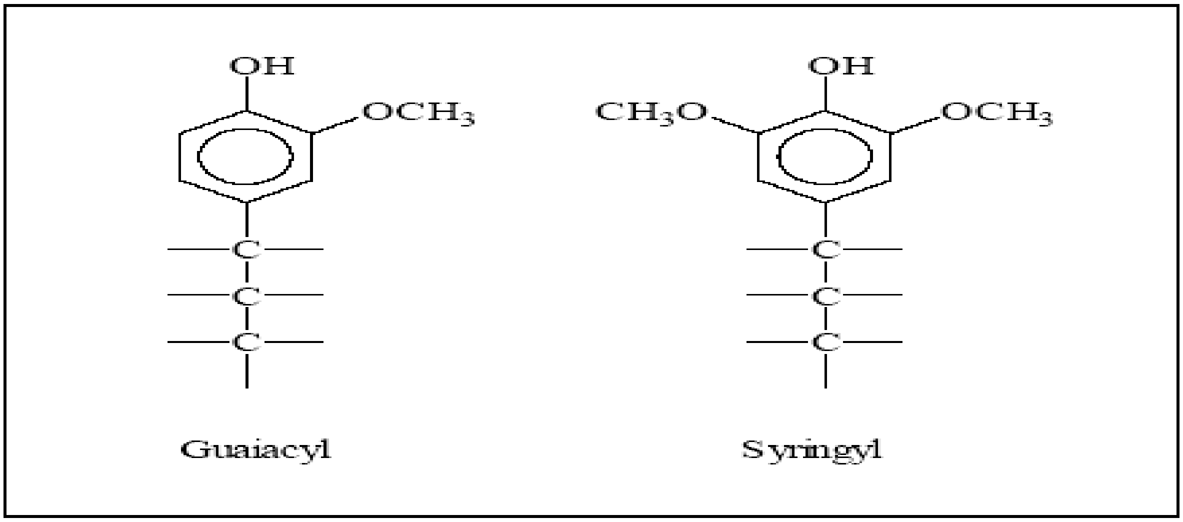


Figure 2.9: Schematic illustration of building units of lignin (Mazlan, 1998)

2.3.3. Extractives

Extractives are organic substances in the wood that can be extracted using solvents. It is a group of cell wall chemicals mainly consisting of fats, fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, waxes, and many other minor organic compounds (Roger *et al.*, 2005). They only represent between 4-10 % of the total weight of dry wood, and the contents of extractives vary among wood species, geographical site and season. The extractives can be found mostly in resin canal and ray parenchyma cells and small amount in middle lamella and cell walls of tracheids (Mazlan, 1998). In general, hardwoods have higher extractives content than softwoods (Table 2.0).

2.3.4. Hemicelluloses

Hemicelluloses rank second to cellulose in abundance in agricultural waste residues such as wheat straw, corn hulls, and oil palm biomass (Reddy *et al.*, 1984; Zhang and Whistler, 2004; Anis, 2000). It is usually defined as polysaccharides part of plant tissue, which are accessible to the actions of dilute acids and alkalis (Sun and Sun, 2002). In the cell wall, hemicelluloses have the main role of sticking and stabilizing the different components of the wall. They are natural plant rubbers; therefore they have good flexibility and mechanical properties (Gaspar *et al*., 2005).

Hemicelluloses are chemically complex and consist of homopolymer or heteropolymers backbone, such as arabinans, galactans, mannans, and xylans units (Sun and Sun, 2002). However, homopolymer of hemicelluloses are rare and only reflect special cases (Hatfield and Minor, 1996). The polymer chains of hemicelluloses have short branches and are amorphous. Because of the amorphous morphology, hemicelluloses are partially soluble or swellable in water. However, most of the hemicelluloses fraction is soluble in water after alkaline extraction (Doner and Hicks, 1997). Hemicelluloses have a much lower degree of polymerization (100–200) (Xiao *et al*., 2001) compared with that of cellulose (9,000–15,000) (Roger *et al*., 2005).

Figure 2.10 showed formula of the sugar components of hemicelluloses. The principle sugar components are D-xylose, D-mannose, D-glucose, D-galactose, L-arabinose, D-glucuronic acid, 4-O-methyl-d-glucuronic acid (MeGlcA), and D-galacturonic acid (Xiao *et al*., 2001). Although considerable differences in sugar composition and degree of substitution can be observed amongst various hemicelluloses present in softwoods and hardwoods, there are still similarities with respect to their physico-chemical properties and response to treatment.

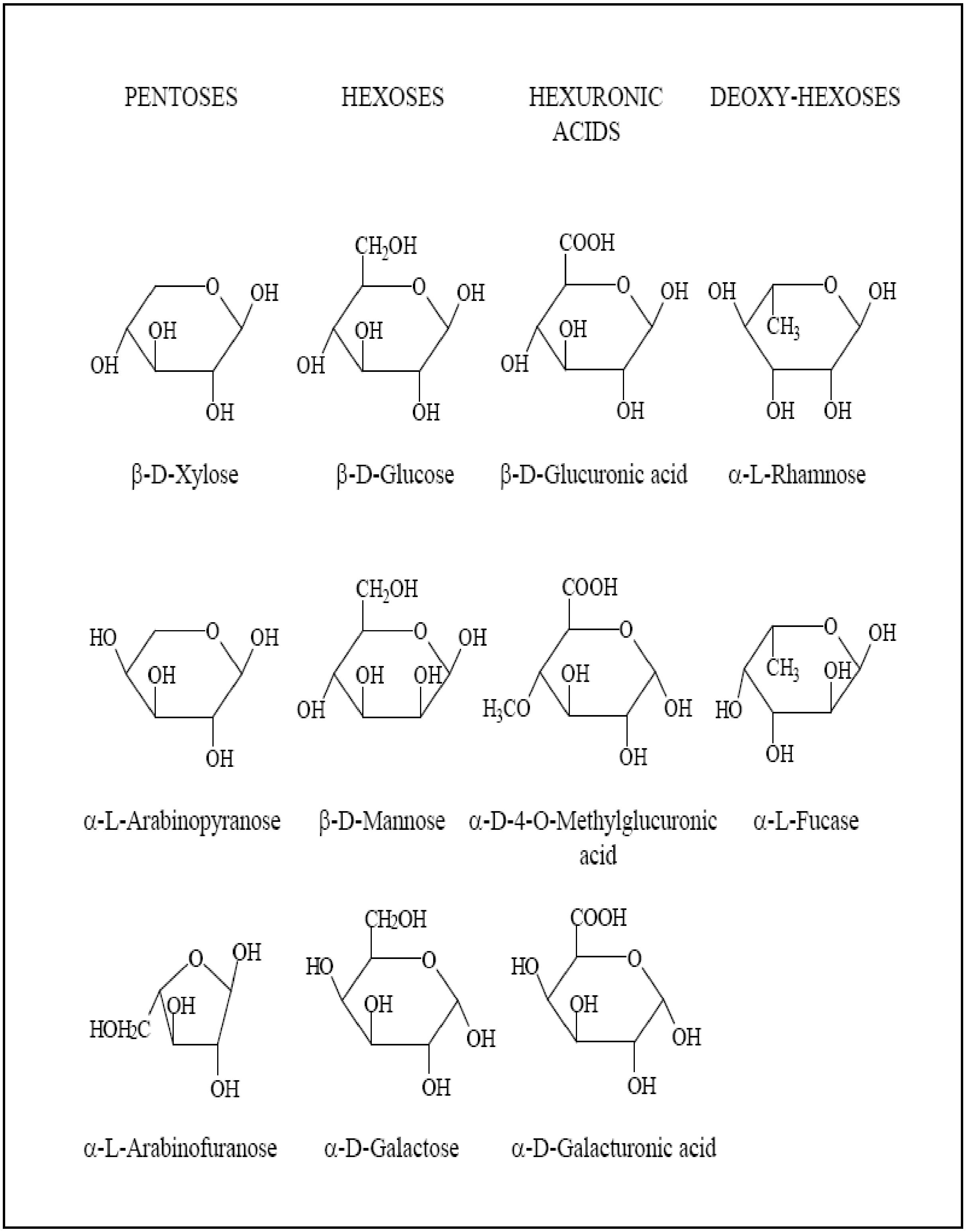
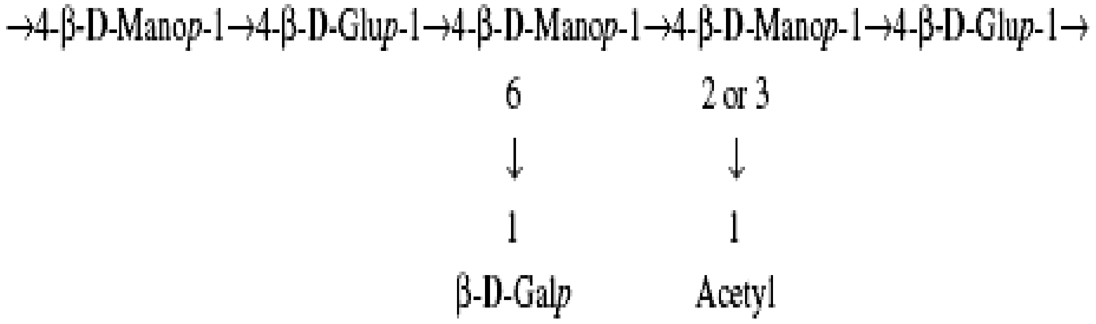


Figure 2.10: Schematic illustration of sugar units of hemicelluloses (Mazlan, 1998)

2.3.4.1. Softwood hemicelluloses

The composition and structure of the hemicelluloses in the softwoods differ in a characteristic way from those in the hardwoods (Sjostrom, 1981). Softwood hemicelluloses consist of glucomannan, galactoglucomannan, arabinan, and a small amount of arabino-(4-O-methylglucorono)-xylan (Popa and Spiridon, 1998). Galactoglucomannans are the major hemicellulose component in softwoods (20%) with a linear or possibly branched with β-(1→4) linkages (Figure 2.11). Glucose and mannose make up the back bone polymer with branches containing galactose (Roger *et al*., 2005). Galactoglucomannan can be roughly divided into two types; one with low galactose content, and the other with high galactose content (Laine, 2005). For softwood hemicelluloses, the acetyl groups are fewer in the backbone chain. Acetyl group are substituted at the C-2 and C-3 positions of the backbone polymer with an average of every three to four hexose units (Roger *et al*., 2005). However, softwood xylan has additional branches consisting of arabinofuranose units linked by α-(1,3)-glycosidic bonds to the backbone (Mazlan, 1998).



$$\begin{array}{ccccc}
\rightarrow 4\text{-}\beta\text{-D-Mano}p\text{-}1 & \rightarrow 4\text{-}\beta\text{-D-Glu}p\text{-}1 & \rightarrow 4\text{-}\beta\text{-D-Mano}p\text{-}1 & \rightarrow 4\text{-}\beta\text{-D-Mano}p\text{-}1 & \rightarrow 4\text{-}\beta\text{-D-Glu}p\text{-}1\rightarrow \\
 & & 6 & 2\text{ or }3 & \\
 & & \downarrow & \downarrow & \\
 & & 1 & 1 & \\
 & & \beta\text{-D-Gal}p & \text{Acetyl} &
\end{array}$$

Figure 2.11: Partial structure of a softwood arabino 4-*O*-methyglucoronoxylan (Roger *et al*., 2005)

2.3.4.2. Hardwood hemicelluloses

The most abundant hemicelluloses constituent of hardwoods is *O*-acetyl-(4-*O*-methylglucurono)xylan or often called xylan. Hardwood hemicelluloses also contain a minor amount of glucomannans, rhamnose and galacturonic acid integrated to the main xylan chain (Valentine and Luliana, 1998; Goksu, 2005). Depending on the hardwood species, the xylan content varies within the limits of 15-30% of the dry wood (Sjostrom, 1981).

In xylan, the backbone chain consists of xylose units which are linked by β-(1→4)-glycosidic bonds and branched by α-(1→2)-glycosidic bonds with 4-O-methylglucuronic acid groups (Roger *et al.*, 2005). Analysis of partial hydrolyzed xylan indicates that 4-O-methylglucuronic acid units linked to the C2 position are randomly distributed along the xylan backbone (Valentine and Luliana, 1998). In addition, O-acetyl groups sometime replace the OH-groups in position C2 and C3 (Figure 2.12).

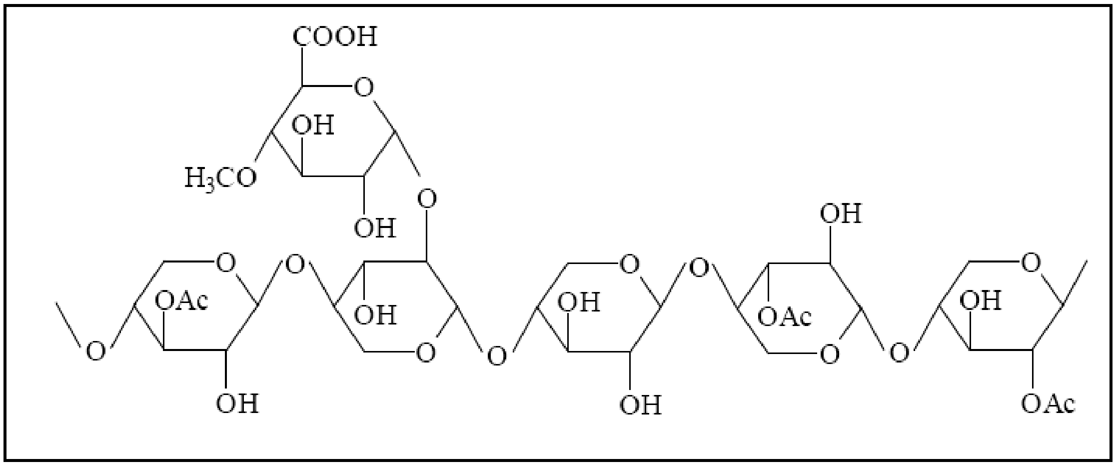


Figure 2.12: Partial xylan structure from hardwood chain (Mazlan, 1998)

2.4. Hemicelluloses extraction

Extraction of hemicelluloses can be made either from original wood or from extractive and lignin free wood (holocellulose). Previous literatures had shown that most of hemicelluloses were extracted from holocelluloses of biomass. This is because alkaline extraction from holocellulose gave higher yield of hemicelluloses compared to direct extraction from the biomass (Sun *et al*., 1996; Fang, *et al.*, 1999; Anis, 2000; Balaban and Ucar, 1999). It is known that ester and ether links which bind lignin to hemicelluloses obstruct the detachment of hemicelluloses chains from the cell wall matrix (Ebringerova and Heinze, 2000; Gubitz *et al*., 1998). Therefore, treatment of biomass by delignification liberates hemicelluloses from ether lignin-hemicelluloses linkage and resulted in higher yield of hemicelluloses. Removal of lignin prior to extraction would also result in lighter color of hemicelluloses (Doner and Hicks, 1997).

Nevertheless, it is difficult to isolate lignin-free hemicelluloses unless harsh treatment is applied during the isolation of hemicelluloses by delignification (Gabrielii *et al*., 2000). Usually, traditional hypochlorite bleaching process was applied for delignification where sodium chlorite is used as the bleaching agent (Fang *et al*., 1999; Balaban and Ucar, 1999). Nowadays, the use of elemental chlorine for delignification is becoming progressively more environmentally unacceptable. This is due to realization that chlorinated chemicals would lead to release of toxic and recalcitrant chlorinated aromatics such as dioxins (Perez, 2002; Sun and Tomkinson, 2002).

Buchala *et al*., (1972) also reported that up to 8% of the total hemicelluloses from different oat tissues were lost on delignification. Higher yield of hemicelluloses can be obtained prior to delignification, especially for softwood. Nevertheless, some hemicelluloses can be extracted directly in appreciable amounts such as arabinogalactans and hardwood xylans (Balaban and Ucar, 1999). Therefore, direct extraction from biomass will help to avoid possible negative effects of delignification such as oxidative changes, degradation and losses of hemicelluloses (Balaban and Ucar, 1999; Gabrielli *et al*., 2000).

Delignification would also result in degradation of hemicelluloses. Studies on chemical properties of hemicelluloses extracted directly with alkaline from de-waxed wheat straw and hemicelluloses extracted from holocellulose were done by Sun *et al*., (1995) and Sun *et al*., (1996). According to them, hemicellulosic fractions extracted from de-waxed straw before delignification had a high degree of polymerization with molecular-average weights between 28 000 and 40 900 Da (Sun *et al*., 1995). Mean while, hemicelluloses extracted from wheat straw holocellulose were degraded to 900 and 13100 Da (Sun *et al*., 1996).

Generally, methods for hemicelluloses extraction can be divided into methods using a high energy input (steam treatment), and methods in which chemicals are added for the dissolution and the temperature is usually only modestly raised (alkaline extraction) (Margaretha, 2003).

2.4.1 Steam Treatment/autohydrolysis

When wood is treated with steam, the temperature is raised and water is added. The increase in temperature leads to cleavage of the acetyl group in hemicelluloses, forming