EFFECT OF HYDROTHERMAL TREATMENT ON THE EXTRACTABILITY AND DIGESTIBILITY OF OIL PALM (*Elaeis guineensis Jacq.*) FRONDS DERIVED HEMICELLULOSE

by

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SABIHA HANIM BINTI SALEH

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TABLE OF CONTENTS

CHAPTER 3 : MATERIALS AND METHODS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviations	Caption
cfu/ml	Cell forming unit per ml
DP	Degree of polymerization
EFB	Empty fruit bunches
FFB	Fresh fruit bunches
FFPRI	Forestry and Forest Products Research Institute
FOS	Fructo– oligosaccharides
FT-IR	Fourier Transform Infra-Red Spectroscopy
GOS	Galacto–oligosaccharides
GPC	Gel permeation chromatography
HPLC	High performance liquid chromatography
IOS	Isomalto–oligosaccharides
log Ro	Severity factor
ML	Middle lamela
M_n	number-average molecular weight
MPOB	Malaysian Palm Oil Board
MW	Molecular weight
M_w	weight-average molecular weight
M_w/M_n	polydispersity
NDO’s	Non-digestible oligosaccharides
OPF	Oil palm fronds
OPT	Oil palm trunk

OS	Oligosaccharides
P	Primary wall
pl	Isoelectric point
RI	Refractive index
R_o	Severity
RS	Reducing sugar
S	Secondary wall
S_1	Outer layer
S_2	Middle layer
S_3	Inner layer
SEC	Size exclusion chromatography
SEM	Scanning Electron Microscopic
SOS	Soybean-oligosaccharides
STAF	OPF residue from autoclave treatment
STAH	Hydrolysate from autoclave treatment
STAHe	Hydrolysate of STAH after enzymatic hydrolysis
STEXk	Steam exploded OPF obtained by soaking OPF chips in 0.5 % KOH solution at room temperature for 24 h prior to steam explosion.
STEXw	Steam exploded OPF obtained by soaking OPF chips in water at room temperature for 24 h prior to steam explosion
Tb	Base temperature
TDM	Total dry matter
TEM	Transmission electron microscopy
T_r	Reaction temperature
TS	Total sugar

UV	Ultra Violet
WI-STEXk	OPF residue from water extraction of STEXk
WI-STEXw	OPF residue from water extraction of STEXw
WSe-STEXk	Hydrolysate of water-soluble fraction (WS-STEXk) after enzymatic hydrolysis
WSe-STEXw	Hydrolysate of water-soluble fraction (WS-STEXw) after enzymatic hydrolysis
WS	Water-Soluble
WS-STEXk	Water-Soluble fraction from water extraction of STEXk.
WS-STEXw	Water-Soluble fraction from water extraction of STEXw
XOS	Xylo-oligosaccharides

APPENDICES

KESAN PENGOLAHAN HIDROTERMAL KE ATAS KEBOLEHEKSTRAKSI DAN KEBOLEHADAMAN TERBITAN HEMISELULOSA PELEPAH KELAPA SAWIT (*Elaeis guineensis Jacq.*)

ABSTRAK

Pelepah kelapa sawit (*Elaeis guineensis Jacq.*) (OPF), hasil sampingan dalam industri minyak kelapa sawit merupakan salah satu sumber bahan mentah di Malaysia. Pengekstrakan hemiselulosa daripada OPF telah dijalankan melalui dua pengolahan hidrotermal; letupan stim dan pemanasan dengan autoklaf. Campuran kepingan OPF-air atau OPF-KOH (1:10, w/v) diletup stim pada takat musnah 2.96–3.84 (180–210 °C selama 4 min) sebelum diekstrak dengan air pada 70 °C selama 2 jam. Dalam pengolahan menggunakan autoklaf, campuran kepingan OPF-air (1:10, w/v) diautohidrolisis pada takat musnah 1.92-2.52 (121 °C selama 20-80 min). Peletupan stim pada takat musnah 3.84 (210 °C selama 4 min) dan takat musnah 2.40 (121 ^{o}C selama 60 min) di dalam autoklaf menghasilkan hemiselulosa larut air OPF (WS-STEXw_210, WS-STEXk_210, STAH_60) sebanyak 15.75 %, 26.50 % dan 24.77 % masing-masing. Hemiselulosa larut air ini kemudiannya dihidrolisis dengan pelbagai kepekatan endo 1,4-ß-xilanase untuk menghasilkan xilo-oligosakarida (XOS). Hidrolisis enzimatik menggunakan 8U xilanase/100 mg substrat pada 40 ^{o}C dan pH 5.0 selama 24 jam menghasilkan jumlah XOS sebanyak 41.63 %, 26.06 % dan 17.49 % masing-masing. Pertumbuhan empat spesis bakteria; *L. acidophilus, L. bulgaricus, , L. casei* dan *B. animalis* di dalam medium pertumbuhan mengandungi XOS telah dikaji. Kehadiran XOS hasil hidrolisis enzimatik WS-STEXw_210 telah mempengaruhi pertumbuhan *B. animalis* dan *L. casei* yang mana pertumbuhan

tertinggi dipamer oleh *B. animalis,* diikuti oleh *L. casei* sedangkan XOS tidak mempengaruhi pertumbuhan *L. acidophilus* dan *L. bulgaricus*.

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ABSTRACT

Oil palm (*Elaeis guineensis Jacq.*) fronds (OPF) are produced worldwide as a by-product of the palm oil industries and represent another abundant raw material in Malaysia. Extraction of hemicellulose from OPF was conducted with two hydrothermal treatments; steam explosion and heating with autoclave. The mixture of OPF chips-water or OPF-KOH (1:10, w/v) were steam exploded at severity of 2.96-3.84 (180-210 °C for 4 min) before extracted with water at 70 °C for 2 h. In the treatment using autoclave, the mixture of OPF-water (1:10, w/v) was autohydrolysed in an autoclave at severity of 1.92-2.52 (121 °C for 20-80 min). Steam explosion at severity of 3.84 (210 °C for 4 min) and severity factor of 2.40 (121 °C for 60 min) produced water-soluble hemicelluloses (WS-STEXw_210, WS-STEXk_210, STAH_60) of 15.75 %, 26.50 % and 24.77 %, respectively. These water-soluble hemicelluloses were then hydrolysed with the various concentration of endo-1,4-ß-xylanase for production of xylo-oligosaccharides (XOS). Enzymatic hydrolysis using 8U xylanase / 100 mg substrate at 40 °C and pH 5.0 for 24 h produced XOS of 41.63 %, 26.06 % and 17.49 %, respectively. The growth of four bacterial species; *L. acidophilus, L. bulgaricus, L. casei*, and *B. animalis* in the growth medium containing XOS were studied. The present of XOS produced from enzymatic hydrolysis WS-STEXw_210 has affected the growth of *B. animalis* and *L. casei* where the highest growth was shown by *B. animalis*, followed by *L. casei* whereas XOS did not affect the growth of *L. acidophilus* and *L. bulgaricus*.

CHAPTER 1

INTRODUCTION

1.1 Background

Agriculture has played and will continue to play an important economic role in Malaysia. Malaysian agriculture is traditionally based on crop production, particularly rubber, palm oil, cocoa, pepper, rice and pineapple. Oil palm (*Elaeis guineensis Jacq.*) fronds (OPF) are produced world-wide as a by-product of the palm oil industries and represent another abundant raw material in Malaysia. The productive life of the oil palm tree is approximately 25 - 30 years and a tree yields about 120 kg fronds per tree. Therefore, the average yield per hectare (ha) of OPF is about 80 tonnes of dry matter (Lim, 1986). OPF are available daily throughout the year when the palms are pruned during harvesting of fresh fruit bunches for the production of oil. In Malaysia, the oil palm industry generates 36 million tonnes of pruned and felled fronds per year (Wan Zahari *et al.*, 2004) and is generally considered as waste. For that reason OPF is used as raw material for this research work.

OPF consist of three main components: cellulose, hemicellulose and lignin. Fractionation of hemicellulose into the main components could be of interest in obtaining separate streams useable for different product application (Kabel, 2002). Hydrothermal or autohydrolysis treatment performed under mild operational conditions has been employed in this study to extract hemicellulose from OPF. Hydrothermal technology covers a range of treatments; including both water and steam-based processes. Hydrothermal treatment is an environmental friendly process to fractionate hemicellulose. It allows the selective depolymerisation of the

hemicellulose backbone. Usually, mild operational conditions are preferred in order to achieve a high extent of hemicellulose solubilisation while sugar - degradation reactions are avoided.

In this study, the OPF was subjected to hydrothermal treatment to facilitate separation of the water-soluble fraction (hemicelluloses) and water-insoluble fraction (OPF residue). The water–soluble fraction may consist of hemicellulose as a major product, monosaccharide sugars and other by-products, while the water-insoluble consist of mainly cellulose, residual hemicellulose and lignin. Two types of hydrothermal treatments were carried out, that were steam explosion pre-treatment and treatment using an autoclave. Steam explosion pre-treatment was employed in this study to enhance the extraction process during water extraction. Hydrothermal treatment using autoclave was also employed in this study to simulate the normal conditions applicable for sterilization of process food. Autoclaving method besides generally cheaper and easy in handling, it is also less severe than steam explosion pre-treatment to minimise the undesired by-products.

Hemicellulose extracted from OPF can be hydrolysed by xylanase to produce xylo-oligosaccharides (XOS). XOS are newly developed functional oligosaccharide, having beneficial properties such as low cariogenicity, non-digestibility, improving the effects of intestinal flora, dietary fibre like action, water retention and antifreezing activity (Koga & Fijikawa, 1985). The growing demand for functional foods and the potential for product development open promising markets for XOS in many fields, including pharmaceuticals, agricultural, food and feed applications. One of the most important features of XOS as a food ingredient is their ability to

stimulate the growth of intestinal *Bifidobacteria* and *Lactobacillus* strains (Moura *et al*., 2007).

Several methods have been proposed to produce XOS from suitable feedstock, including direct enzymatic treatments, chemical fractionation followed by enzymatic hydrolysis of hemicellulose isolates, and hydrolytic degradation of hemicellulose to XOS by dilute solution of mineral acids, steam or water (autohydrolysis) (Vazquez *et al*., 2000; Nabarlatz *et al*., 2007; Akpinar *et al*., 2009). Figure 1.1 summarizes the approach addressed for xylo-oligosaccharides production from OPF derived hemicellulose in this study. If the final distribution of molecular weight is unfavourable for the desired application, further depolymerization of hemicellulose can be achieved by enzymatic treatment. Endo-1,4-β-xylanases (EC 3.2.1.8), mainly from *Trichoderma* sp. is chosen because the main backbone of hemicellulose is xylan. This enzyme degrades hemicellulose to short-chain XOS of varying lengths.

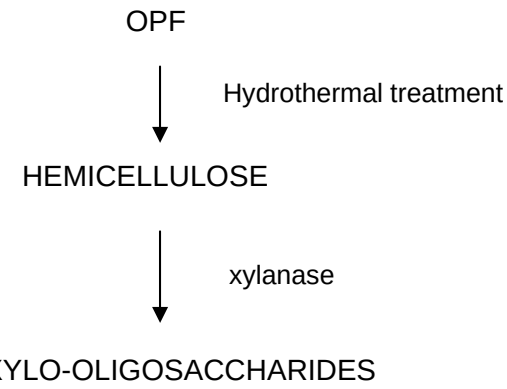

Figure 1.1: The approaches addressed for xylo-oligosaccharides production from oil palm fronds derived hemicellulose.

XOS produced from OPF derived hemicellulose is further studied by fermentation with *Bifidobacteria* and *Lactobacilli* strains. There is an increasing interest in

generating non-digestible oligomers, so-called prebiotics which would selectively promote the growth of potentially beneficial bacteria. *Bifidobacteria* and *Lactobacilli* are indigenous bacteria to the human intestinal tract, where their presence is commonly associated with several health benefits, and therefore constitute common targets for prebiotic action (Leahy *et al*., 2005). *Bifidobacterium* strains are largely decribed as capable of efficiently fermenting xylose-based oligosaccharide. The so-called bifidogenic effect of XOS, i.e. their specific capacity to stimulate bifidobacterial growth, was first observed by Okazaki *et al.* (1990) and further confirmed by several other authors (Jaskari *et al*., 1998; Van Laere *et al*., 2000 & Crittenden *et al.*, 2002). Members of the *Lactobacillus* genus are also considered beneficial within the intestinal microbiota, whose growth should be stimulated by prebiotic compounds such as XOS and others.

1.2 Aim and objective of research

The aim of this study is to extract hemicellulose employing hydrothermal treatment and digestion of OPF derived hemicelluloses for production of xylo-olisaccharides, and followed by studies involving the suitability of xylo-oligosaccharides as potential prebiotics. There are three phases in achieving this aim.The first phase is to extract hemicellulose from OPF by hydrothermal treatment. The second phase involves hydrolysation of water-soluble fraction for production of XOS using xylanase. The third phase is to study the suitability of the obtained XOS to enhance the growth of *Bifidobacteria* and *Lactobacillus* species. Thus, the specific objectives of this study are:

1) To study the effects of hydrothermal treatments on water-soluble fraction (hemicelluloses) and water-insoluble fraction (OPF residue) of OPF.

2) To characterize OPF derived hemicellulose such as monosaccharides composition, oligosaccharides concentration, total sugar content, molecular weight distribution, Klason lignin, furfural and acetic acid content.

3) To study the digestibility of OPF derived hemicellulose water-soluble fraction using xylanase.

4) To assess the suitability of xylo-oligosaccharides produced from OPF derived hemicellulose as potential prebiotic.

1.3 General overview of the contents

The following is a brief account of the remaining chapters of this thesis. Chapter two reviews theories that have been proposed to describe the anatomy of wood, chemical components of wood, OPF structure, xylan structure, *Bifidobacteria* and *Lactobacillus* sp. This chapter also deals with the literature review that highlighted the previous work related to the hydrothermal treatment in the process of hemicellulose extraction. The enzyme involved in producing XOS from OPF derived hemicellulose and the functional properties and purification process of XOS are also discussed in this chapter. Chapter three describes in detail the materials and methods that are used in this study. In this chapter, the research methodology is divided into four parts. Phase one describes the method of two hydrothermal treatments; autoclaving treatment uses autoclave, and steam explosion pre-treatment uses steam explosion equipment. Part two describe the method used for enzymatic hydrolysis by xylanase. Part three describes the method used to analyse the untreated OPF and treated OPF (OPF residue). A study of probiotics growth on XOS is described in phase four. This phase also describes the statistical analysis using SPSS. Chapter Four discusses the results obtained during the experimental work and elaborates

further on the findings, with comparisons and support from other researchers. Chapter five summarizes the work and provides a brief conclusion to the thesis. Finally, the recommendations for future research are given in Chapter six. References and appendices, followed by a list of publications and seminars, are listed in the last part of this thesis.

CHAPTER 2

LITERATURE REVIEW

2.1 Oil palm

An oil palm (*Elaeis guineensis Jacq*.) (Figure 2.1) was brought to Malaysia in 1870 from Africa as an ornamental plant but found its way into a commercial plantation, and finally grew to become one of the most important commercial crops in Malaysia (Husin *et al*., 1986). In 2006, Malaysia is the world's leading palm oil producer and exporter, accounting for 47 % of global production and 89 % of exports (Sumathi *et al*., 2007). The production target for palm oil is 8.5 million tonnes by 2000 and 10.5 million tonnes by 2010. The export target for 2010 is 53 % higher than in 2000. To achieve those targets, 1.2 million hectares of new plantings area will be required.

Figure 2.1: Photograph of oil palm tree

Oil palm produces about 55 t/ha/yr of total dry matter (TDM). Currently the main product is the oil; palm oil and palm kernel oil which is averaging about 4.3 t/ha/yr. This constitutes about 8 % of the TDM whereas 92 % has not been converted into high value–added products. In Malaysia, the oil palm industry generates more than 1.3 million tonnes of oil palm trunks and 2.4 million tonnes empty fruit bunches (Jaafar & Sukaimi, 2001) and 36 million tonnes of pruned and felled fronds (Wan Zahari *et al*., 2004).

Currently more effort is directed towards the re-use of such by-products, considering economic values and environment. The by-products from oil palm industry such as fronds, empty fruit bunches mesocarp fibres, shells and trunks represent another abundant raw material in Malaysia. These by-products can be utilized to produce value-added products and make the oil palm industry a “zero-waste” industry. Research on the utilization of OPF should be emphasized because at least 70 % of the oil palm by products consists of fronds, especially pruned fronds (Husin *et al.*, 1985).

2.1.1 Oil palm fronds

Oil palm fronds are the stem part produced continuously from the oil palm plant (Figure 2.2). The OPF grow in tightly clustered bunches or heads. This is a readily available by-product of oil plantations, that are cut down during harvesting of fresh fruit bunches (FFB), senescence and felled palms during replanting (Dahlan, 1992). The productive life of the tree is about 25 - 30 years and tree yield about 120 kg fronds per tree. Therefore, the average yield per ha is about 80 tonnes of dry matter per year (Lim, 1986). Usually 2 - 3 OPF are cut to get the FFB from the oil palm plant because FFB are compactly packed and hidden in the leaf axils (Dahlan, 2000).

Figure 2.2: Photograph of oil palm fronds

The OPF are generally considered as a waste by-product of plantation management practise. Currently OPF are shredded and allowed to decompose in the plantation, thus contributing to organic matter recycling and soil conservation. The felled OPF also provides an ideal shelter for snakes, rats and other pests (Dahlan, 2000). Large sums of money are spent for the disposal of this by - product to make way for replanting and to avoid insect disease and pollution problems.

2.2 Wood structure

In wood cell walls, different layers can be recognized and their concentric arrangement is caused by the differences in the chemical composition and different orientations of the structural elements. The components of the cell walls are divided into structural (i.e. cellulose) and substructural, i.e. hemicelluloses and lignin. Figure 2.3 show a model of the construction of wood cell walls (Persson, 2000).

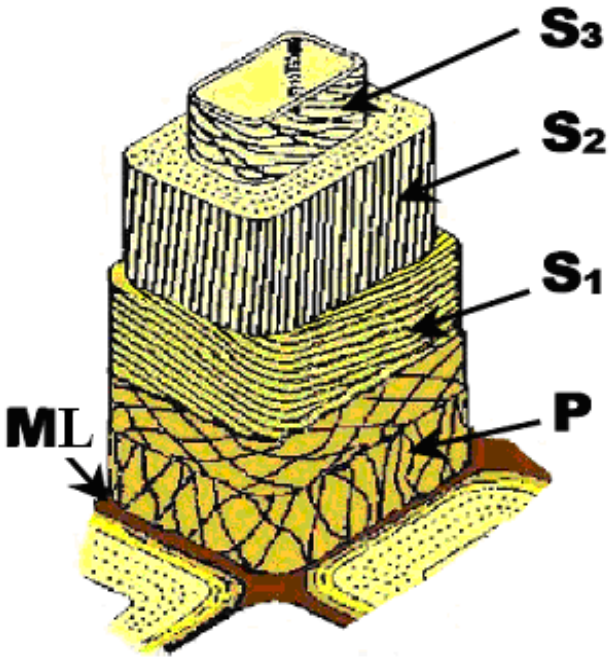

Figure 2.3: Schematic illustration of the cell wall of wood cells which generally applies to many cells in both softwood and hardwoods. ML = Middle lamella; P = Primary wall; S_1, S_2, S_3 = Outer, Middle and Inner layers of the secondary wall, respectively (Persson, 2000).

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Between the individual cells, there is a thin layer called the middle lamella (ML), which glues the cells together to form the tissue. In general, plant cell walls are subdivided in primary wall (P) and secondary wall (S). The distribution of cellulose, hemicellulose and lignin varies considerably among these layers (Fengel & Wegener, 1989). The primary cell wall is a thin layer that is permeable and flexible in physiologically active tissues (sapwood) but may become highly lignified in heartwood cells. The primary wall can be divided into an outer and an inner surface. The arrangements of the microfibrils in the primary wall are increasingly disperse

from inner to outer surface. The secondary wall is formed by a sequence of three layers, outer layer (S_1), middle layer (S_2), and inner layer (S_3). In the outer layer of the secondary wall S_1, the microfibrils are oriented in a cross-helical structure (S helix). The middle layer of the secondary wall (S_2), which is thickest layer, has relatively consistent orientation of microfibrils. In contrast, the microfibrils of in the inner layer of the secondary wall (S_3) may arrange in two or more orientations.

Pure hemicellulose is also present between the cellulose fibrils and the matrix (Kerr, 1975). Pores in the matrix are small, and permit the transfer of water, salts and small organic molecules only. Although the hemicelluloses are at least partially water soluble, they cannot diffuse out of the cell wall due to their molecular size.

2.2.1 Cell wall structure of oil palm fronds (OPF).

OPF contain 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, fibres, phloem, and parenchymatous tissues (Figure 2.4A). According to Abdul Khalil *et al.* (2006) xylem and phloem tissues are clearly distinguishable. 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 (Figure 2.4B). According to previous study, 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 permit possible transfer of gas bubbles between proto- and metaxylem vessels (Tomlinson *et al.,* 2001).

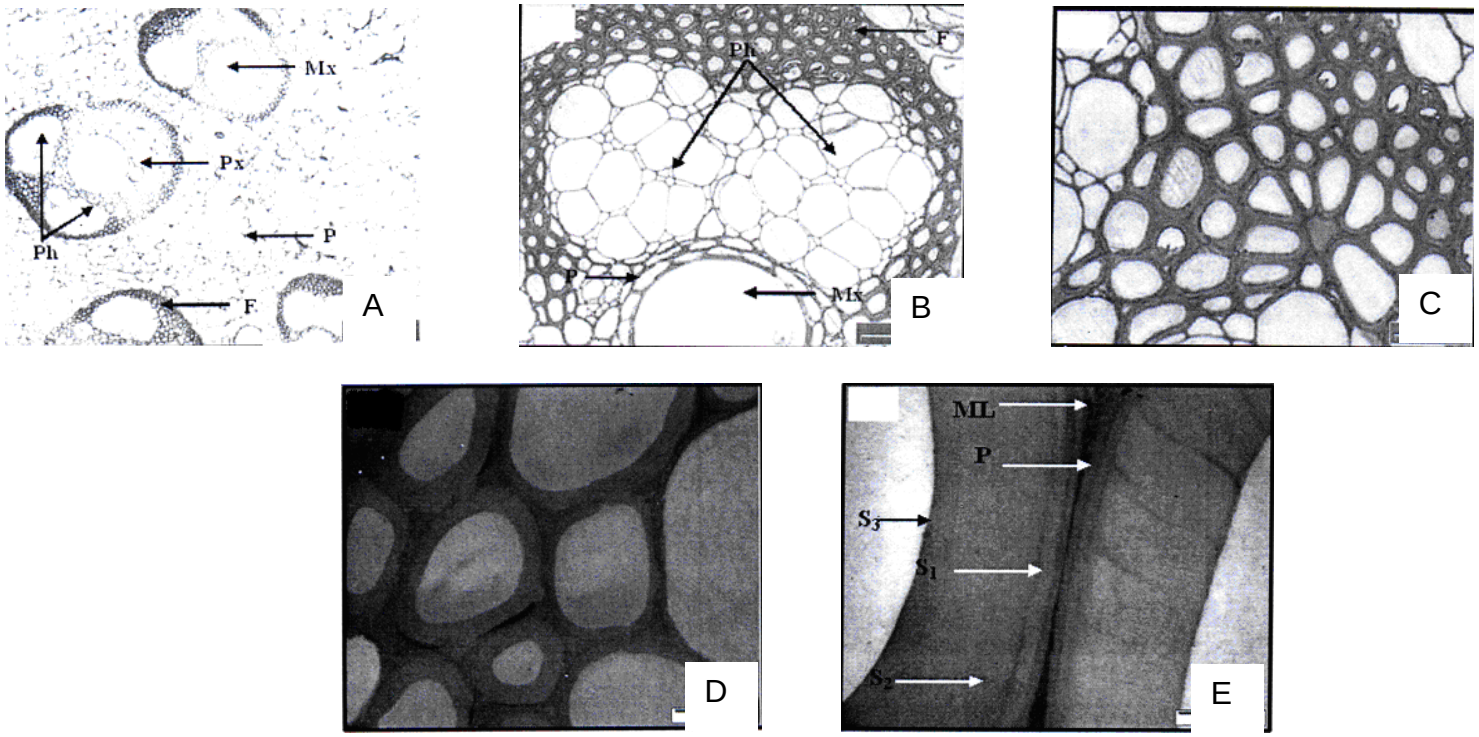

Figure 2.4: (A) Transverse section of OPF at low magnification (4x), (B) Transverse section of OPF at high magnification (20x), (C) Transverse section of OPF after being stained with toluidine at high magnification (40x), (D) Transmission electron micrograph of urathin section of OPF after being stained with uranyl acetate and lead citrate at low magnification (3400x), (E) Transverse section of multi layered structure of OPF at high magnification (17000x). F = Fibre; P = Parenchyma; Mx = Metaxylem; Ph = Phloem ML = Middle lamella; P= Primary Wall; S_1, S_2 & S_3 = Secondary wall sublayers (Abdul Khalil *et al.*, 2006).

A transmission electron microscopy (TEM) view of transverse sections of OPF is shown in Figure 2.4C (Abdul Khalil *et al.*, 2006). The electron microscopic observation is restricted mainly to the wall of OPF fibre within the vascular bundles. The TEM electron micrographs have confirmed that the layered structure of OPF wall contains primary (P) and secondary (S_1, S_2 and S_3) wall layer (Figure 2.4D). The primary wall appears as a solid boundary of the cell (Figure 2.4E). The middle lamella, which glues the cells together, shows a clear transition to the adjacent primary wall layers. The S_1 layer of OPF is well-defined and could be distinguished from the adjoining S_2 layer, as it is the brightest layer compared to other layers. The observation using TEM micrograph also provides evidence for the presence of distinct S_3 layer in the cell wall of OPF.

2.3 Chemical structure of wood

The chemical components of wood can be divided into four major components. They are cellulose, hemicellulose, lignin and extractives. Generally, the first three components have high molecular weights and contribute a significant mass, while the latter component is of small molecular size, and it is available in a smaller quantity. Hemicellulose will be discussed further in Section 2.4.

2.3.1 Cellulose

The cellulose content of wood varies between species in the range of 40 – 50 %. Cellulose is a linear polymer chain which is formed by joining the anhydroglucose units into glucan chains. These anhydroglucose units are bound together by β-(1, 4)-glycosidic linkages (Sjostrom, 1993). Due to this linkage, cellobiose is established as the repeat unit for cellulose chains. By forming intramolecular and intermolecular

hydrogen bonds between OH groups within the same cellulose chain and the surrounding cellulose chains, the chains tend to arrange in parallel and form a crystalline supermolecular structure. Cellulose can appear in crystalline form, called crystalline cellulose (Beguin & Aubert, 1994; Huang & Chen, 1996; Perez *et al*., 2002). Then, bundles of linear cellulose chains form a microfibril which is oriented in the cell wall structure.

In addition, there are a small percentage of non-organized cellulose chains, which form amorphous cellulose. In this conformation, cellulose is more susceptible to enzymatic degradation (Beguin & Aubert, 1994). Cellulose appears in nature associated with other plant substances and this association may affect its biodegradation. Cellulose is insoluble in most solvents including strong alkali. It is difficult to isolate from wood in pure form because it is intimately associated with the lignin and hemicelluloses (Pettersen, 1984). Wood cellulose is more resistant to acid hydrolysis than hemicellulose. The degree of polymerization of cellulose (i.e. the number of glucose-units, DP) depends on the type of treatment (Nada *et al*., 2007). The DP is variable and may range from 700 to 10,000 DP or more.

2.3.2 Lignin

Lignin is the most abundant polymer in nature. It is present in the cellular cell wall, conferring structural support, impermeability, and resistance against microbial attack and oxidative stress (Perez *et al*., 2002). Structurally, lignin is an amorphous heteropolymer, non water soluble and optically inactive; it consists of phenylpropane units joined together by different types of linkages and is very difficult if not impossible to isolate in a natural state (Sjostrom, 1993). Lignin gives mechanical strength to wood by gluing the fibres together between the cell walls (Boudet, 2000;

Douglas, 1996), and if it is lacking, the plant will no longer grow upright (Zhong *et al*., 1997). Lignin also serves as disposal mechanism for metabolic waste. Lignin comprises 18 – 30 % by weight of the dry wood, most of it concentrated in the compound middle lamella and the layered cell wall.

2.3.3 Extractives

Extractives are the organic substances which have low molecular weight and are soluble in neutral solvents. Resin, fats, waxes, fatty acids and alcohols, terpentines, tannins and flavanoids are categorized as extractives. The amount of extractives in wood varies from 5 – 20 % by weight and includes a wide variety of organic chemicals (Rowe, 1989). The extractive can be found mostly in resin canal and ray parenchyma cells and small amount in middle lamella and cell walls of tracheids. The extractives contribute to wood properties such as colour, odour and decay resistance (Kallioinen *et al*., 2003; Pandey, 2005). Some extractives are toxic and this is an advantage for the wood to resist attack by fungi and termites (Arango *et al*., 2006; Schwarze, 2007).

2.4 Hemicellulose

Hemicellulose is a complex carbohydrate polymer and make up 25 – 30 % of total wood dry weight. It is a polysaccharide with a lower molecular weight than cellulose. They are also easily hydrolysed to monomeric sugars, uronic acid and acetic acids. Many different hemicelluloses have been isolated from wood (Krawczyk *et al*., 2008; Gaspar *et al*., 2007; Jacobs *et al*., 2002; Bikova & Treimanis, 2002; Sun *et al*., 2001).

The backbone of the chains of hemicellulose can be a homopolymer (generally consisting of single sugar repeat unit) or a heteropolymer (mixture of different

sugars). Formulas of the sugar component of hemicellulose are listed in Figure 2.5. Among the most important sugar of the hemicellulose component is xylose. Hemicellulose includes xylan, mannan, galactan, and arabinan as the main heteropolymers. The classification of these hemicellulose fractions depends on the types of sugar moieties present. The principal monomer present in most of the hemicellulose is D-xylose, D-mannose, D-galactose, and L-arabinose. The polymer chains of hemicellulose have short branches and are amorphous, consisting of a few hundred sugar residue. Hemicellulose is partially soluble in water because of the amorphous morphology (Coughlan & Hazlewood, 1993).

Hemicellulose is plant heteropolysaccharides whose chemical nature varies from tissue to tissue and from species to species. These polysaccharides are formed by a wide variety of building blocks including pentoses (xylose and arabinose), hexoses (glucose, mannose and galactose) and uronic acids (4–*O*–methyl-glucuronic acids) (Fengel & Wegener, 1989).

Generally they fall into four classes:

(a) unbranched chains such as (1,4)-linked xylans or mannans

(b) helical chains as (1,3)-linked xylans

(c) branched chains such as (1,4)-linked galactoglucomannas

(d) pectic substances such as polyrhamnogalacturonans.

Hemicellulose is structurally more related to cellulose than lignin and is deposited in the cell wall at an earlier stage of biosynthesis. Despite the complexity of these polysaccharides, their structure seems to be generally rod-shaped with branches and side chains folded back to the main chain by means of hydrogen bonding. This rod

like structure facilitates their interaction with cellulose, resulting in a tight association that gives great stability to the aggregate (Rydholm, 1965).

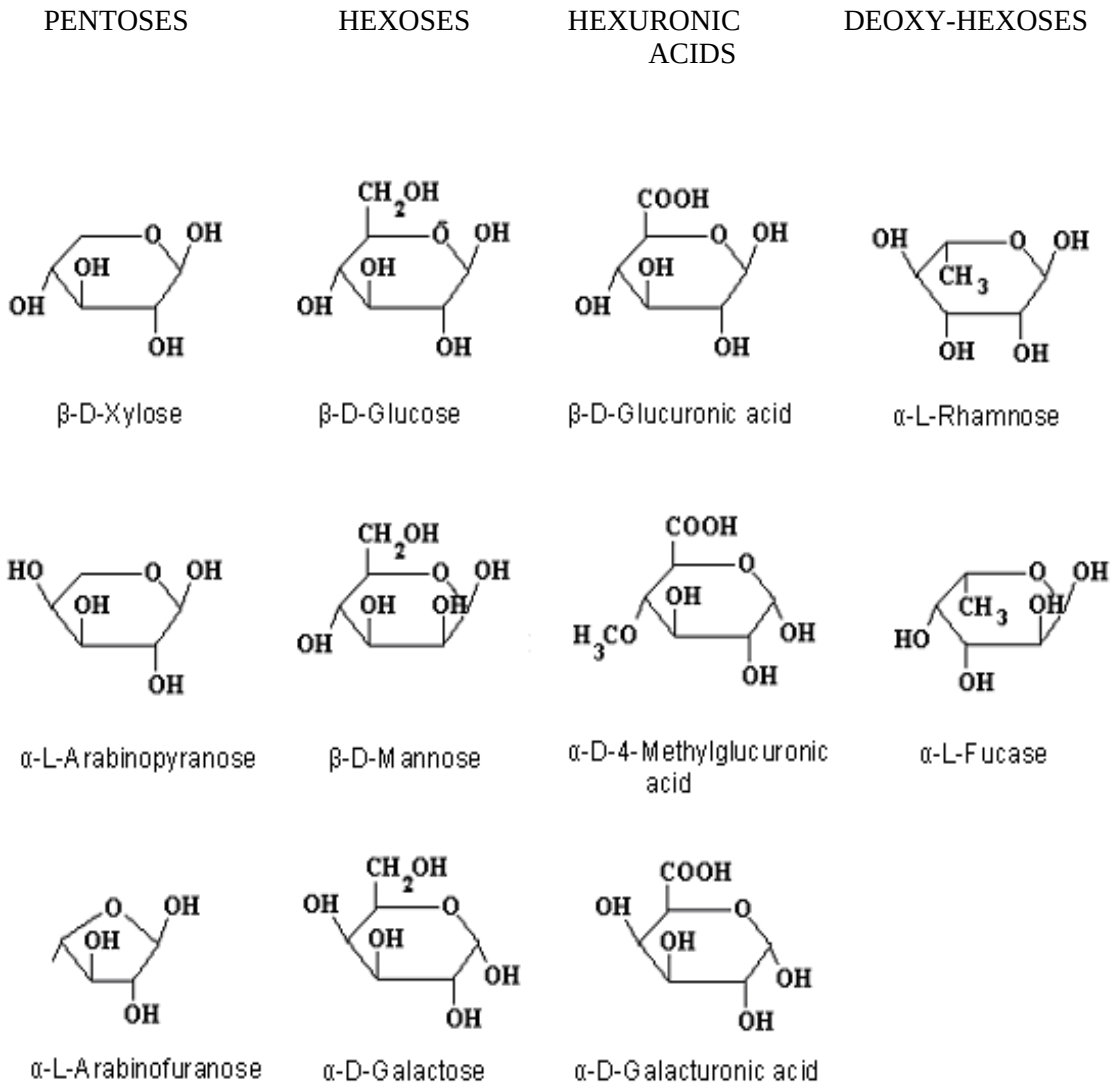

Figure 2.5: Chemical structure of sugar units of hemicelluloses (Fengel & Wegener, 1989)

The hemicellulose content of softwood and hardwood differ significantly (Fengel & Wegener, 1989). Some hemicelluloses particularly heteroxylans, also show a considerable degree of acetylation (Atalla, 1988). Hardwood hemicellulose is mostly composed of highly acetylated heteroxylans, generally classified as 4–*O*-methylglucuronoxylans. Hexosans are also present but in very low amount as glucomannan. Owing to their acidic characteristics and chemical properties, hardwood xylans are relatively labile to acid hydrolysis and may undergo autohydrolysis under relatively mild conditions. In contrast, softwoods have a higher proportion of partly acetylatedglucomannas and galactoglucomannas, and xylans correspond to only a small fraction of their total hemicellulose content (Garrote & Parajo, 2002). As a result, softwood hemicelluloses (mostly hexosans) are more resistance to acid hydrolysis than hardwood hemicelluloses (mostly pentosans).

2.4.1 Xylan

Xylan, one of the major components of hemicellulose found in cell wall is the second most abundant polysaccharides next to cellulose. Xylans are heteropolymers whose chemical nature varies from tissue to tissue and from species to species. It is consisting principally of D-xylose, a five carbon sugar as its monomeric unit and traces of L-arabinose (Bastawde, 1992). Xylan is the major hemicellulose in hardwood from Angiosperms, but is less abundant in softwood from gymnosperms; it accounts for approximately 15 – 30 % and 7 -12 % of the total dry weight, respectively (Whistler & Richards, 1970; Wong *et al.*, 1988).

The xylan from hardwood is *O*-acetyl-(- 4-*O*-methylglucurono) xylan. The backbone of this hardwood xylan consists of β-(1, 4)-D-xylopyranose residues with, on average, one α-(1,2)-linked 4-*O*-methyl glucuronic acid substituent per 10 - 20 such

residues. In addition, *O*-acetyl groups sometime replace the OH groups in position C2 and C3 (Figure 2.6A). In the case of softwood plants, xylan is mainly arabino-4-*O*-methyl glucuronoxylan which in addition to 4-*O*-methyl glucuronic acid is also substituted by α–arabinofuranoside units linked by α-1,3-linkage to the xylan backbone (Figure 2.6B) (Fengel & Wegener, 1989).

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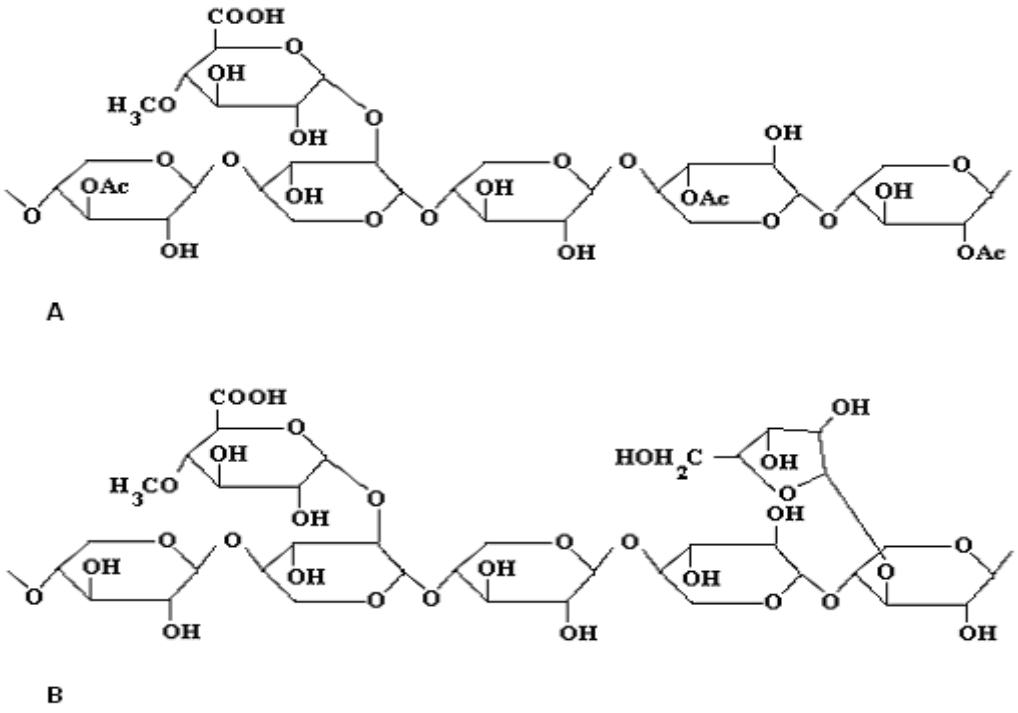

A - Partial xylan structure from hardwood chain
B - Partial xylan structure from softwood chain

Figure 2.6: Schematic illustration of xylans (Fengel & Wegener, 1989)

Xylan appears to be a major interface between lignin and other carbohydrate components in plant cell walls. Physical access to xylosic linkages in xylan is restricted by the surrounding lignocellulosic components as well as substituents on

its backbone. Therefore, the raw materials are conventionally pretreated before enzymatic degradation (Dekker, 1985). Since the main component of the hemicellulose mentioned is xylan, fractionation of xylan may result in both xylose and a variety of xylo-oligosaccharide.

2.4.2 Xylo-oligosaccharides

Xylo-oligosaccharides (XOS) are sugar oligomers made up of xylose units. XOS are non-digestible oligosaccharides (NDOs), which are not degraded by the low-pH gastric fluid, nor by human and animal digestive enzymes and will therefore reach the large bowel intact (Okazaki *et al.*, 1990). XOS are reported to enhance growth of *Bifidobacteria* and they are frequently defined as prebiotics (Fooks *et al.*, 1999). XOS can be used as ingredients of functional foods, cosmetics, pharmaceuticals or agricultural products (Vazquez *et al*., 2000).

2.4.2(a) Properties of xylo-oligosaccharides and their effects on health

For food applications, xylobiose (DP = 2) is considered to be a XOS, even if for other purposes the concept ‘oligo’ is associated with higher degree of polymerization (DP>5). The sweetness of xylobiose is equivalent to 30 % that of sucrose and the sweetness of other XOS such as xylotriose and xylotetrose is moderate and possess no off-taste (Vazquez *et al*., 2000). As food ingredients, XOS have an acceptable odour, and are non-cariogenic (Kazuyoshi *et al*., 1998) and low-calorie, allowing their utilization in anti-obesity diets (Taeko *et al.*, 1998).

XOS are moderately sweet, stable over a wide range of pH 2.5 – 8.0, temperatures up to 100 °C and have organoleptic characteristics suitable for incorporation into foods. XOS are advantageous over other NDOs such as fructo-oligosaccharides, particularly

in the acidic range, even at the relatively low pH value of the gastric juice. Water activity of xylobiose is reported to be higher than xylose, but almost the same as glucose. Antifreezing activity of xylobiose on water at temperatures higher than −10 °C is the same as that of xylose, but greater than that of glucose, sucrose and maltose (Bhat, 1998). As food ingredients, XOS have an acceptable odour, and are non-cariogenic (Kazumitsu *et al.*, 1997; Kazuyoshi *et al*., 1998) and low-calorie, allowing their utilization in anti-obesity diets (Taeko *et al*., 1998). In food processing, XOS show advantages over inulin in terms of resistance to both acids and heat, allowing their utilization in low pH juices and carbonated drinks (Modler, 1994).

One of the most important features of XOS as food ingredients is their ability to stimulate the growth of intestinal *Bifidobacteria* (Dohnalek *et al*., 1998; Jeong *et al.*, 1998). *In vitro* assays proved that *Bifidobacterium* spp. and *B. adolescentis* are able to utilize both xylobiose and xylotriose, whereas a mixture containing xylobiose as the main component was utilized by *B. adolescentis*, *B. infantis* and *B. longum*. XOS are readily utilized by *B. animalis*, and the oral ingestion of XOS promotes the proliferation of *B. animalis* in intestines (Suwa *et al*., 1999). Contrarily, *Staphylococcus*, *Escherichia coli* and many *Clostridium* spp. cannot utilize XOS (Suwa *et al.*, 1999; Okazaki *et al.*, 1990). A few *Lactobacillus* species also can utilize XOS such as *L. fermentum and L. brevei* but not as well as *Bifidobacteria. Bacteroides* utilize XOS, but in a lower degree compared with glucose (Okazaki *et al.*, 1990).

The growing commercial importance of these NDOs is based on their beneficial health properties, particularly the prebiotic activity. The healthy effects of XOS are mainly related to their effects on the gastrointestinal flora. Subramaniyam & Prema (2002) reported that XOS favour the selective growth of *Bididobacterium spp*, which have important biological effects since they : (i) suppressing activity of entero putrefactive bacteria, preventing the formation of products such as toxic amines (Campbell *et al*., 1997; Fujikawa *et al*., 1991), (ii) repression of the proliferation of pathogenic bacteria (Suwa *et al*., 1999; Okazaki *et al.*, 1990) due to the production of short-chain organic acids such as lactic acid or acetic acid (Loo *et al*., 1999; Wolf *et al*., 1994; Okazaki *et al.*, 1990) leading to decreased pH in the gastro-intestinal tract (Wolf *et al*., 1994; Imaizumi *et al*., 1991; Okazaki *et al.*, 1990), and (iii) promotion of the digestion and absorption of nutrients. These properties result in the ability to prevent gastrointestinal infections, to reduce the duration of diarrhoea episodes (Dohnalek *et al*., 1998) and to maintain the fecal water content within normal levels (Okazaki *et al.*, 1990), with delayed gastric emptying (Ziemer & Gibson, 1998). As an additional favourable effect of XOS, a modest enhancement of cecal epithelial cell proliferation by this kind of compound has been reported (Howard *et al*., 1995). Based on the above reasons, XOS 'affect functions in the body so as to have positive cellular or physiological effects', fullfilling the requirements for prebiotics, and because of this, XOS are used as active ingredients of 'functional foods', which are similar in appearance to conventional foods that are consumed as part of a normal diet and have demostrated physiological benefits and/or reduce the risk of chronic disease beyond basic nutritional functions (Clydesdale, 1997).

2.4.2(b) Production of xylo-oligosaccharides

Specifically for XOS production from lignocellulosic material, three different approaches have been used (Pellerin *et al*., 1991): i) enzyme treatments of native lignocellulosic material containing hemicelluloses; ii) chemical fractionation of lignocellulosic material to isolate or solubilize hemicelluloses, with further enzymatic hydrolysis to XOS; iii) hydrolytic degradation of hemicelluloses to XOS by steam, water or dilute solutions of mineral acids or alkali.

The direct production of xylo-oligosaccharides from xylan-containing lignocellulosic materials must be carried out from a susceptible feedstock. The production of xylo-oligosaccharides by combined chemical-enzymatic methods consists basically in two steps. In the first one, the xylan can be obtained by treatment of the lignocellulosic materials with alkali (i.e. solutions of NaOH, KOH, etc.), being this treatment favored by the pH stability of this polymer. In some cases, the raw material has been pretreated with oxidizing agents, salts or alcohols to remove lignin or pectic substances.

Once the hemicellulose has been isolated or degraded to a soluble form, further DP reduction can be accomplished by hydrolysis with xylanases (Masayu *et al*., 1993). For enzymatic production of XOS, enzymes complexes with low exo-xylanase and/or β-xylosidase activity are desired, in order to avoid the production of xylose. The enzyme can be directly added to the reaction media (Pellerin *et al*., 1991), immobilized (Suwa *et al.*, 1999) or produced *in situ* by micro-organism. With this approach, low-DP XOS can be produced. For food–related applications, the preferred DP range is 2 – 4 (Loo *et al*., 1999).

Alternatively, XOS can be produced from lignocellulosic materials in a single step by reaction with steam or water through hydronium catalyzed degradation of xylan, according to the procedure known as autohydrolysis, hydrothermolysis or water prehydrolysis. In the first stages of the reaction, the catalytic species are obtained from water autoionization, but side reactions (for example, cleavage of acetyl groups to give acetic acid) contribute to the generation of hydronium ions in further reaction stages. The hydrolytic degradation of hemicelluloses is facilitated when an acid is added externally (acid hydrolysis), but in this case, the oligosaccharides behave as reaction intermediates and the main reaction products are monosaccharides. Besides the degradation of xylan, several side-processes occur in such kinds of treatment, including extractive removal, solubilization of acid soluble lignin, and neutralization of ash, which contributes to the presence of undesired compounds in the final product. In this case, the purification of XOS becomes important (Vazquez *et al*., 2000).

Usually, mild operational conditions are preferred in order to achieve high extent of hemicellulose solubilization to give XOS with DP typically ≤ 20 while sugar - degradations are avoided. If the final distribution of molecular weights is unfavourable for the desired application, further DP reduction can be achieved by enzymatic treatments (Motohiro *et al*., 1986). XOS are naturally present in fruits, vegetables, bamboo, honey and milk and can be produced at industrial scale from hemicellulose-rich materials.

Figure 2.7 summarizes the different approaches addressed for XOS production from hemicellulose-rich materials (Vazquez *et al*., 2003). Chemical and enzymes