ISOLATION AND FUNCTIONAL PROPERTIES OF HEMICELLULOSES FROM OIL PALM TRUNKS

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ABSTRACT

Approximately 80% (dry weight) of the total hemicelluloses (xylan) was extracted from oil palm trunks (OPT). Analysis of OPT gave the following composition (dry basis): 42%-45% cellulose, 26%-29% hemicellulose and 16%-18% lignin. Optimisation of the aqueous extraction was done using the following parameters: 3.0 M alkali concentration, 40°C temperature, 4 hr soaking time, 50 g sample with <0.32 mm fibre/dust sieve size. Extracted hemicelluloses were found to have a higher quantity of hemicellulose A (50%) as compared to hemicellulose B (30%). Xylose was found to be the major sugar in each of the fractions, with glucose, arabinose and mannose as minor constituents. The carbohydrate composition of the hemicellulose fraction consisted of xylan, to which other carbohydrates were attached. It was found that hemicellulose B had a higher molecular weight than hemicellulose A, while the intrinsic viscosity for both hemicelluloses was in the range of 0.6-0.7 dL g^{-1}. Both hemicelluloses were not completely soluble in water at elevated temperature, and their water-holding capacity was less than 10%. OPT hemicelluloses exhibited pseudoplastic flow behaviour ('shear thinning') and were affected by shear rate and temperature. The extracted hemicelluloses also had a low digestibility using xylanase enzyme.

INTRODUCTION

Generally, the oil palm has an average economic life of about 25 years, and during replanting an average of 0.74 t (dry basis) per hectare of oil palm trunks (OPT) is generated (Chan *et al.*, 1980). OPT consist of three main components: cellulose (42%-45%), hemicelluloses (25%-28%) and lignin (16%-18%), beside extractives (8%-10%). Hardwood xylans can be extracted directly from lignified wood or fibre with aqueous potassium hydroxide. The yields vary widely for different types of woody materials, probably reflecting the difference in the structure of the cell wall (Timell, 1967). Different extraction procedures also influence the yield of hemicelluloses. The best pre-treatment procedures for lignocellulosic material extraction are those in which physical and chemical modifications are combined (Ghosh and Singh, 1993). All the components can be fractionated, isolated and purified to obtain value-added products.

Hemicelluloses are low-molecular weight polysaccharides, associated in plant cell walls with cellulose and lignin. Hemicelluloses are usually defined as polymers that are solubilised from plant cell walls by alkali (Darvill *et al.*, 1980). They have great industrial potential from their physical and chemical properties and biological activities. Most of the hemicellulose fraction is soluble in water after alkaline extraction. Isolation of these polysaccharides is difficult because of the intricate physical and chemical bonding within the lignin-hemicellulose-cellulose complex of the plant materials.

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Hemicelluloses are heteroglycans built up from a relatively few sugar residues, the most common of which are D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose and D-galacturonic acid. Currently, several chemicals (such as xylitol and gluconic acid) derived from hemicelluloses are of industrial importance.

In this study, the hemicelluloses were isolated using different parameters such as temperature, time and alkali concentration. Their functional properties were characterised based on the sugar content in each portion.

MATERIALS AND METHODS

Materials

OPT were obtained from Kahang Industrial Estate in Kluang, Johor, Malaysia and they were chipped using a chipper, dried (at a temperature of 105°C using a forced-draft oven until 12% moisture content), ground and sieved into a size less than 0.32 mm. The samples were stored at room temperature until further analysis, and their moisture content was maintained below 10%.

Alkali Extraction

Extraction was carried out on the fresh (non-delignified) OPT fibre/dust samples of <0.32 mm sieve size. Approximately 50 g of samples were soaked in different concentrations of potassium hydroxide (0.5-3.5 M) solution for different soaking times (2-24 hr) and at different temperatures (25°C-60°C) with stirring to extract the hemicelluloses. The extraction was done in triplicate. The ratio of solid to liquid used was 1:10. After 4 hr of extraction, the hemicelluloses were vacuum-filtered using Whatman filter paper No. 4 through a Buchner funnel. The residual fibre/dust was kept for Klason lignin analysis. The filtrate was then neutralised with acetic acid to pH 5.5-6.0, and allowed to stand for 24 hr at 4°C to precipitate the fraction A (HA) of the hemicelluloses. The mixture was centrifuged at 12 000 g for 15 min, and the resultant fraction was freeze-dried before further analysis. The supernatant was then mixed with four volumes of 95% ethanol, and kept for 24 hr at 4°C. The precipitate was centrifuged and the resultant fraction was identified as hemicellulose B (HB). This was then freeze-dried prior to further analysis. The flow chart of the extraction process is shown in *Figure 1*.

Hemicellulose Hydrolysis

Approximately 300 mg samples of both HA and HB were hydrolysed with 3.0 ml of 72% H_2SO_4 for
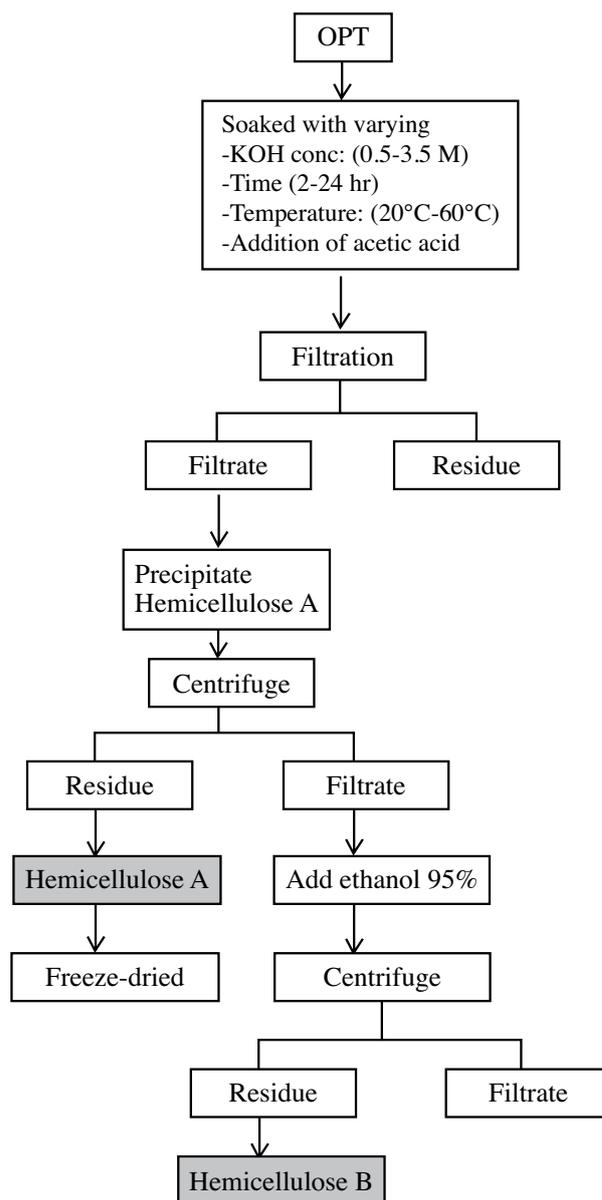

Figure 1. Flow chart for alkaline extraction of hemicelluloses from oil palm trunks (OPT).

1 hr at room temperature, before diluting the samples with 84 ml of distilled water and autoclaving them for 1 hr. The hydrolysis was done in triplicate. After cooling, the solution was neutralised with a saturated barium hydroxide solution to pH 5-6, and the precipitate was removed by centrifugation. Filtration was carried out to remove the precipitate of barium sulphate. The supernatant was then used in the analysis for monosaccharide composition.

Determination of Monosaccharides

Monosaccharide composition was analysed using HPLC with a Sugar-Pak column (6.5 mm × 300 mm, Waters) and a refractive index detector after hydrolyzing the hemicelluloses with H_2SO_4. The column temperature was maintained at 90°C

with a flow rate of 0.5 ml min^{-1}, while 0.001M CaEDTA was used as the mobile phase. Prior to the analysis, the samples were neutralised and filtered through SEP C18 followed by 0.45 µm PTFE membranes. The analysis was done in triplicate.

Solubility in Water

Solubility of the hemicelluloses in water was determined in triplicate using the Browning (1967) method. Approximately 1.0 g of extracted hemicelluloses (HA and HB) was soaked in water (100 ml) for periods of 1, 2 and 3 hr. The samples were then filtered using filter paper, and dried to constant weight.

Solubility was calculated as follows:

$$\text{Solubility in water, \%} = \frac{(A-B) \times 100}{A}$$

where: A = initial weight of the oven-dried test sample in g.
B = weight of oven-dried sample in g after soaking.

Water-holding Capacity

Water-holding capacity (WHC) was determined in triplicate following the method by McConnel *et al.* (1974). Samples (0.5 g) were put into tarred 50-ml polypropylene centrifuge tubes to which 10 ml distilled-deionised water were added. The tubes were capped before the contents were vigorously mixed. They were then held for 24 hr at 20°C before centrifuging at 13 300 g for 1 hr. The tubes were then weighed, freeze-dried and re-weighed.

Flow Behaviour

Viscosity was determined in triplicate using a viscometer (Rheology International) fitted with a closed concentric cylinder system. For the determination, approximately 5.0 g of extracted hemicellulose (HA and HB) sample were suspended in 20 ml cupriethlenediamine (CED) for 2 hr at room temperature. The solution was transferred into the cylinder and the viscosity was monitored at room temperature with increasing shear rate.

Enzyme Digestibility

Digestibility of the hemicelluloses was studied in triplicate using xylanase enzyme (Sigma) from *Trichoderma viride* under optimum conditions (temperature of 48°C, duration of 48 hr, pH of 5.5 and agitation at 100 rpm). The amount of reducing sugars after digestion by the enzyme was determined using the reducing sugar method of Bruner (1964). The samples were analysed using a UV spectrophotometer at 540 nm.

Determination of Klason Lignin Content

Determination of the lignin content of the extracted hemicellulose samples was performed according to the TAPPI T222 method. Determination was done in triplicate. Approximately 2 ml of 72% H_2SO_4 (v/v) were added to 0.2 g of a dried sample in a 250 Erlenmeyer flask. The mixture was gently stirred for 60 min at room temperature. To the mixture were slowly added approximately 56 ml of distilled water, and the samples were then autoclaved at 121°C for 60 min. Pre-weighed glass microfibres were used to filter off the undissolved material, which is known as the Klason lignin. After filtration, the residue was washed with hot water until a neutral pH was reached, and then it was dried at 105°C overnight. The cooled sample was stabilised in a desiccator and weighed.

The lignin content was calculated as follows:

$$\text{Klason lignin (\%)} = \frac{(B) - (C)}{A} \times 100$$

where:
A = weight of oven-dried sample.
B = weight of dry glass microfibre + lignin.
C = weight of dry glass microfibre.

RESULTS AND DISCUSSION

Yield of Hemicelluloses

Results on the extraction using KOH at different concentrations are shown in *Figure 2*. The yield of HA (22.8%) was significantly higher than that of HB (5.7%). According to Nacos *et al.* (2006), the first precipitation using acetic acid usually precipitates hemicelluloses with a higher molecular weight, whereas the next precipitation using ethanol precipitates hemicelluloses with a lower molecular weight. Hemicelluloses have limited solubility in cold alkaline solutions, but warm alkaline solutions lead to hemicellulose degradation (Hoije *et al.*, 2005). During the extraction process, hydroxyl ions in KOH solution will cause the swelling of cellulose and the disruption of hydrogen bonds between cellulose and hemicelluloses. This tends to increase hydrophilicity and hence the solubility of the polymers (Xiao *et al.*, 2001). Extraction of hemicelluloses also involves the hydrolysis of ester linkages of the hemicelluloses. According to Xiao *et al.* (2001), in particular, all of the ester-linked substances of the hemicelluloses can be cleaved by alkali except for the α-ether bonds between lignin and hemicelluloses.

Klason Lignin Content

The lignin contents in the OPT samples extracted with different alkali concentrations are shown in
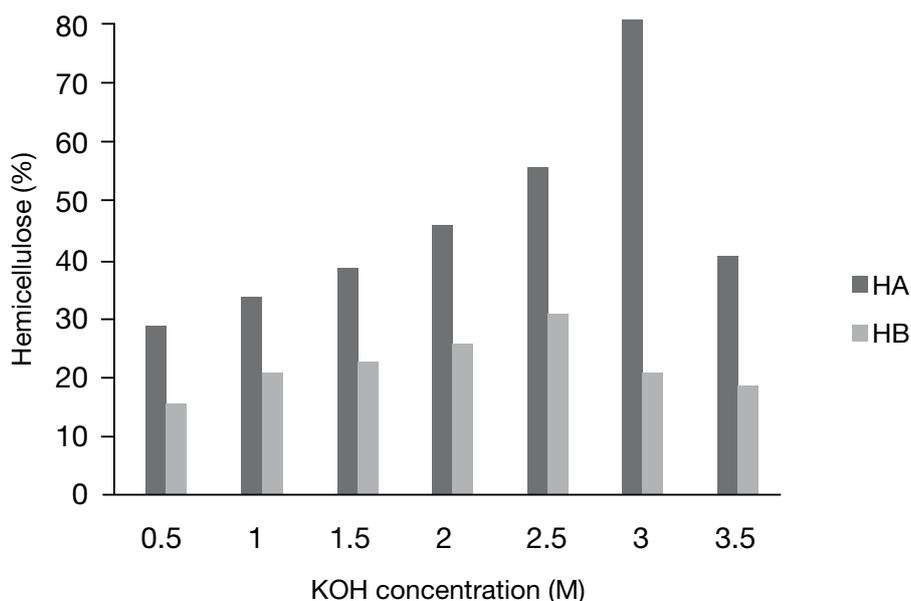
Figure 2. Effect of KOH concentrations on the extracted hemicelluloses.
Soaking time: 4 hr; temperature: 40°C.
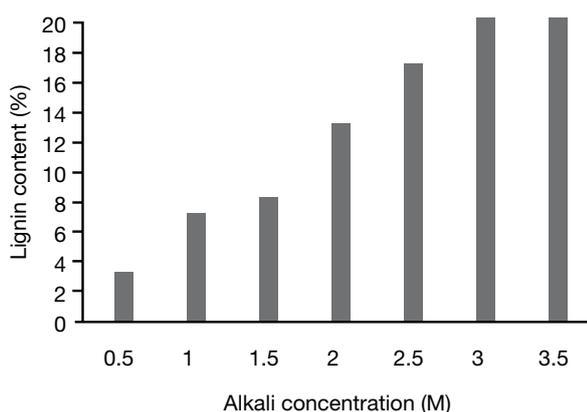*(HA: hemicellulose A; HB: hemicellulose B)*

Figure 3. Effect of different alkali concentrations on the amount of lignin extracted from oil palm trunks (OPT).

Figure 3. Results show that by increasing the alkali concentration up to 3.0 M, the yield of lignin was at the optimum level, and that the yield will not increase further if the alkali concentration was raised. It was found that 3.0 M alkali concentration was the maximum level that extracted the highest amount of lignin from the samples. The same situation was also reported by Vilpponen *et al.* (1993) who reported that lignin can be extracted from wood fibre at a higher alkali concentration.

Monosaccharide Composition

The monosaccharide (xylose, arabinose and glucose) contents obtained from the hydrolysis of both HA and HB are shown in *Table 1*. The composition of the three sugar components was different between hemicelluloses A and B. For xylose, the composition was 6.4% higher in HB

TABLE 1. MONOSACCHARIDE COMPOSITION OF THE EXTRACTED HEMICELLULOSES

Monosaccharide component	Percentage (%)	
	Hemicellulose A[a]	Hemicellulose B[a]
Xylose	39.50	45.90
Arabinose	2.40	3.79
Glucose	2.66	5.94

Note: [a] mean data based on three replicates.

than in HA, while for arabinose and glucose, the contents were 1.39% and 3.28% respectively higher in HB. The different monosaccharide composition in each fraction was due to the differences in the chemical structure of HA and HB. However, on the basis of sugar composition alone, it is difficult to draw conclusions about the branching patterns of the hemicelluloses (Geng *et al.*, 2003).

The results show that xylose was the predominant monosaccharide for both HA and HB, followed by glucose and arabinose (HA: 2.66% glucose and 2.40% arabinose; HB: 5.94% glucose and 3.79% arabinose). The content of xylose in HA and HB was 39.5% and 45.9%, respectively. The results show that the hemicelluloses from OPT were composed mainly of arabinoglucoxylan. HB had a higher content of each of the sugar components compared with HA. The ratio of xylose to arabinose was 16.4 for HA and 12.1 for HB. According to Wedig *et al.* (1987), the ratio of xylose to arabinose shows the degree of linearity or branching of hemicelluloses. A high xylose-to-arabinose ratio indicates a high degree of polymerisation with little bonding with the monosaccharide constituents.

A low xylose-to-arabinose ratio suggests a short-chain polymer with a large amount of branching with other monosaccharides. From this study, the xylose-to=arabinose ratios of HA and HB were approximately 16.4 and 12.1, respectively, which indicate that HB may be more highly branched than HA. This observation corresponds well with previous reports that the lower the arabinose content (indicating a lower degree of branching of the hemicellulose chain), the lower the solubility of the polymer (Ebringerova *et al.*, 1990).

Water Solubility of Hemicelluloses

The extracted hemicelluloses were not completely soluble in water at room temperature (*Table 2*). The differences in xylose/arabinose ratios between HA and HB may have contributed to the differences in water solubility. The results show that HA which is suspected of being less branched had lower solubility in water as compared with HB. According to Saulnier *et al.* (1995), highly branched hemicelluloses show the highest solubility in water after extraction. Bendahaou *et al.* (2007) also reported that water-soluble hemicelluloses were more highly branched than water-insoluble hemicelluloses.

Water-holding Capacity of Hemicelluloses

WHC is defined as the amount of water that can be taken up by a unit weight of dry fibre and is the point at which there is no free water (Southgate, 1976). WHC for the extracted hemicelluloses are shown in *Table 3*, where WHC for HA is higher

TABLE 2. SOLUBILITY OF HEMICELLULOSES IN WATER (substrate concentration: 1% at room temperature)

Time (hr)	Solubility (%)	
	Hemicellulose A[a]	Hemicellulose B[a]
1	66.2	65.8
2	66.5	65.5
3	68.5	86.4

Note: [a] mean data based on three replicates.

TABLE 3. WATER-HOLDING CAPACITY OF HEMICELLULOSES

Sample	WHC (%)[a]	WHC (g H_2O/g sample)[a]
Hemicellulose A	35.42	8.76
Hemicellulose B	28.12	6.23
Birchwood xylan[b]	33.02	6.95

Note: [a]mean data based on three replicates.
WHC – water-holding capacity.
[b]Data from Charles *et al.* (1993).

than for HB. The differences in WHC of the isolated hemicelluloses can indicate the differences in their chemical composition. According to Charles *et al.* (1993), the chemical composition of fibre plays an important role in its ability to hold water. Cellulose and lignin tend to have low WHC values, while hemicelluloses and pectin have high WHC values (Rasper, 1979). WHC depends both on the physicochemical properties and the composition of the extracted hemicelluloses (Labuza, 1968). The high WHC values for sugar beet fibre [14.8 g/g soluble fibre (SF)] and orange fibre (28.3 g/g SF) appear to be related to their high concentration of soluble fibre (Charles *et al.*, 1993).

Rheology of Hemicelluloses

Both fractions of hemicelluloses exhibited pseudoplastic flow behaviour as shown in *Figure 4*. This behaviour is also known as non-Newtonian or shear thinning. From this study, it was found that the increase in shear rate reduced the viscosity of both hemicelluloses. The viscosity of both fractions was also reduced by an increase in temperature (40°C). Results show that the suspected less branched HA was more affected by temperature and shear rate as compared with the suspected highly branched HB. According to Holdsworth (1971), the viscosities of both fractions were affected by an increase in temperature and shear rate due to molecular rearrangement in the samples.

Enzymatic Digestibility of Hemicelluloses

A study on the digestibility of the extracted hemicelluloses was carried out using xylanase enzyme under optimum conditions. Digestion of hemicelluloses is a complex affair because hemicelluloses are a composite of various sugars and glucosidic linkages. Results (*Figure 5*) show that the maximum percentage of hydrolysis was less than 20% for both hemicelluloses. This means that the extracted hemicelluloses from OPT have a low digestibility by the enzyme.

CONCLUSION

OPT hemicelluloses extracted by the aqueous extraction method comprised two different fractions, namely A and B. The best combination of extraction parameters were 4 hr of soaking time, a soaking temperature of 40°C, an alkali concentration (KOH) of 3.0 M and a sample size of 0.32 mm. About 28% of the hemicelluloses could be extracted, which comprised 22.8% hemicellulose A and 5.7% hemicellulose B. The extracted hemicelluloses were characterised in terms of solubility, WHC and enzyme digestibility. WHC was less than
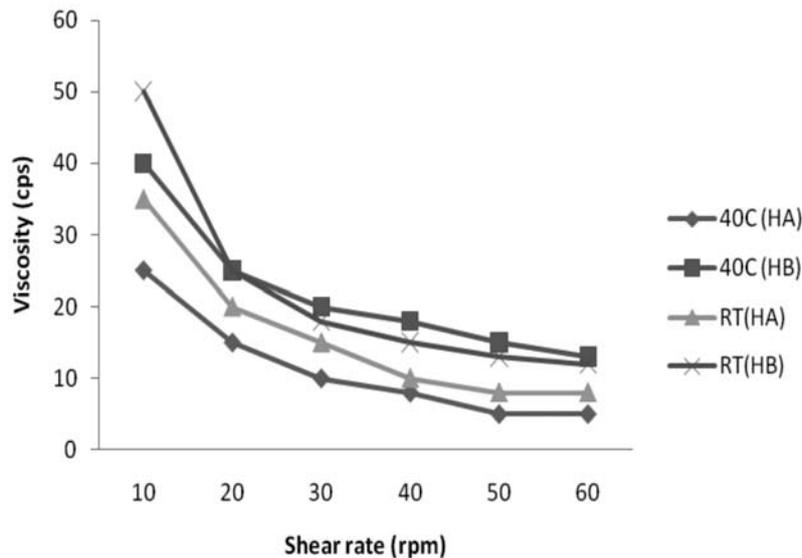
Figure 4. *Effect of temperature and shear rate on the viscocity of hemicelluloses at 4% concentration. (HA: hemicellulose A; HB: hemicellulose B; RT: room temperature).*
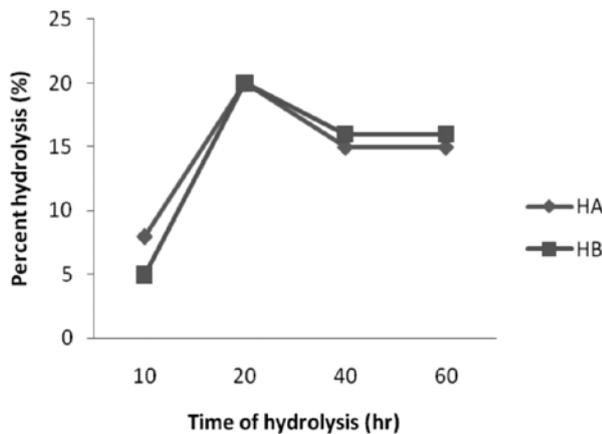

Figure 5. *Hydrolysis of hemicelluloses using xylanase enzyme. (HA: hemicellulose A; HB: hemicellulose B). Substrate concentration: 1% (w/v), temperature: 500C, agitation rate: 100 rpm*

20%. Both fractions (HA and HB) showed low enzyme digestibility. Both fractions also showed pseudoplastic flow behaviour and were affected by shear rate and temperature.

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