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CHARACTERIZATION OF HEMICELLULOSES FROM OIL PALM EMPTY FRUIT BUNCHES OBTAINED BY ALKALINE EXTRACTION AND ETHANOL PRECIPITATION

(Pencirian Hemisellulosa Daripada Tandan Kosong Kelapa Sawit Diperolehi dari Pengekstrakan Alkali dan Pemendakan Etanol)

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Abstract

Hemicelluloses from oil palm empty fruit bunches were extracted using alkaline extraction and fractionated by ethanol precipitation. Extraction of hemicellulose from oil palm empty fruit bunches were carried out with different KOH concentration (1 M, 3 M, 5 M), temperature (30 °C, 40 °C, 50 °C) and time (2 hours, 4 hours, 6 hours). The alkaline extraction produced two hemicellulosic fractions namely precipitate (HA) and alkali soluble hemicelluloses (HB). The alkali soluble hemicelluloses were then sub-fractionated by precipitation in 0.2 and 4 volumes ethanol for obtaining HB1 and HB2, respectively. Three hemicellulosic fractions (HA, HB1 and HB2) were further characterized in the form of total sugar, monosaccharides and Klason Lignin content. The optimal alkaline extraction was obtained at temperature of 40 °C, 3 M KOH concentration and extraction time 4 hrs. It was found that the alkaline extraction and ethanol precipitation is the suitable method to maximize the hemicelluloses yield.

Keywords: oil palm empty fruit bunches, alkaline extraction, hemicellulose

Abstrak

Hemisellulosa daripada tandan kosong kelapa sawit telah dieskstrak menggunakan pengesktrakan alkali dan terpisah oleh pemendakan etanol. Pengesktratan hemisellulosa daripada tandan buah kepala sawit dijalankan dengan pelbagai kepekatan KOH (1 M, 3 M, 5 M), suhu (30 °C, 40 °C, 50 °C) dan masa (2 jam, 4 jam, 6 jam). Pengesktrakan alkali menghasilkan dua pecahan hemisellulosa iaitu mendakan (HA) dan hemisellulosa yang larut alkali (HB). Hemisellulosa yang larut alkali masing- masing kemudiannya di sub-pisahkan oleh pemendakan dalam isipadu etanol 0.2 dan 4 masing-masing bagi menghasilkan HB1 dan HB2. Tiga pecahan hemisellulosa (HA, HB1 dan HB2) selanjutnya dicirikan oleh jumlah gula, kandungan monosakarida dan Klason Lignin. Pengesktrakan alkali optimum telah diperolehi pada suhu 40 °C, KOH 3 M dan masa 4 jam. Secara kesimpulannya pengesktrakan alkali dan pemendakan etanol merupakan kaedah yang sesuai untuk memaksimumkan hasil hemisellulosa.

Kata kunci: tandan kosong kelapa sawit, pengesktratan alkali, hemisellulosa

Introduction

Oil palm (*Elaeis guineensis)* were originated from West Africa and currently is one of the leading perennial oleaginous food crops cultivated widely in Malaysia, Indonesia and Thailand [1, 2]. Malaysia alone produce 47 %

oil palm which can be considered as second largest producer and exporter of oil plam. According to Idris et al. [3] oil palm cultivation in Malaysia covered more than 3.88 million hectares of land and operates more than 368 palm mills. Eventhough, palm oil industry has boosted the national economy in Malaysia, it also generated abundant of by-products. For every tonne of palm oil produced from fresh fruit bunch (FFB), approximately 1 tonne of oil palm empty fruit bunch (EFB), 0.7 tonnes of palm fibers, 0.3 tonnes of palm kernels and 0.3 tonnes of palm shells were generated in palm oil mills during the processing of palm oil from FFB [4]. Out of these by-products, EFB considered the biggest biomass waste and if not handled properly, would pose a severe environmental menace. Part of EFB is either incinerated for burn ash or recyled back to the plantation as mulching or used as solid fuel in the boilers to generate steam and electricity for the mills [5]. Increasing the utilization of this biomass is necessary and one such possibility would be to extract hemicelluloses to convert them to highly value-added products such as biodegradable film. The EFB biomass contains cellulose, hemicelluloses and lignin. It is estimated that EFB biomass comprised of 24 % hemicellulose which known as a valuable in pulp additives [6] and natural barrier for packaging film [7].

Hemicelluloses are the second most abundant polysaccharides in nature. Hemicelluloses, which are found in wood together with cellulose and lignin, give the best combination of mechanical support and transport properties. It is difficult to separate hemicelluloses from cellulose and lignins due to the component in lignocelluloses are bound tightly together without significant modification to its structure [8]. This could be among the main explanation why the material properties of hemicelluloses have not been exploited. Many treatments have been done to extract hemicelluloses from the plant such as steam explosion and ultra-filtration, heat treatment, classical alkaline extraction, ultrasound extract assistant and organosolv process. But among this processes, alkaline extraction has been the most promising method to accomplish complete utilization of lignocelluloses with minimal harm to the environment [9].

The purpose of the present work is to optimize the alkaline extraction conditions and ethanol precipitation of EFB on the yield of hemicelluloses. The effect of KOH concentration, temperature and time on hemicelluloses yield was investigated. The chemical properties of hemicelluloses fractions were examined in detail by means of sugar analysis and Klason Lignin. Furthermore, FT-IR was used to determine the chemical structure of sample.

Materials and Methods

Material

Oil palm EFB was obtained from a local palm oil factory in Johor, Malaysia. It was first soaked with distilled water overnight to remove dirt. Then EFB was oven-dried at 105 °C overnight to avoid fungus growth. Finally, dried EFB were ground to pass a 0.08 mm size screen using a mill blade. The samples were stored in the plastic bag prior to use. All chemicals used were of analytical grade unless otherwise stated. Xylose, glucose and arabinose were used as a standard for monosaccharide determination.

Hemicelluloses extraction

The extraction of hemicelluloses was carried out according to the method reported by Anis [10] with some modification. Oil palms EFB were soaked in different concentrations (1 – 5 M) KOH solution for different soaking time (2 – 6 hours) and at different temperatures (30 – 50 °C) with solid liquid ratio of 1:10. After extraction, the crude hemicelluloses was collected by filtration, acidified with 50 % acetic acid until pH 4.7 – 5.0 and allowed to stand for 24 hours at 4°C. The mixture was centrifuged at 3000 rpm for 15 min and the precipitates were washed with 95 % ethanol to remove the residual acid. The precipitate was dried in an oven at 60 °C for 24 hours to evaporate the ethanol and was designated as hemicelluloses (HA). The supernatant were successively sub-fractionated by graded ethanol volume solutions of 0.2V and 4V. Then both supernatant were centrifuged at 3000 rpm for 15 min. After centrifuged, the precipitates were dried in an oven at 60 °C for 24 hours to evaporate the ethanol. The precipitate obtained from the supernatant was designated as hemicelluloses B1 (0.2 volumes) and B2 (4 volumes). Hemicelluloses yield in solid extract were quantified by determining the total sugar content.

Hemicellulose characterization

Total sugar content was measured according to phenol-sulphuric acid method [11]. The total sugar content was evaluated using UV-Vis spectrophotometer (Perkin Elmer, USA) at 480 nm. Deionized water was used as a blank.

Klason lignin content in the hemicellulosic fractions was determined following TAPPI method T222. The monosaccharides content of hemicellulose fraction analysis was performed by acid hydrolysis. The hemicelluloses fraction was pre-hydrolyzed with 1.5 mL of 72 % H_2SO_4, before diluting the samples with 40 mL of distilled water and autoclaving them at 121°C for 1 hour. The samples were neutralized using $CaCO_3$ to pH 5.5 – 6.0.

The monosaccharides sugar was determined by high performance liquid chromatography (HPLC) (Agilent Technologies 1200 Series, Germany) using Aminex HPX 87P column (300 mm x 7.8 mm). The HPLC was equipped with a refractive index detector (G 1362A, Agilent Technologies 1200 Series, Germany) and column oven (Agilent Technologies 1200 Series, Germany). The sample was filtered through a 0.20 µm nylon syringe filter (Minisart, Sartorius AG, Germany) prior directly injected to the HPLC system. The sample was eluted using deionised water as the mobile phase at temperature of 80 °C with a flow rate of 0.5 mL/min [12]. The FT-IR spectra were obtained at a resolution of $4cm^{-1}$ using potassium bromide (KBr) pellet technique. The spectra were recorded between 500 and 4000 cm^{-1}.

Statistical analysis
Statistical analyses were performed using the software SPSS 16.0 program, via one way analysis of variance (ANOVA). Tukey's multiple range test was applied to determine the significantly different ($P < 0.05$) samples.

Results and Discussion

Effect of concentration of KOH on the hemicelluloses yield
To investigate the effect of KOH concentration on hemicelluloses yield, different KOH concentration (1 M, 3 M and 5 M) were used for extraction at 40 °C for 4 hours. Alkali extraction was found to have the most efficient method for isolating large amounts of hemicellulose polysaccharides. Hydroxyl ions cause swelling of cellulose, hydrolysis of ester linkage and disruption of storage linkages, such as intermolecular hydrogen bonds between hemicelluloses and cellulose, bring a portion of hemicellulose material into solution [13]. As shown in Figure 1a, the yield of hemicelluloses; HA (3.46 % to 4.67 %), HB1 (8.97 % to 7.43 %) and HB2 (17.32 % to 29.37 %) increased from 1 M to 3 M. Hemicelluloses yield increased with increasing of KOH concentration, however up to 5M KOH, the yield of all hemicelluloses fractions decreased indicating that appreciable amounts of hemicelluloses were leached out. It was found that HB2 has higher hemicelluloses yield compared to HA and HB1. Therefore, 3 M KOH was chosen for the following experiments.

Effect of temperature on the hemicelluloses yield
The hemicelluloses yield obtained at different temperature are shown in Figure 1b. It was observed that all hemicelluloses fractions increased after temperature 30 °C. The yields of hemicelluloses increased from 2.3 % to 4.7 %, 13.7 % to 24.4 % and 10.9 % to 29.4 % for HA, HB1 and HB2 respectively. Hemicellulose has a finite solubility in cold alkaline temperatures, but presumably, warm alkaline solutions lead to enhance hemicelluloses degradation; above 40 °C further extraction was occurred. It shows that high temperature is necessary for extracting hemicellulose in order to help the process of penetrating the lignified cell wall [14]. However at 50 °C, hemicellulose yield for HA (4.7 % to 2.6 %), HB1 (24.4 % to 8.5 %) and HB2 (29.3 % to 26.9 %) were decreased. Hemicellulose yield decreased at 50 °C due to degradation of hemicellulose by end-wise peeling reactions [15]. Therefore, a reaction temperature of 40 °C was considered as an optimum temperature for all hemicelluloses fractions.

Effect of time on the hemicelluloses yield
The effect of time on hemicelluloses yield during alkaline extraction was examined after an ideal concentration and temperature were obtained. Extraction periods of 2, 4 and 6 hrs were used at 40 °C and 3 M KOH. As observed from Fig. 1c), an increasing of extraction time from 2 hours to 4 hours, the hemicellulose yield were increased for HA and HB2. Both hemicellulosic fractions, HA and HB2 have higher hemicelluloses yield at 4 hours extraction. The results indicated that increasing extraction time may enhance the yield of hemicellulose. This is in accordance with other research finding [16]. Hydrolytic cleavage may degrade polysaccharides and induces the change of polysaccharides molecule structure at elevated time [17]. Therefore, the period of time 4 hours was chosen as an ideal time for the following experiments to save time and energy.

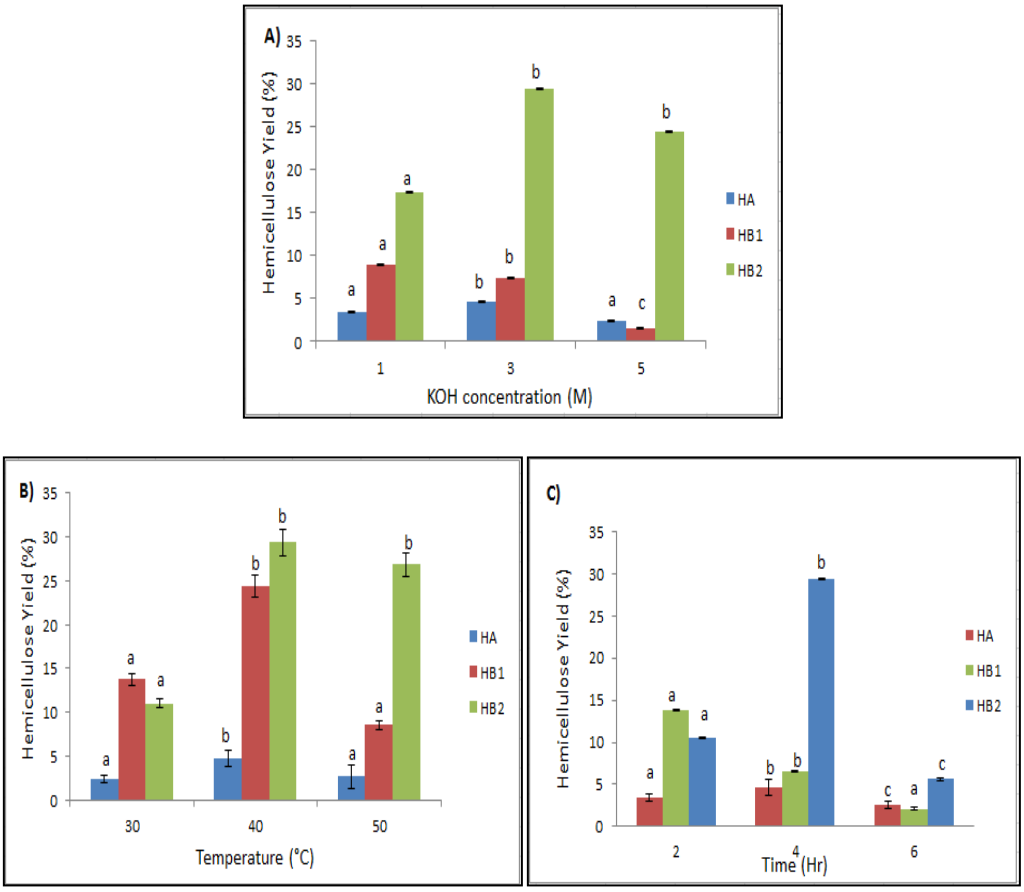

Figure 1. Effect of KOH concentration (a), temperature (b) and time (c) on the hemicelluloses yield (%). Letters (a - c) represent statistical comparisons made between different samples (HA, HB1 and HB2). Means with different letters indicate statistically significant difference from each other ($p < 0.05$).

Characterization:

Fourier transform infrared analysis

Figure 2 displays the FTIR spectra of untreated EFB, HA1, HB1 and HB2, recorded as the transmittance (%) versus wavenumber in the range of 500-4000 cm^{-1}. The O-H and aliphatic C-H stretching frequencies can be seen in region from 4000 to 1900 cm^{-1} [18]. Stretching band at 3440 cm^{-1} is attributed for O-H stretching band which corresponds to hydroxyl groups on anhydrogalactose bonds, this band showed a decrease in the intensity of HA spectra with alkaline treatment. This is due to the removal of –OH groups by reaction with KOH. HB1 and HB2 showed similar characteristics because they were also subjected to alkaline treatment. The band at 2941 cm^{-1} is attributed to C-H stretching vibration in cellulose and hemicellulose. The absorption at 1575 – 1665 cm^{-1} in all spectra is associated with water, since the hemicelluloses has strong affinity for water [19]. Untreated EFB has characteristic of ring vibrations overlapped with stretching vibrations of C-OH side groups and the C-O-C glycosidic bond vibration band at 1200 – 1000 cm^{-1} [20]. The disappearance peak observes at 1277 cm^{-1} in HA, HB1 and HB2 sample suggested that lignin was removed during alkaline extraction. This peak represents C-O linkage in guiacyl aromatic methoxy groups [21]. Combination of deformation of syringyl and cellulose was assigned at 1165 cm^{-1} band. The decrease in intensity at wavenumber 1165 cm^{-1} was greater than that at wavenumber 1277 cm^{-1} after alkaline extraction. This suggests that syringyl was more solubilized by alkaline extraction than guaicyllignin [22]. Band at ~1035 cm^{-1} is responsible for typical of xylan-rich polysaccharides which indicated a dominant xylan of the hemicellulosic

fractions [23]. Bands at 1420, 1360 and 1052 cm^{-1} are indication of hemicelluloses existence in the sample. In addition with band at 900 $cm^{-1}$is a characteristic of β-glycosidic linkage between the monosaccharide units, indicating β-linked hemicelluloses [23]. These spectral changes are characteristics of hemicellulose, which further demonstrates the removal of lignin.

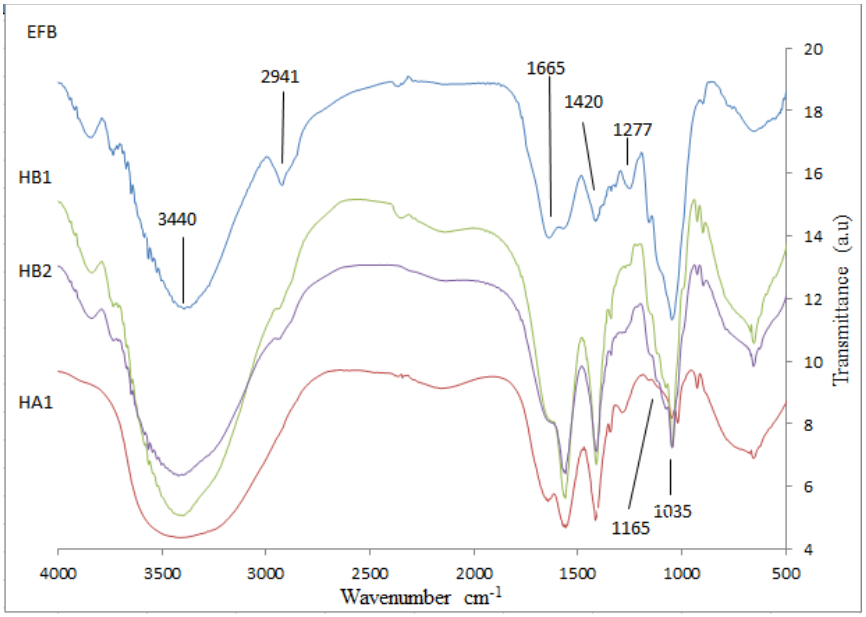

Figure 2. FTIR spectra of EFB, HA, HB1 and HB2

Klason lignin content

It was observed that a significant reduction in lignin content of HA has been achieved as compared to the original untreated OPEFB through the alkaline extraction process (Table 1). Treatment using KOH permitted the swelling of the particles that resulted in easy lignin degradation. It proved that alkaline extraction can significantly breaks the bonds between lignin and hemicelluloses [24]. After alkali extraction, the lignin content of HB1 and HB2 were decreased. This is in accordance with the finding reported by Zhao et al. [25].

Table 1. Monosaccharide compositions and lignin content in untreated EFB and hemicellulosic fractions

Sample	Monosaccharide Compositions (mg/ml)				Lignin Content (%)
	Glucose	Xylose	Arabinose	Ratio Xyl/Ara	
Untreated EFB	-	-	-	-	24.50 ± 0.01
HA	0.051 ± 0.033	0.490 ± 0.017	0.142 ± 0.027	3.456	12.06 ± 0.05
HB1	nd	0.546 ± 0.105	0.119 ± 0.004	4.591	8.96 ± 0.05
HB2	0.277 ± 0.043	1.130 ± 0.042	4.131 ± 0.023	0.273	6.08 ± 0.03

nd = not detected. Each data point were average at least two replicate measurements

Monosaccharide analysis by HPLC

The neutral monosaccharides composition content of the three precipitated hemicellulosic fractions; HA, HB1 and HB2 are shown in Table 1. Sugar analysis of hemicellulosic fractions showed that the two predominant monosaccharides in EFB were xylose and arabinose. It is suggested that the fraction may be a mixture of xylan with a small amount of arabinoxylans-type polysaccharides [26, 27]. The degrees of linearity or branching of hemicellulosic polymers were indicated by the ratio of xylose to arabinose (Xyl/Ara). A low Xyl/Ara ratio suggest that a short-chain polymer with a large amount of branching with other monosaccharide constituents. As shown in the Table 1, the ratio of Xyl/Ara gradually increased from 3.456 to 4.591. These result indicated that the hemicellulosic fractions released in alkaline extraction and ethanol precipitation has more linear structures [28].

Conclusion

This study presented extraction of hemicellulose from empty fruit bunches with various conditions. The hemicelluloses yield of HB2 was found to be higher than HA and HB1. As conclusion, alkaline extraction and ethanol precipitation are suitable methods for extracting hemicelluloses from oil palm empty fruit bunches.

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