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FRACTIONATION OF HEMICELLULOSE FROM RICE STRAW BY ALKALINE EXTRACTION AND ETHANOL PRECIPITATION

(Pemisahan Hemiselulosa daripada Hampas Batang Padi melalui Pengekstrakan Beralkali dan Pemendakan Etanol)

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Abstract

Hemicelluloses were extracted from rice straw using alkaline extraction and ethanol precipitation. The effects of different extraction conditions; temperature (45 – 65 °C), pH (4.5 – 6.5) and concentrations of NaOH (0.25 – 1 M) on the hemicellulose yield were investigated. Two hemicelllulosic fractions namely Ha_1 and Ha_2 were obtained by precipitation of alkaline soluble hemicellulose in 0.2 volumes and 4 volumes ethanol respectively. The Ha_1 and Ha_2 were further characterized to obtain total sugar, monosaccharides and Klason Lignin content. The highest hemicellulose yield for Ha_1 was 4.00 % obtained at 55 °C and pH 5.5 with 0.25 M of NaOH, whereas the highest hemicellulose yield for Ha_2 was 19.88 % obtained at 55 °C and pH 5.5 with 0.5 M of NaOH. This study revealed that hemicellulose yield is dependent on the pH, temperature and concentration of NaOH in the alkaline extraction.

Keywords: hemicelluloses, rice straw, alkaline extraction, ethanol precipitation

Abstrak

Hemiselulosa daripada hampas batang padi telah diekstrak melalui pengekstrakan beralkali dan pemendakan etanol. Kesan perbezaan kondisi pengekstrakan; suhu (45 – 65 °C), pH (4.5 – 6.5), dan kepekatan NaOH (0.25 – 1 M) ke atas hasil hemiselulosa dikaji. Dua pecahan hemiselulosa, Ha_1 and Ha_2 telah diperolehi melalui pemendakan hemisellulosa larut alkali dalam isipadu etanol 0.2 dan 4 masing-masing. Ha_1 and Ha_2 dicirikan oleh jumlah gula, kandungan monosakarida dan Klason Lignin. Hasil hemiselulosa tertinggi untuk Ha_1 adalah 4.00 % yang diperolehi pada suhu 55 °C, pH 5.5 and 0.25 M NaOH, manakala untuk Ha_2 adalah 19.88 % diperolehi pada suhu 55 °C, pH 5.5 and kepekatan 0.5 M NaOH. Kajian ini membuktikan hasil hemisellulosa bergantung kepada pH, suhu dan kepekatan NaOH dalam pengekstrakan beralkali.

Kata kunci: hemiselulosa, hampas batang padi, pengekstrakan beralkali, pemendakan etanol

Introduction

The main cereal crop in the whole wide world is rice, which is being produced in more than 148 million hectares with huge range of ecosystem. The scientific name of paddy is *Oryza sativa*. It is an important cereal crop that produce a lot of residues which is rice straw [1]. Rice straw is the rice plant stalk that is left as waste product on the field after harvesting of the rice grain [2]. A lot of rice straw are disposed by biomass burning that leads to air pollution.Therefore, a lot of research work has been performed to find ways of utilizing these agricultural waste. Hemicellulose is the second most abundant polysaccharide in nature, representing approximately 20-30% of lignocellulosic biomass [3]. Hemicellulose polymers are complex components in the plants cell wall. With cellulose and lignin, hemicellulose form hydrogen bonds and covalent bond respectively.

The hemicelluloses are used in various industrial applications such as food additives, biodegradable films and in medicinal applications [4]. Various methods were used to isolate hemicellulose from the agricultural waste. Among these methods, alkaline extraction is most widely used. Alkaline solution hydrolyzes the ester linkage between hemicellulose and other components, resulted in isolating hemicellulose in the aqueous media. Usually the isolation of hemicellulose is done by using alkaline solution such as NaOH for softwood and KOH for hardwood [5]. The aim of this study was to optimize the alkaline extraction conditions and ethanol precipitation of hemicelluloses from rice straw. The effects of the concentration of NaOH, temperature and pH on hemicellulose percentage yield were investigated.

Materials and Methods

Materials

Rice straw was obtained from local sources (Perlis, Malaysia). It was dried and cut into small pieces. The straw was ground to pass a 0.08 mm size screen and stored at room temperature. All other chemicals used were of analytical grade unless otherwise stated.

Extraction of hemicelluloses

Extraction of hemicellulose was carried out by mixing the rice straw with 0.25 M sodium hydroxide (NaOH) with ratio of 1:25 (w/v). The mixture was stirred at 400 rpm and heated at constant temperature of 55 °C for 2 hours. After filtration, the pH of the solution was adjusted to pH 5.5 by adding 6 M hydrochloric acid (HCl). Then the mixtures were kept at 4.0 °C for 24 hours. The mixture was then centrifuged at 3500 rpm for 15 minutes. Ethanol 0.2 volumes and 4 volumes containing 10 % of acetic acid were added to the liquid fraction to precipitate Ha_1 and Ha_2 respectively. Both hemicellulose fractions (Ha_1 and Ha_2) were obtained and dried at 40.0 °C for 24 hours. In order to optimize the alkaline extraction conditions, the extraction was carried out at various concentration of NaOH (0.25 M, 0.5 M and 1 M) temperature (45 °C, 55 °C, and 65 °C) and pH (4.5, 5.5 and 6.5).

Analytical method

Total sugar content was measured according to phenol-sulphuric acid method [6]. The hemicellulose fraction with unknown concentration was added with 0.5 mL of 5.0 % phenol solution and the mixture was shaken. A volume of 2.5 mL concentrated H_2SO_4 was quickly added to the mixture (phenol-hemicellulose) and shaken once again. These solutions were then analysed where the absorbance of the mixtures were recorded at 480 nm using UV-Visible spectrophotometer. The hemicelluloses yield (%) was calculated based on total sugar content in the hemicelluloses fraction. The monosaccharides sugar was determined by using a 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 hemicellulose fraction was filtered through a 0.20 µm nylon syringe filter (Minisart, Sartorius AG, Germany) prior directly injected to the HPLC system. The hemicellulose fractions were eluted using deionised water as the mobile phase at column temperature of 80 °C and a flow rate of 0.5 mL/min. Klason lignin content was determined following TAPPI method T222. The FTIR spectra of the hemicellulose fractions were obtained on a FT-IR spectroscopy (Nicolet 500) using a KBr disc containing 1% finely ground samples. Spectra were recorded between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1}.

Statistical analysis

ANOVA was used to analyse the data of experiment and Duncan's multiple range test was applied in order to determine the significant difference ($P<0.05$) of samples using Statistical Package for Social Science, Version 10 (SPSS Inc., Illinois). The result was expressed as mean ± SD (standard deviation).

Results and Discussion

Effect of NaOH concentration

Alkaline extraction was carried out to investigate the effects of NaOH concentrations; 0.25 M, 0.5 M and 1 M on hemicellulose yield, while other parameters were fixed as followed : pH 5.5 and temperature 55 °C. As shown in

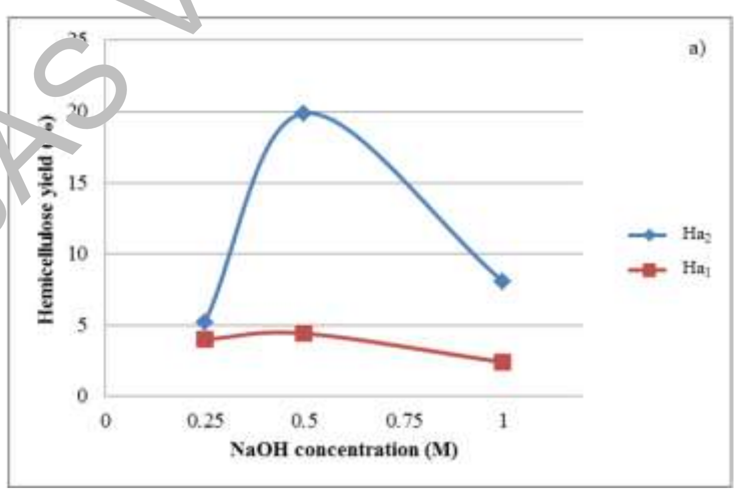
Figure 1(a), hemicellulose yield for both Ha_1 and Ha_2 increased as NaOH concentration increased from 0.25 M to 0.5 M but then decreased tremendously when the NaOH concentration was increased to 1 M. This indicates that hemicellulose extraction was best performed at mild conditions. Hemicellulose yield for both, Ha_1 and Ha_2 were highest at 0.5 M NaOH concentration. Statistical analysis for Ha_1 showed that there were no significant differences between 0.25 M NaOH and 0.5 M NaOH ($P > 0.05$), while there were significant differences among 0.25 M NaOH and 1 M NaOH. Thus 0.25 M NaOH concentration was chosen for further analysis for Ha_1. On the other hand, the statistical analysis for Ha_2 showed that there were significant differences between 0.25 M, 0.5 M and 1 M NaOH ($P < 0.05$).Therefore, 0.5 M NaOH concentration was chosen for further analysis for $Ha_{2.}$

Effect of temperature

The effects of different temperatures (45 – 65 °C) on the yield of hemicellulosic fractions; Ha_1 and Ha_2 were studied while the other parameters were fixed; pH 5.5 with 0.25 M NaOH for Ha_1 and pH 5.5 with 0.5 M NaOH for Ha_2. Statiscal analysis of hemicellulose yield, Ha_1 and Ha_2 showed that the significant difference existence between temperature of 45 °C and 55 °C and among hemicellulose extracted at 55 °C and 65 °C ($P < 0.05$), however there is no significant difference between hemicellulose yield extracted at 45 °C and 65 °C ($P > 0.05$). Based on Figure 1 (b), the best temperature for alkaline extraction was at 55°C which gave the highest hemicellulose yield. The presence of high lignin content hindered the yield of hemicellulose in Ha_1 resulted in lower hemicellulose yield than Ha_2. The hemicellulose yield for both Ha_1 and Ha_2 significantly increased from 45 °C to 55 °C and as temperature of extraction reached 65 °C, the yield of hemicelluloses significantly decreased. The yield of hemicellulose decreased at 65 °C due to less degradation of macromolecular hemicellulose.The minor degradation occurred mainly because of the linkages between glycosyl units were cleaved at high temperature [6].

Effect of pH

pH was another factor which affected the efficiency of hemicellulose extraction. The effect of pH for hemicellulosic fractions, Ha_1 and Ha_2 were studied at different pH (4.5 – 6.5) while the other parameters were fixed; 0.25 M NaOH and temperature of 55 °C for Ha_1 while 0.5 M NaOH and temperature of 55 °C for Ha_2. Statiscal analysis for Ha_1 and Ha_2 showed that the significant difference existence between pH of 4.5 and 5.5 and between pH 5.5 and 6.5, ($P < 0.05$), however there is no significant difference between pH 4.5 and 6.5 ($P > 0.05$). There was an increasing trend in hemicellulose yield from pH 4.5 to 5.5 and decreasing trend from pH 5.5 to 6.5 for both Ha_1 and Ha_2. This indicated that minor degradation occurred when the pH was slightly acidic (pH 4.5) and at neutral condition (pH 6.5). Figure 1 (c) showed that the suitable pH to yield the maximum concentration for both Ha_1 and Ha_2 was at pH 5.5. Thus, pH 5.5 was favorable to isolate hemicellulose from rice straw using alkaline extraction.



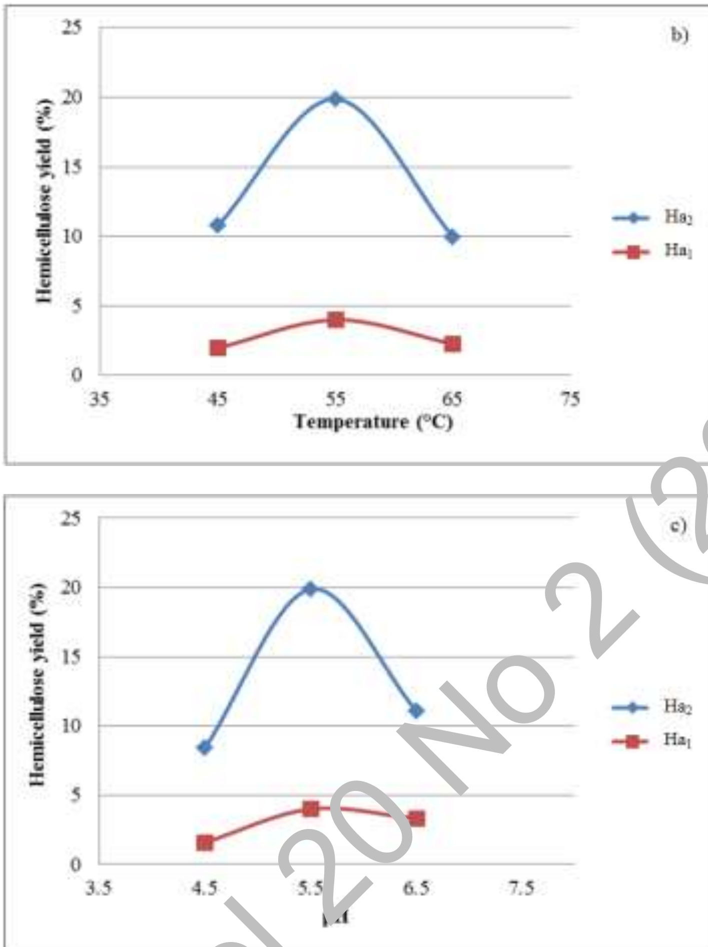


Figure 1. Effects of NaOH concentration (a), temperature (b), and pH (c) on hemicellulose yield (%) Ha_1 and Ha_2.

Klason Lignin content

The Klason lignin content for both hemicellulose fractions, Ha_1 and Ha_2 is shown in Table 1. The result showed that Ha_1 contain more lignin (11.72 %) than Ha_2 (4.22 %). Table 1 showed that as the volume of ethanol decreased (0.2 v) in Ha_1, the Klason lignin content increased and as the volume of ethanol increased (4v) in Ha_2, the Klason lignin content decreased. The increased volume of ethanol (4v) increased the polarity of plant cell wall in Ha_2 exposed it more to the solvent thus promote the lignin removal [7].

Table 1. Monosaccharide composition and Klason Lignin content for Ha_1 and Ha_2

	Glucose (mg/mL)	Arabinose (mg/mL)	Xylose (mg/mL)	Ara/Xyl ratio	Klason Lignin (%)
Ha_1	0.57 ± 0.44	0.10 ± 0.02	0.27 ± 0.02	0.36	11.72 ± 5.07
Ha_2	0.70 ± 0.18	0.96 ± 0.07	0.05 ± 0.05	21.08	4.22 ± 1.32

Data means and standard deviation n=3. Ha_1 represent hemicellulose high lignin prepared by alkaline extraction from rice straw with 0.2v ethanol precipitation. Ha_2 represent hemicellulose low lignin prepared by alkaline extraction from rice straw with 0.4v ethanol precipitation.

Monosaccharide analysis by HPLC

As shown in Table 1, both Ha_1 and Ha_2 composed of glucose, arabinose and xylose. The predominant sugar component for Ha_1 was glucose while for Ha_2 was arabinose. Ha_2 consisted of higher glucose concentration mainly because the presence of glucans which associated with xylans in primary cell wall, which were less associated with lignin. The xylose concentration higher in Ha_1 as compared to Ha_2. The lower amount of arabinose in Ha_1 suggested that the hemicellulose was closely assosiated to the cellulose surface [8]. Hemicellulose fraction, Ha_2 contained higher amount of Ara/Xyl (21.08) whereas Ha_1 showed lower Ara/Xyl ratio (0.36). Higher amount of Ara/Xyl means that the hemicellulose obtained more linear arabinoxylan while lower Ara/Xyl ratio consisted of branched arabinoxylan [9].

FTIR Spectra

Figure 2 shows the FTIR spectra of hemicellulosic fractions (Ha_1, Ha_2) and rice straw within the region of 500 – 4000 cm^{-1} including region 850 – 1200 cm^{-1} which is typical region for hemicellulose [10]. Both hemicellulose fractions show similar features. All of the spectra showed similarity as they all have hemicellulose band (850 –1200 cm^{-1}), which concluded that the alkaline extraction did not change the macromolecular structure of hemicellulose. Absorption band at 1610 cm^{-1} corresponded to the water bending mode presence for only Ha_1 and Ha_2 since hemicellulose have strong affinity towards water whereas, this band could not found in rice straw due to the presence of cellulose which hindered the water affinity [11]. Low intensity absorption band at region 850 – 1200 cm^{-1} in Ha_1 compared to Ha_2 shows low purity of hemicellulose fraction due to the higher lignin content for Ha_1 compared to Ha_2. The absoption band at region between 1126 – 1032 cm^{-1} is a typical band for lignin [12]. Higher intensity absorption band at region 1126 – 1032 cm^{-1} in Ha_1 compared to Ha_2 proved that lignin content was higher in Ha_1. The presence of absorption band at 1710 cm^{-1} in Ha_1 is due to the carboxyl groups as the hydroxyl groups in lignin undergo oxidation [13]. Absorption band at 3049 cm^{-1} is assigned to the C-H in an aromatic vibration as hemicellulose contained a small amount of lignin [10]. Absorption band at 1043 cm^{-1} is due to the C-O-C stretching glycosidic linkages in xylans. And the absorption band at 1740 cm^{-1} refer to acetyl carbonyl. As shown in Figure 2, broad absorption band at 3400 and 2400 cm^{-1} is due to the stretching vibrations of OH and CH respectively and absorption band at 1200 cm^{-1} refer to the stretching vibrations of C-O. The C-H bending vibration shows absoption bands at 1467 and 1427 cm^{-1} while the OH bending vibration shows absoption bands at 1340 cm^{-1} [14].

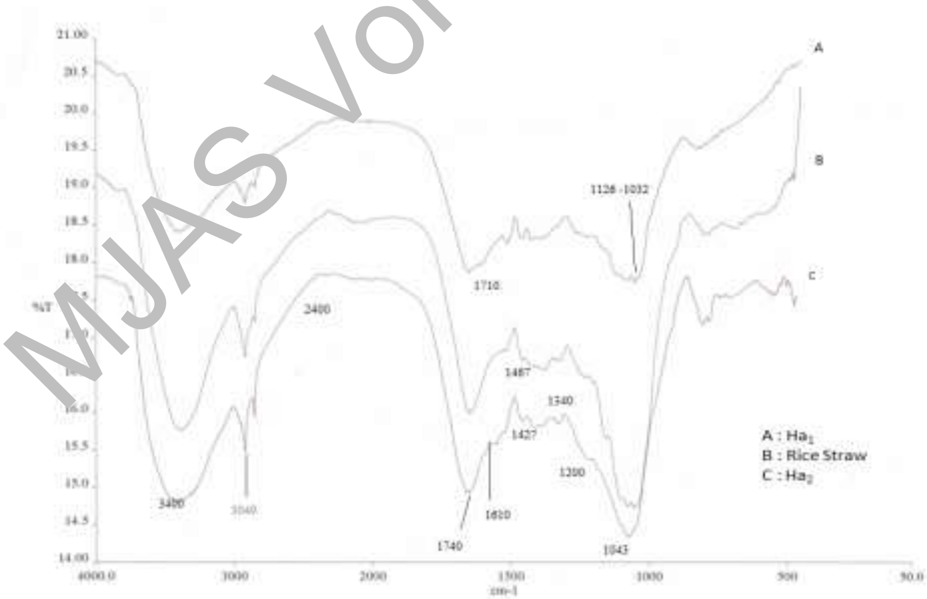


Figure 2. FTIR spectra of Ha_1, Ha_2 and rice straw

Conclusion

This study showed that the maximum hemicellulose percentageyield was obtained by alkaline extraction. The highest hemicellulose yield for Ha_1 was 4.00 % obtained at 55 °C and pH 5.5 with 0.25 M NaOH, whereas the highest hemicellulose percentage yield for Ha_2 was 19.88 % obtained at 55 °C and pH 5.5 with 0. 5M of NaOH. Hemicellulose yield Ha_2 was found to be higher than Ha_1, showing that lignin content affected the percentage yield of hemicellulose.

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