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DETERMINATION OF GLUCOSE CONTENTS IN KENAF

(Penentuan Kandungan Glukosa dalam Kenaf)

Fatin Afifah Ahmad Kuthi[1], Nurulhuda Mohd Yunus[1], Goh Kae Horng[1], Khairiah Haji Badri[1,2]*

[1]*School of Chemical Sciences and Food Technology, Faculty of Science and Technology*
[2]*Polymer Research Center, Faculty of Science and Technology*
Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

**Corresponding author: kaybadri@ukm.edu.my*

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Abstract

Kenaf is one of the potential plant fibres used in research on the extraction and recovery of glucose. Kenaf fiber is comprised of a kenaf core (KC) and kenaf bast (KB). This study was performed to explore a hot water pre-treatment (HWP) using boiling water immersion for 2 hours on both KC and KB. Alkaline treatment using 6% (w/v) sodium hydroxide (NaOH) aqueous solution was subsequently performed for 3 hours at room temperature. The percentages of α-cellulose and hemicellulose in both KC and KB were found to increase after HWP. On the contrary, the percentages of α-cellulose and hemicellulose decreased upon NaOH treatment. The percentage of Klason lignin was reduced when KC and KB had undergone the HWP and NaOH treatments. A Fourier transform infrared spectroscopy analysis (FTIR) revealed the removal of lignin from both parts of kenaf fibre after the NaOH treatment. This was confirmed when the peaks at 1735 cm^{-1} and 1246 cm^{-1} disappeared after the treatment. Glucose as part of carbohydrates was determined in the fibre and filtrate using phenol sulfuric (PS) and dinitrosalicylic acid (DNS) analyses respectively. PS analysis indicated that the amount of glucose in KC-HWP (0.17 mg/g) was higher than in untreated KC (0.13 mg/g) and KC-NaOH (0.09 mg/g). The untreated KB showed the highest glucose content (0.36 mg/g), followed by KB-HWP (0.29 mg/g) and KB-NaOH (0.18 mg/g). Meanwhile, DNS analysis disclosed that the glucose concentrations in the KC filtrate for both treatments were 0.05 mg/mL. In contrast, the DNS analysis for KB showed a slightly lower glucose concentration in the KB-HWP filtrate (0.062 mg/mL) compared to the KB-NaOH (0.064 mg/mL). The glucose production was highly related to the composition of α-cellulose in the kenaf fibers.

Keywords: carbohydrate, kenaf fibre, compositional analysis, phenol sulfuric, dinitrosalicylic acid

Abstrak

Kenaf merupakam salah satu serabut tumbuhan yang berpotensi dalam penyelidikan yang melibatkan pengestrakan dan perolehan glukosa. Serabut kenaf terdiri daripada dua bahagian iaitu teras (KC) dan kulit (KB). Kajian ini meneroka pra-rawatan rendaman air mendidih (HWP) selama 2 jam bagi kedua-dua KC dan KB. Seterusnya, rawatan alkali menggunakan 6% (w/v) larutan akues natrium hidroksida (NaOH) dijalankan selama 3 jam pada suhu bilik. Peratus kandungan α-selulosa dan hemiselulosa bagi KC dan KB didapati meningkat selepas pra-rawatan air panas. Sebaliknya, peratusan α-selulosa dan hemiselulosa menurun selepas rawatan NaOH. Peratus kandungan lignin Klason berkurang apabila KC dan KB menjalani rawatan HWP dan NaOH. Analisis spektroskopi inframerah transformasi Fourier (FTIR) menunjukkan bahawa berlaku penyingkiran lignin pada kedua-dua bahagian kenaf yang dirawat NaOH. Ia dapat dibuktikan apabila puncak pada nombor gelombang 1735 cm^{-1} dan 1246 cm^{-1} hilang selepas rawatan NaOH dijalankan. Glukosa yang merupakan sebahagian daripada karbohidrat ditentukan kandungannya di dalam serabut dan filtrat masing-masing menggunakan kaedah fenol sulfurik (PS) dan asid dinitrosalisilik (DNS). Berdasarkan analisis PS, kandungan glukosa dalam KC-HWP (0.17 mg/g) adalah lebih tinggi berbanding sampel KC tidak terawat (0.13 mg/g) dan KC-NaOH (0.09 mg/g). Berbeza dengan serabut KB di mana sampel KB tidak terawat menunjukkan kandungan glukosa yang tinggi iaitu 0.36 mg/g diikuti dengan KB-HWP (0.29 mg/g) dan KB-NaOH (0.18 mg/g). Analisis DNS menunjukkan kepekatan filtrat KC-HWP dan KC-NaOH adalah sama iaitu 0.05 mg/mL. Sebaliknya,

analisis DNS bagi KB mempamerkan kepekatan glukosa yang sedikit rendah dalam filtrat KB-HWP (0.062 mg/mL) berbanding filtrat KB-NaOH (0.064 mg/mL). Perolehan glukosa sangat bergantung kepasa kandungan komposisi α-selulosa serabut kenaf.

Kata kunci: serabut kenaf, analisis komposisi, karbohidrat, fenol sulfurik, asid dinitrosalisilik

Introduction

Kenaf (*Hibiscus cannabinus, L. family Malvacae*) has been planted intensively to replace tobacco plantations in Malaysia. Kenaf is a non-wood lignocellulosic material that originated from Africa [1]. Kenaf contains two parts, i.e. the outer part, which is known as the bast and the inner part called the core. The percentages of lignocellulosic material are different in both kenaf core and bast as shown in Table 1 [2]. Kenaf core (KC) is used as a source of pulp for the paper industry [3], cosmetics, industrial chemicals and biopolymers [4]. Meanwhile, kenaf bast (KB) is used to produce ropes, canvas, bags and carpets [5, 6].

Table 1. Chemical composition of kenaf core and bast [2]

Constituent (%)	Kenaf Bast	Kenaf Core
Holocelluose	76.9	77.6
α-cellulose	69.8	45.3
Lignin	9.2	19.0
Alcohol-benzene extractives	3.4	3.0
Hot water solubilty	15.9	7.5

Cellulose is a homopolysaccharide composed of β-D-glucopyranose, $C_6H_{10}O_5$ units which are linked together by (1→4)-glycosidic bonds with a molecular weight between 50,000 to one million kDa [7, 8]. Cellulose molecules are completely linear and have a strong tendency to form intra- and intermolecular hydrogen bonds. Bundles of cellulose molecules are thus aggregated together in the form of microfibrils in which crystalline regions alternate with amorphous regions [9].

Hemicelluloses are heterogeneous polysaccharides. Like cellulose, most hemicelluloses function as supporting materials in cell walls. The main feature that differentiates hemicellulose from the cellulose is that hemicellulose has branches with short leteral chains consisting of different sugars. Due to its branching sructure, hemicellulose lacks crystalline regions. Hemicellulose mainly consists of five carbon sugar monomers, xylose and six carbon sugar monomers such as glucose. In contrast to cellulose, the polymers present in hemicellulose are hydrloysable [10, 11].
Lignin can be divided into klason lignin (acid insoluble lignin) and acid soluble lignin. Lignin has a complex and large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell wall and is resistant to microbial attack [12]. Lignin consists of three main C6-C3 (phenypropanoid) units known as syringyl alcohol, guaiacyl alcohol and p-coumaryl alcohol [13].

Measurements of carbohydrate content is one of the basic analytical operations. The most common methods used for measurement are the phenol sulfuric (PS) analysis and dinitrosalisylic (DNS) analysis. The phenol sulfuric method is widely used due to its sensitivity and simplicity [14]. The analytical method that can be used in determining the reduction in sugar usually involves colorimetric detection based on the oxidation of the carbonyl group. In a DNS analysis, dinitrosalicylic acid will react with the free carbonyl group in the reducing sugar under an alkaline environment. This will allow the formation of an aromatic compound (3-amino-5-nitrosalicylic acid) with the highest absorption at 540 nm [15].

In this paper, we focus on the compositional analysis of the kenaf core and bast before and after treatment by comparing the determination method of glucose content using either the phenol sulfuric analysis or the DNS assay analysis. The fibre was first pre-treated with hot water, followed by treatment with sodium hydroxide (NaOH) at 6%

(w/v). There are numerous methods that can be used to determine glucose content. In this study, the PS and DNS methods were chosen because they can provide a quick check on the sugar content [16]. These methods are not only reliable but also time and cost efficient [15].

Materials and Methods

Materials

The kenaf fibre used was generously supplied by Kenaf Natural Fibre Industry Sdn Bhd (KFI), Kelantan, Malaysia. The kenaf core (KC) and kenaf bast (KB) were refined to a size of 100 to 160 μm and 125-250 μm respectively. The chemicals used for α-cellulose, hemicellulose and lignin extraction and acid hydrolysis were ethanol, C_2H_5OH (John Kollin Chemicals, United Kingdom), sodium chlorite, $NaClO_2$ (Sigma Aldrich, USA) and hydrogen peroxide, H_2O_2 (Friendemann Schmidt Chemical, Germany). Acetic acid (CH_3COOH), sodium hydroxide pellet (NaOH), sulfuric acid (H_2SO_4) and toluene (C_7H_8) were purchased from Systerm Sdn Bhd, Selangor, Malaysia. The chemicals used for phenol sulphuric analysis, hydrochloric acid (HCl) and sodium carbonate ($NaCO_3$) were obtained from Systerm Sdn Bhd, Selangor, Malaysia. Chemicals used for the dinitrosalicylic (DNS) assay such as 3,5-dinitrosalicylic acid ($C_7H_4N_2O_7$), phenol crystal (C_6H_6O) were supplied by Fluka Analytical, Subang Jaya, Selangor, Malaysia, while sodium bisulfate ($NaHSO_3$) and potassium sodium tartrate ($KNaC_4H_4O_6{\cdot}4H_2O$) were purchased from Systerm Sdn Bhd, Selangor, Malaysia.

Method

The kenaf core (KC) and kenaf bast (KB) fibres were dried at 105 °C until a constant weight was obtained. Hot water pre-treatment (HWP) was carried out at a boiling temperature for 2 hours with a fibre to hot water ration of 1:20 [17, 18]. The sample was filtered and dried at 105 °C until it reached a constant weight. The pre-treated sample was then soaked in 6% (w/v) NaOH aqueous solution for 3 hours at room temperature. The ratio of fibre to NaOH aqueous solution was 1:20 [19, 20]. The sample was filtered and rinsed with distilled water for several times until it reached pH 7. The filtered cake was dried at 105°C for 24 h in a conventional oven until a constant weight was obtained. These steps were followed and repeated for KB fibre.

Characterisation: Chemical composition analysis

The untreated and treated kenaf fibres (KB and KC) were analysed to determine their moisture content, solubility and composition [21-26]. Standard methods of moisture content was followed, where the samples were dried in a conventional oven for 24 hours at the temperature of 105 °C (TAPPI T208 om-84). Hot water solubility (TAPPI T 207 om-81) was carried out by soaked the samples in a boiling water for 3 hours. Alcohol-benzene solubility was carried out to remove the extractives materials such as oil, wax and inorganic substances according to TAPPI T204 cm-97. Holocellulose content was determined according to Wise et al. [25] where the sample was bleached with natrium chlorite ($NaClO_2$) and and acetic acid (CH_3COOH) alternately. The α-cellulose content was determined via treatment of holocellulose sample with 17.5% (w/v) NaOH to removed hemicellulose from the fibres under 20 °C water bath (TAPPI T203 om-83). The KC and KB fibres was treated with 72% (v/v) H_2SO_4 for 1 hour followed by dilution of the acid to 3% (v/v) by addition of distilled water. Subsequently, the solution was heated at boiling temperature for 4 hours to determine the Klason-lignin content (TAPPI T222 om-83).

Fourier transform infrared spectrophotometer

The FTIR spectroscopy analysis was conducted on a spectrophotometer model Perkin Elmer Spectrum BX using the KBr pellet method. The functional group was analysed at wavenumbers ranging from 4000 to 400 cm^{-1} for the untreated, pre-treated and treated samples.

Phenol sulfuric analysis

100 mg of the untreated KC and KB were put into separate test tubes. Each sample was hydrolysed with 5 mL 2.5 N hydrochloric acid for 3 hours at 100 °C. The solution was cooled to room temperature and neutralised with 10% (w/v) sodium carbonate ($NaCO_3$) solution. Deionised water was added to a final volume of 100 mL. The solution was centrifuged and 0.1 mL of supernatant was placed into the test tube. Deionised water was added until the final volume reached 1 mL. 1 mL of 5% (w/v) of phenol solution was added, followed by 5 mL of 96% (v/v) sulfuric acid. The test tubes were placed in a water bath at 30 °C for 20 minutes. The colour was read at 490 nm on a UV-Vis Spectrophotometer (model Secomam Prim Advanced Visible Spectrophotometer) and 1 mL of deionised water

was set as the blank. A standard calibration curve was prepared using a glucose solution at concentration of 0.00 – 0.02 mg/mL. The absorbance reading was recorded. These steps were repeated for the HWP and NaOH samples.

Dinitrosalicylic acid assay: Preparation of dinitrosalicylic acid reagent

This reagent was prepared by dissolving 10 g of 3, 5-dinitrosalicylic acid and 10 g NaOH in deionised water. After a homogenous mixture was produced, 2 g of crystal phenol and 1 g $NaHSO_3$ were added in order to avoid the dinitrosalicylic acid from getting oxidised. Then, 133 g of $KNaC_4H_4O_6{\cdot}4H_2O$ was added, followed by deionised water until the total DNS reagent reached up to 1 L. The solution was mixed well until the entire reagent dissolved, and it was then stored in a dark place [27].

Determination of reducing sugar

300 µL of filtrate from the HWP pre-treated samples and 900 µL DNS solution were mixed using a vortex mixer in an Eppendorf tube. A blank solution was prepared by mixing 300 µL distilled water with 900 µL DNS reagent. These solutions were heated using a heating block at 95 °C for 5 min. After the solutions were cooled, a dilution process was carried out by adding 50 µL of each solution to 950 µL distilled water. These samples were analysed using a UV-vis spectrophotometer at 540 nm. The concentration of each sample was calculated using a standard D-glucose calibration curve at 0.05-1.00 mg/mL [28]. The absorbance was measured on a UV-Vis Spectrophotometer (model Secomam Prim Advanced Visible Spectrophotometer) at a fixed wavelength of 540 nm. These steps were repeated for filtrates obtained from the NaOH treatment of both KC and KB.

Results and Discussion

Compositional analysis

Table 2 shows the changes in the chemical compositions of KC and KB before and after treatment. The percentage of α-cellulose and hemicellulose increased upon pre-treatment with hot water. The hot water pre-treatment also showed a decrement in the percentage of lignin. According to Farm et al. [29], hot water pre-treatment helps in dissociating lignin and this then increases the cellulose content. The HWP caused the structure of KC and KB to open up or swell (Figure 1).

Table 2. Chemical composition of KC and KB

Constituent	Kenaf Core (KC), (%)			Kenaf Bast (KB), (%)		
	Untreated	HWP	NaOH	Untreated	HWP	NaOH
α-cellulose	30.2	30.5	22.9	56.5	57.8	66.8
Hemicellulose	31.9	42.3	17.2	28.0	28.3	11.8
Klason Lignin	22.0	20.5	14.0	10.9	10.7	9.40
Moisture content	5.80	4.80	6.30	8.00	2.20	7.20
Hot water solubility	11.8	13.0	33.5	14.8	10.5	11.5
Alcohol-benzene extractives	7.90	11.6	13.7	9.50	10.5	12.7

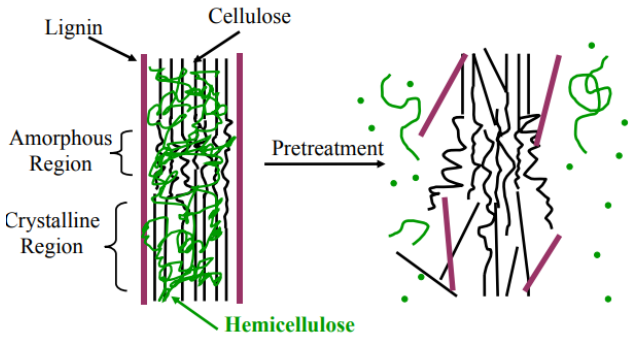

Figure 1. Pre-treatment effect on minimizing degradation of the fiber [30]

The composition of lignocellulosic materials decreased after the NaOH treatment. Hydrolysis via alkaline treatment resulted in the hydrogen bonding of the structure being disturbed, which leads to surface roughness [32, 33]. In this treatment, the extractive materials that covered the outer surface of the fibre such as wax and oils were removed. NaOH treatment causes the degradation in the ester linkage of lignin with carbohydrate chains (glycosidic linkages). During this treatment, there will be an alteration of lignin structure along with a partial decrystallisation of cellulose and hemicellulose [10, 33]. According to Trivedi et al. [34], alkaline treatment is also known as a delignification process as it can also solubilise some amount of hemicellulose. A saponification reaction occurred, leading to the disturbance of intermolecular ester bonds that cross-link xylan in hemicellulose.

The percentage of moisture content had decreased after HWP and increased after NaOH treatment. Cellulose is hydrophilic in nature, whereas lignin is hydrophobic [34]. According to Pettersen [35], wood materials absorb water, causing its cell walls to swell and become saturated with water. Kenaf fibre swells when exposed to moisture. Once the fibre swells, micro-cracking occurs and at later stage, wrecks the fibre. Thus, the hydrophilicity of kenaf fibre further contributes to major water penetration into the micro cracks. Theoretically, fibre with higher lignin content is expected to exhibit the lowest values of water absorption. This is due to the fact that lignin is hydrophobic, thus can provide resistance against the hydro-degradation (or hydrolysis) of kenaf fibre. The delignification process during alkaline treatment makes the water molecules to be easily absorbed by the fibre. As the NaOH solution penetrates into the kenaf fibre, the degradation of cellulose becomes active. The proposed mechanism of cellulose in alkaline media as shown in Figure 2 is also supported by Nosbi et al. [36].

Hot water solubility estimates the external components such as inorganic compounds, gum, sugar and coloring wood present [37]. In this analysis, the percentage of hot water solubility in NaOH samples was much higher compared to the HWP samples. When kenaf fibre was treated with alkaline solution, lignin was removed and water molecules were absorbed easily through the cellulose fibre [38].

Alcohol-benzene solubility indicated the presence of components such as wax, fats, resin, non-volatile hydrocarbons, carbohydrates with low molecular weight, salts and other water soluble materials [18, 37]. From the analysis, the percentage solubility in alcohol-benzene increased when treated with hot water and NaOH solution. This might be due to the increasing solvent absorption of the sample upon delignification of the fibres.

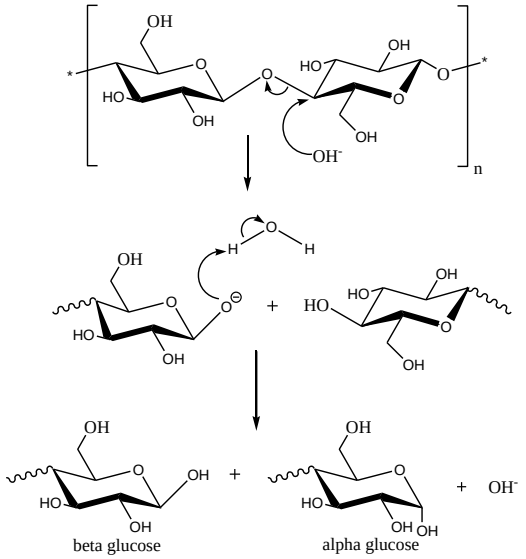

Figure 2. Hydrolysis of cellulose linkage in alkaline medium

Identification of functional group

Figures 3 and 4 shows the FTIR spectra for untreated and treated KC and KB, respectively. Peaks at 3400 and 2927 cm^{-1} represent the hydroxyl (OH) group and C-H stretching, respectively. These peaks belong to the cellulose, hemicellulose and lignin [39-41]. The peak at 1731 cm^{-1} indicates the presence of acetyl group (C=O) in the hemicellulose and lignin, while the peak at 1242 cm^{-1} is the stretching of the aryl group (C-O) in lignin [31, 42, 43]. The peak at 1650-1590 cm^{-1} is associated to the vibration of the C=C in the aromatic ring. The guaiacyl unit and syringyl units in lignin are present at 1245 cm^{-1} and 1375 cm^{-1} respectively. The guaiacyl unit comprises of two methoxy groups, whereas syringyl unit has one methoxy group [44, 45]. Upon alkaline hydrolysis, the peak of acetyl group (C=O) and peak of aryl group (C-O) disappeared. This indicates that the alkaline treatment has a greater tendency in removing lignin [33]. The absorption band around 897 to 898 cm^{-1} refers to the C-O-C stretching of β-glycosidic linkages [46, 47].

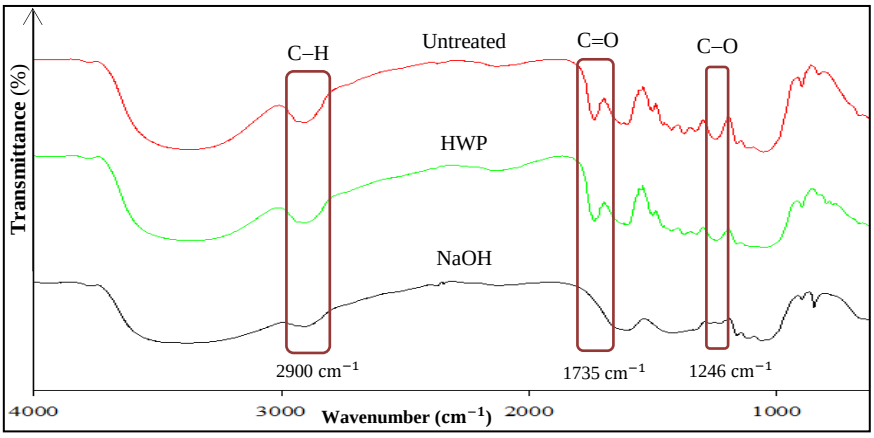

Figure 3. FTIR spectra of untreated and treated kenaf core (KC) fibre

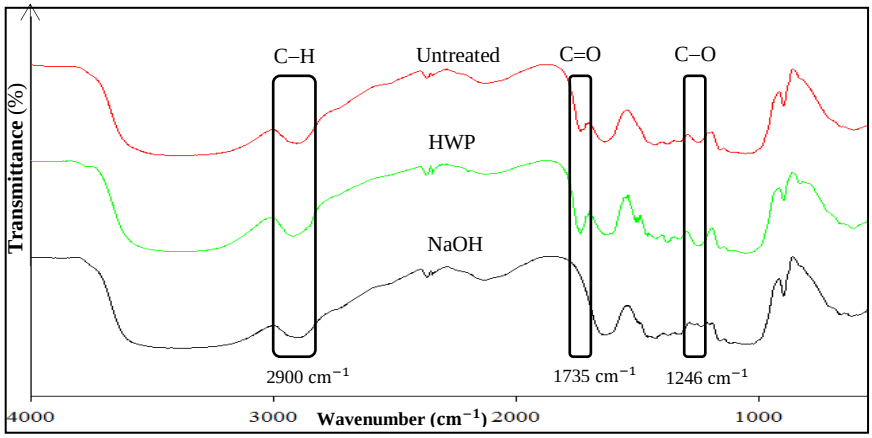

Figure 4. FTIR spectra of untreated and treated kenaf bast (KB) fibre

Examination of glucose content in kenaf fibres via phenol sulfuric analysis

The glucose concentration was determined based on the calibration curve. The increment of glucose concentration of KC after HWP are due to the dissociating of lignin and the increment of cellulose content by the treatment [29]. The HWP caused the structure of KC to swell and this aid the scissoring of the α-cellulose into glucose. Through this analysis (Table 3), the glucose recovery for KC and KB showed decrements after NaOH treatment. The reduction in glucose content is probably due to the possible formation of furfural during the acid hydrolysis. When

acid hydrolysis is running at a high temperature and high concentration, it will further degrade the glucose to furfural [48].

Table 3. Glucose concentration of untreated, HWP and NaOH treated KC and KB obtained from phenol sulfuric analysis

Fibre Sample	Glucose Concentration (mg/g)	
	KC	KB
Untreated	0.13	0.36
HWP	0.17	0.29
NaOH treated	0.09	0.18

Glucose content of kenaf treated filtrate *via* dinitrosalicylic acid assay

Table 4 shows that the reduced sugar resulting from hot water treatment is similar to the one obtained from the NaOH treatment. The result showed that the cellulose in fibres are readily hydrolysed in alkaline medium compared to HWP. During the NaOH treatment, the phenolic benzyl ether bonds in lignin were easily hydrolysed in the alkaline solution [49]. This would inhibit the reading of glucose in the filtrate from the NaOH treatment.

Table 4. Glucose concentration of untreated, HWP and NaOH treated KC and KB obtained from DNS assay analysis

Sample	Glucose Concentration (mg/mL)	
	KC	KB
HWP filtrate	0.050	0.062
NaOH filtrate	0.050	0.064

Conclusion

The percentage of α-cellulose and hemicellulose increased upon hot water pre-treatment and decreased after NaOH aqueous solution treatment for KC. Meanwhile, the α-cellulose content in KB increased from untreated to HWP to NaOH, and hemicellulose increased upon hot water pre-treatment and decreased after NaOH treatment. The percentage of Klason lignin reduced when the kenaf fibres undergone HWP and NaOH treatments. The Fourier Transform Infrared Spectroscopy analysis (FTIR) revealed the removal of lignin from KC and KB after NaOH treatment. These were confirmed when the peaks at 1735 cm^{-1} and 1246 cm^{-1} disappeared after the treatments. Phenol sulfuric analysis showed a decrement in glucose concentration on fibres after NaOH treatment. The DNS analysis showed no significant changes on the filtrate after NaOH treatment due to hindrance from the furfural and lignin in the liquid sample. The experimental investigation showed that hot water pre-treatment (HWP) enhances sugar recovery compared to alkaline treatment.

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