

## Assessment of Natural Cellulosic Powder from Pepper Pericarp Waste (*Piper nigrum* L.) after Alkalization and Bleaching Treatment: Effect of Alkali Concentration and Treatment Cycle

(Penilaian Serbuk Selulosa Semula Jadi daripada Sisa Perikarpa Lada (*Piper nigrum* L.) selepas Rawatan Alkali dan Pelunturan: Kesan Kepekatan Alkali dan Kitaran Rawatan)

AIN NADIAH SOFIAH AHMAD KHORAIRI<sup>1</sup>, NOOR-SOFFALINA SOFIAN-SENG<sup>1,2,\*</sup>, RIZAFIZAH OTHAMAN<sup>3</sup>, NOORUL SYUHADA MOHD RAZALI<sup>1,2</sup> & KHAIRUL FARIHAN KASIM<sup>4</sup>

<sup>1</sup>Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia

<sup>2</sup>Innovation Centre for Confectionery Technology (MANIS), Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia

<sup>3</sup>Department of Chemical Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia

<sup>4</sup>School of Bioprocess Engineering, Universiti Malaysia Perlis, 01000 Kangar, Perlis, Malaysia

Received: 11 March 2021/Accepted: 7 September 2021

### ABSTRACT

Pepper (*Piper nigrum* L.) pericarp is an agriculture waste in the production of white pepper. It is underutilised agro-industrial waste which could be a promising natural source of cellulose. Hence, finding an optimum way to remove the non cellulose components without degrading the cellulose structure is essential. In this work, the effects of alkaline concentration (4, 5, and 6% NaOH) and number of soaking cycle (3 & 4 cycles) on the characteristics of cellulose from pepper pericarp were investigated. The obtained cellulose powder was characterized for its yield,  $\alpha$ -cellulose content, particle size, zeta potential, morphology, whiteness index, crystallinity degree and thermal stability. The white powder cellulose after 4<sup>th</sup> cycle treatment with 4% NaOH appeared to have the highest yield (23.63%),  $\alpha$ -cellulose (65.97%), crystallinity structure (51%) and better thermal stability at 334 °C. FTIR spectrum at band around 1732 cm<sup>-1</sup> indicates a partial removal of non-cellulosic material at all alkalization condition due to the presence of remaining lignin and hemicellulose. These may contribute to formation of negative surface charge on all cellulose samples which may potentially enhance the functionality of the material as emulsifier. Based on two-way ANOVA test, concentration and cycle of alkaline treatment significantly ( $p < 0.05$ ) influenced the yield, particle size and zeta potential, meanwhile  $\alpha$ -cellulose significantly influence by NaOH concentration only ( $p < 0.05$ ). The findings showed that manipulating the synthesis condition of cellulose powder influenced its properties which could be further used in various applications.

Keywords: Cellulose powder; concentration alkaline solution; morphology; white pepper pericarp

### ABSTRAK

Perikarpa lada (*Piper nigrum* L.) adalah sisa pertanian dalam pengeluaran lada putih. Bahan buangan agroindustri yang kurang digunakan ini mungkin boleh menjadi sumber semula jadi selulosa. Oleh itu, mencari kaedah yang optimum untuk membuang komponen bukan selulosa tanpa merosakkan strukturnya adalah sangat penting. Dalam kajian ini, kesan kepekatan alkali (4, 5 dan 6% NaOH) dan bilangan kitaran rendaman (3 & 4 kitaran) terhadap ciri serbuk selulosa daripada perikarpa lada dikaji. Serbuk selulosa dicirikan berdasarkan hasil, kandungan  $\alpha$ -selulosa, saiz zarah, potensi zeta, morfologi, indeks keputihan, tahap kehabluran dan kestabilan terma. Serbuk putih selulosa dengan 4% NaOH pada 4 kitaran rendaman alkali mempunyai hasil tertinggi (23.63%),  $\alpha$ -selulosa yang tinggi (65.97%), struktur kehabluran (51%) dan kestabilan terma yang lebih baik pada 334 °C. Spektrum FTIR pada sekitar jalur 1732 cm<sup>-1</sup> menunjukkan penyingkiran separa bahan bukan selulosa pada semua keadaan rawatan alkali. Ini mungkin menyumbang kepada cas permukaan negatif pada sampel selulosa yang berpotensi dapat meningkatkan fungsi bahan sebagai pengemulsi. Berdasarkan ujian ANOVA dua hala, kepekatan dan kitaran rawatan alkali secara signifikan

( $p < 0.05$ ) mempengaruhi hasil, saiz zarah dan potensi zeta, sementara  $\alpha$ -selulosa hanya dipengaruhi secara signifikan oleh kepekatan NaOH sahaja ( $p < 0.05$ ). Secara keseluruhan, penemuan kajian ini membuktikan bahawa manipulasi keadaan sintesis selulosa mampu untuk mempengaruhi sifatnya sehingga dapat digunakan dalam pelbagai aplikasi.

Kata kunci: Kepekatan cecair alkali; morfologi; perikarpa lada putih; serbuk selulosa

## INTRODUCTION

Pepper plant (*Piper nigrum* L.) produce both black and white pepper and are among the most widely used spices in the world. The global production of pepper product increased consistently throughout the years with annual growth rate of 9.2% (International Pepper Community 2018). International Pepper Community (IPC) also reported a total global production of 532,759 tons in 2018 and 80% of these spices are produced by Asian countries which includes Vietnam, Indonesia, India, China, and Malaysia. The huge world demand of white pepper has increased over the last decade and it is predicted to grow in pace with the world's population of 9.8 billion by 2050 (Aziz et al. 2019; Chien & Mansel 2017; Entebang et al. 2020). White peppers are produced via water retting process which removed the pericarp of mature and ripe berries leaving only the inner seed (Devi et al. 2018). It has milder flavour and aroma than black pepper, which makes it preferable in some countries (Rosnah & Chan 2014).

Due to this, lots of studies has been conducted to increase the productivity of existing technique and to improve the quality of white pepper produced (Aziz et al. 2018; Rosnah & Chan 2014; Sreekala et al. 2019). But there is a lack of research on controlling and managing waste generated from white pepper industry which has been an unresolved issue among the farmers. On average, about 25-28% of white pepper is produced from mature pepper berries and this low recovery rate is because of the drying process and the removal of pepper pericarp (Devi et al. 2018). According to Aziz et al. (2019), the cumulative loss was around 8-9 kg in every 100 kg of mature pepper fruits in white pepper production. With no viable options, the generated waste is commonly disposed straight into the environment.

The decorticated pericarp is mainly composed of three segments which include exocarp, mesocarp, and endocarp (Rosnah & Chan 2014). The plant cell wall is made up of network of cellulose covered with non-cellulosic materials like hemicellulose, lignin, protein, and pectic polymers (Held et al. 2015). Raman and Gaikar (2002) also stated that the pericarp is rich with

bioactive compound such as piperine, phenol, flavonoids and cellulose crystallite structure which could be a promising primary source production of biomaterial like cellulose powder. Over the last few decades, studies on agro-industrial waste as alternative to fossil oil-based products have drawn further interests from researchers worldwide. It is widespread in nature and has been used as a medium for the manufacture of cellulose or cellulose nanomaterial derivatives.

Cellulose powders can be extracted either by mechanical or chemical process. Combination of alkaline and chlorite bleaching treatment is the most used pre-treatment method (Ching & Ng 2014). Alkaline treatment helps in eliminating hemicellulose, lignin, and other substances such as pectin and wax, meanwhile bleaching process aids in removal of any residual lignin (Gomes et al. 2007). The alkaline method is favoured due to its simplicity, high efficiency, and recovery (Tran et al. 2020). This treatment is less corrosive than the acidic method, as it is usually performed under milder condition with a bases solution such as sodium hydroxide (NaOH) (Kim et al. 2016). NaOH effectively split the hydrogen bond in the natural fibre and dissolve non-cellulosic component (Santos et al. 2018).

Although the manipulation of alkalization process in lignocellulosic material has been investigated extensively for the past few decades (Gomes et al. 2007; Tran et al. 2020), the quest is still continue owing to the lack of practical method that can be used for a range of biomass. Alkaline treatment is strongly dependent on the types and concentration of the bases, temperature, and the length of the process. It can modify structure and composite properties by enhancing thermal stability, yield, mechanical strength, crystalline structure and in some cases, reduced the absorption of moisture of cellulose fibres (Kathirselvam et al. 2019). Sosiati et al. (2015) also mention that the amount of cellulose microfibrils exposed to the fibre surface is highly affected by the parameter of alkaline treatment used. Mild alkaline condition lowers the dissolution of non-cellulosic materials but, too strong concentration and temperature for a long period of time damage the fibre

layer. Kunusa et al. (2018) reported 6% NaOH at 100 °C for 2 h, produce cellulose from corncob with crystallinity index of 98%, meanwhile 4% NaOH extract the highest yield of corncob cellulose. In other research, Yew et al. (2019) discovered 5% NaOH concentration at longer period of time (24-48 h) increase the surface roughness of cellulose fibre but partly removed the lignin component.

To date, very few data available on the effect of concentration of NaOH together with number of cycle process on physical characteristics of pepper pericarp waste, as compared to many other research on agro-industrial waste like banana peel (Fatmawati et al. 2017; Singanusong et al. 2014) and coconut husk (Abdullah et al. 2021; Din et al. 2021). The discovery of an optimum parameter in alkaline treatment is therefore significant to prove the potential of underutilized pepper pericarp waste as a source of natural cellulose. Hence, the aim of this work was to investigate the influence of alkalization conditions on the characteristics of cellulose fibres from pepper (*Piper nigrum* L.) pericarp waste. The cellulose was extracted in different concentration of sodium hydroxide (NaOH) solution at different number of alkaline treatment cycle, which was then followed by bleaching process. The chemical-physical properties of cellulose powder were analysed by microscopic, spectroscopic, thermal and X-ray diffraction methods.

#### MATERIALS AND METHODS

The fresh pepper berries (*Piper nigrum* L.) were purchased from Herba Bagus farm in Kluang, Johor, which located at the south part of Malaysia. Sodium hydroxide (NaOH) with 99% purity was supplied from QREC Sdn Bhd. Sodium chlorite (NaClO<sub>2</sub>) and acetic acid glacial (CH<sub>3</sub>COOH) were purchased from Sigma-Aldrich. All chemical reagents used were analytical grade.

#### PREPARATION OF PEPPER PERICARP

The pepper pericarp was obtained according to the pepper retting protocol described by Aziz et al. (2018). The pepper berries were immersed in distilled water for 8 days at 28 °C. The temperature was maintained by placing the mixture in a temperature-controlled water bath shaker (Amerex GYROMAX Sk-929, United States). Once the pepper pericarp was softened, it was removed by manual rubbing and dried overnight in a convection oven (UM400, Memmert Universal Ovens, Germany) at 40 °C. The dried pericarp sample was then ground and

sieved until a fine powder was obtained. The yield of pepper pericarp was calculated as shown in (1):

$$\text{Yield pepper pericarp (\%)} = \frac{\text{Dry mass of pepper pericarp (g)}}{\text{Dry mass of fresh pepper berries (g)}} \times 100 \quad (1)$$

#### EXTRACTION OF CELLULOSE

The alkaline and bleaching treatment were carried out according to Zahari et al. (2018) with some modification. The pepper pericarp powder was dissolved in alkaline solution (4, 5 & 6 wt.% NaOH) at a ratio of 1:20 (w/w). The alkaline treatment was conducted under reflux condition at 80-90 °C for 3 h with continuous stirring using magnetic stirrer. Then, the powder was filtered and rinsed with distilled water to discard any alkali-soluble substances. This procedure is considered as one alkaline cycle. To evaluate the alkaline cycles treatment effect on pepper pericarp powder, alkaline treatment was repeated for 3 and 4 times. Based on our preliminary findings, 1 and 2 cycle of alkaline treatment was too weak to remove the non-cellulosic materials in pepper pericarp fibre hence it was eliminated from this study (data not shown). After that, all alkaline treated samples were subjected to bleaching treatment. The samples (5 g) were mixed with 100 mL of equal volume of 1.7% w/v sodium chlorite (NaClO<sub>2</sub>), acetic buffer (2.7g NaOH and 7.5 mL glacial acetic acid in 100 mL distilled water) and distilled water in a reflux environment for 4 h at 80 °C. The mixture was filtered and washed with distilled water until neutral (pH 7). The bleaching treatment was repeated 4 times to obtain white cellulose powder. The yield of solid recovery (cellulose) was determined by using (2). The treatment for dried pepper pericarp (DPP) and cellulose pepper pericarp (CPP) is shown in Table 1.

$$\text{Yield cellulose (\%)} = \frac{\text{Dry mass of white cellulose powder obtained (g)}}{\text{Dry mass pepper pericarp (g)}} \times 100 \quad (2)$$

#### CHEMICAL COMPOSITION

The  $\alpha$ -cellulose content obtained from pepper pericarp was determined according to procedure described by German Association of Cellulose Chemists and Engineers (1951). The extracted cellulose was treated with 17.5% of NaOH and acetic acid. The solid was washed and dried at 105 °C, and the  $\alpha$ -cellulose content was calculated in (3).

$$\alpha\text{-cellulose (\%)} = \frac{\text{Dry mass of } \alpha\text{-cellulose obtained (g)}}{\text{Initial weight of dry sample (g)}} \times 100 \quad (3)$$

TABLE 1. Summary of treatments applied in this study

Samples	Treatment
DPP	Untreated fibres (Dried pepper pericarp)
CPP4%.3C	Cellulose powder with 4% NaOH + 3 cycle of alkali treatment
CPP4%.4C	Cellulose powder with 4% NaOH + 4 cycle of alkali treatment
CPP5%.3C	Cellulose powder with 5% NaOH + 3 cycle of alkali treatment
CPP5%.4C	Cellulose powder with 5% NaOH + 4 cycle of alkali treatment
CPP6%.3C	Cellulose powder with 6% NaOH + 3 cycle of alkali treatment
CPP6%.4C	Cellulose powder with 6% NaOH + 4 cycle of alkali treatment

#### PARTICLE SIZE AND ZETA POTENTIAL MEASUREMENT

The  $\zeta$ -potential and particle size measurements were performed on DPP and CPP using Zetasizer (Malvern Instruments, Nano ZS). Dynamic light scattering (DLS) method with refractive index ratio at 1.47 was used to measure the particle size of all samples.

#### MORPHOLOGICAL CHARACTERIZATION

The transverse-section of a fresh pepper berries was observed using a scanning electron microscope (SEM) (Hitachi Tabletop Microscope; model TM 1000, Japan). The dry pepper berry was cut in half and examined with a 25 $\times$  magnification. Meanwhile, the surface morphology of pepper pericarp powder, and all cellulose samples were analysed using Field Emission Scanning Electron Microscopy (FESEM) (Zeiss Supra 55VP, USA) at 3000 $\times$  magnification and voltage of 10 kV.

#### COLOUR DETERMINATION

The colour changes of treated DPP and CPP were analysed using Minolta colorimeter (Chroma meter CR 400, Japan) with a Hunter Lab colour system ( $L^*$ ,  $a^*$ , and  $b^*$ ). The whiteness value was measured based on Color iMatch Color Calculations Guide (2012) formula,  $WI_{\text{hunter}} = L - 3b$ .

#### FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY ANALYSIS

The functional groups that could have been caused by the treatments on DPP and CPP was carried out using Perkin-Elmer Attenuated Total Reflection (ATR-FTIR)

spectrometer. The infrared spectrum of transmittance (%) against wavelength ( $\text{cm}^{-1}$ ) was recorded within the wavelength range from 400 to 4000  $\text{cm}^{-1}$ .

#### X-RAY POWDER DIFFRACTION (XRD) ANALYSIS

The crystallinity structure of DPP and CPP were analysed with x-ray diffractometer (D8-Advance Bruker AXS GmbH) using monochromatic Cu-K $\alpha$  radiation source ( $\lambda = 0.1539$  nm). Results were obtained in  $2\theta$  scale ranging from 10 to 50 $^\circ$ . The crystallinity index (CrI) was calculated according to Segal method (Segal et al. 1959): crystallinity index ( $\text{CrI}\%$ ) =  $[(I_{002} - I_{\text{am}}) / I_{002}] \times 100$ , where  $I_{002}$  is the intensity value of crystalline cellulose ( $I_{002}$ ,  $2\theta = 22.6^\circ$ ) and  $I_{\text{am}}$  is the intensity value that represents amorphous part of the sample ( $I_{\text{am}}$ ,  $2\theta = 18^\circ$ ).

#### THERMOGRAVIMETRIC (TGA) ANALYSIS

The thermal degradation properties of DPP and CPP were measured with Mettler Toledo thermogravimetric analyser (TGA/SDTA 85-F). The samples were subjected to heat from 25  $^\circ\text{C}$  to 600  $^\circ\text{C}$  with constant nitrogen flow rate of 10  $^\circ\text{C min}^{-1}$  (Mohd et al. 2016).

#### STATISTICAL ANALYSIS

The data for colour, yield, cellulose content, zeta potential, and size measurements were performed in triplicate. The results were expressed in mean  $\pm$  standard deviation. The data were analysed using one and two-way analysis of variance test (ANOVA) and Duncan's multiple range test by IBM SPSS Statistic Software version 20. Treatment is reported as significantly different when  $p < 0.05$ .

## RESULTS AND DISCUSSION

## YIELD OF PEPPER PERICARP WASTE AND CELLULOSE FROM PEPPER PERICARP

The yield of waste obtained from white pepper production and cellulose extracted were recorded in Table 2. Water retting process of pepper berries generated  $4.85 \pm 0.04\%$  pepper pericarp waste. These wastes were then further treated with alkaline followed by bleaching treatment to extract cellulose powder. Based on Table 2, 100 g of pepper pericarp produced roughly around 16-24 g of cellulose powder. At 4% NaOH, highest yield of cellulose pepper pericarp able to be produced, with average of 23.63-24.17% and there is no significant difference with number of alkali cycle (3 & 4 times) used ( $p < 0.05$ ). Then, when the concentration of NaOH (5 & 6 NaOH) and number of alkali treatment (3 & 4 times) increase, the percentage of yield decrease significantly ( $p < 0.05$ ).

Based on two-way ANOVA, the yield of cellulose obtained is highly dependent on the concentration of NaOH and number of alkali cycle ( $p < 0.05$ ). Kathirselvam et al. (2019) also observed an increasing pattern yield of cellulose from *Thespesia populnea* barks at 2% NaOH until 5% NaOH concentration and the yield started to decrease at 6 and 8% NaOH. Alkali solution basically eliminate non-cellulosic material like lignin and hemicellulose from biomass by splitting the linkage in lignin and hydrolyses the glycosidic bonds (Chen et al. 2013). According to Sosiati et al. (2015),

the decline of recovery cellulose is not only due to the removal of lignin and hemicellulose but also indicate the destruction of cellulose structure after high concentration or prolonged alkali process.

 $\alpha$ -CELLULOSE COMPOSITION

The  $\alpha$ -cellulose content of untreated and treated pepper pericarp was presents in Table 2. The DPP contained  $47.88 \pm 3.59\%$  of  $\alpha$ -cellulose and the value increased significantly after chemical treatment ( $p < 0.05$ ) to 58.07-65.97%. This difference is possibly due to the properties of hemicellulose which is more susceptible to NaOH reactions and therefore eliminates part of the amorphous material (Kathirselvam et al. 2019). In comparison among all the cellulose obtained, the highest  $\alpha$ -cellulose content measure was in CPP4%.4C powder, followed by CPP6%.3C and CPP5%.3C with values of  $65.97 \pm 1.08$ ,  $62.77 \pm 0.73$ , and  $61.64 \pm 1.33\%$ , respectively.

The two-way ANOVA test showed that the concentration of NaOH has significant effect on  $\alpha$ -cellulose content ( $p < 0.05$ ), meanwhile no significant effect on the number of alkalization cycle towards  $\alpha$ -cellulose content ( $p > 0.05$ ). Kunusa et al. (2018) reported that cellulose from corncob produce the highest content of  $\alpha$ -cellulose at 4% NaOH, in 100 °C for 2 h as compared to 1, 2, 3 and 5% of NaOH concentration. Extreme alkaline treatment may alter the molecular composition of cellulose powder, where NaOH penetrate into the cellulose structure, consequently, degrade the pure crystalline structure of cellulose (Chen 2013).

TABLE 2. The yield,  $\alpha$ -cellulose content, particle size and  $\zeta$ - potential and whiteness index of DPP and CPP prepared at different concentration of NaOH and alkali cycle. (CPP4%.3C, CPP4%.4C, CPP5%.3C, CPP5%.4C, CPP6%.3C & CPP6%.4C)

Samples	Yield (%)	$\alpha$ -cellulose (%)	Particle size, d (nm)	$\zeta$ - potential (mV)	Whiteness index (%)
DPP	$4.85 \pm 0.04$	$47.88 \pm 3.59^d$	$1184 \pm 130.54^a$	$4.52 \pm 1.29^a$	$16.36 \pm 0.74^c$
CPP4%.3C	$24.17 \pm 0.25^a$	$60.85 \pm 1.02^{bc}$	$549.72 \pm 42.49^b$	$-17.60 \pm 2.83^b$	$76.74 \pm 0.06^b$
CPP4%.4C	$23.63 \pm 0.25^a$	$65.97 \pm 1.08^a$	$420.39 \pm 52.50^c$	$-30.67 \pm 5.48^c$	$77.00 \pm 1.10^b$
CPP5%.3C	$20.02 \pm 0.43^b$	$62.77 \pm 0.73^{ab}$	$367.75 \pm 55.73^c$	$-22.97 \pm 4.60^{bc}$	$82.53 \pm 0.61^a$
CPP5%.4C	$19.30 \pm 0.11^c$	$59.86 \pm 2.87^{bc}$	$344.10 \pm 56.88^{cd}$	$-24.37 \pm 7.69^{bc}$	$66.91 \pm 0.52^d$
CPP6%.3C	$18.58 \pm 0.51^d$	$61.64 \pm 1.33^{bc}$	$343.02 \pm 16.18^d$	$-27.94 \pm 2.25^c$	$71.78 \pm 0.19^c$
CPP6%.4C	$16.35 \pm 0.47^c$	$58.07 \pm 3.35^c$	$239.29 \pm 44.01^d$	$-28.23 \pm 9.02^c$	$72.63 \pm 0.15^c$

<sup>a-c</sup>: Means with different alphabet are significantly different ( $p < 0.05$ )

## MORPHOLOGICAL STUDY

The transverse-section of a pepper berry is presented in the SEM micrographs (Figure 1). Pepper berries are spherical fruits with a diameter of around 6 mm. The fruit composed of a single seed coated with a

thin cell wall known as pericarp which has thickness ranging from 1 - 400  $\mu\text{m}$ . These outer pericarps were removed during pepper retting process to produce white peppers. The pericarp was then undergone alkaline and bleaching treatment to produce cellulose powder and

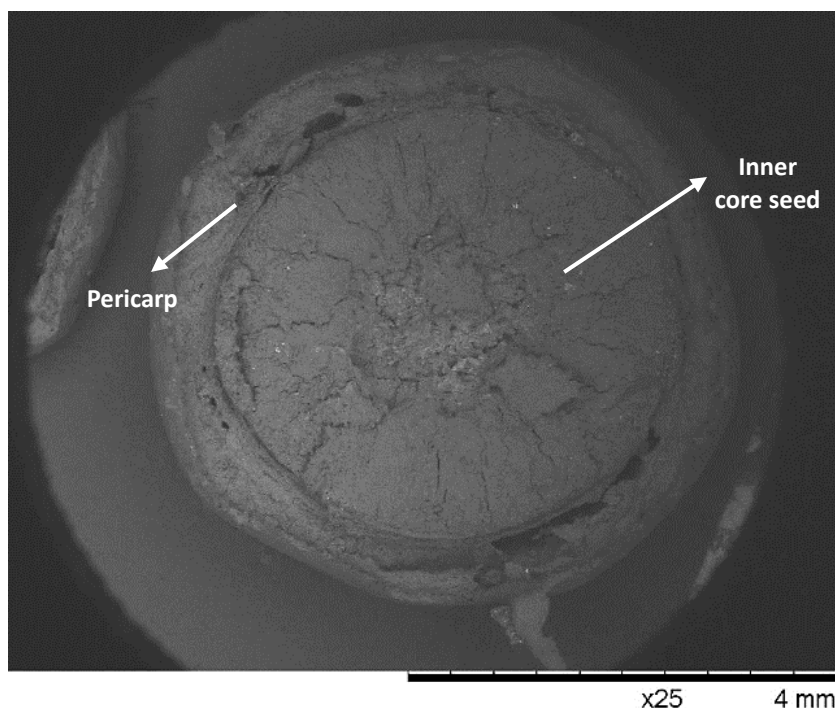


FIGURE 1. Scanning electron micrograph of untreated pepper berry

morphology changes is expected to take place. Figure 2 displays the structure of DPP and CPP obtained from pepper pericarp. The surface area of DPP (Figure 2(a)) was smoother than all treated CPP because of natural wax that covered the fibre (Zahari et al. 2018). After alkaline and bleaching treatment (Figure 2(b)-2(g)), the surfaces of all cellulose samples turn rougher than untreated DPP. The surface structure become creased and peeled-off especially when the number of alkalization cycle was increased (Figure 2(c), 2(e) & 2(g)). These may be due to the removal of outer layers such as hemicellulose, lignin, pectin, and wax which are believed coated the cellulose structure (Din et al. 2020). Moreover, as the concentration of NaOH and number of alkalization cycle increase, the untreated fibres begin to defibrillate into individual fibres (Figure 2(e)).

## PARTICLE SIZE AND ZETA POTENTIAL ANALYSIS

The effects of NaOH concentration and number of alkalization cycle on the particle size and zeta potential

of pepper pericarp cellulose was shown in Table 2. In general, the average particle size decreased with the intense chemical treatment applied. The particle size of untreated pepper pericarp decreases significantly ( $p < 0.05$ ) from 1184 nm into smaller particles ranging from 500 to 240 nm due to defibrillation. The results obtained are consistent with the morphological changes that were discussed earlier. Another notable finding was that the particle size of extracted cellulose was significantly ( $p < 0.05$ ) influenced by the concentration of NaOH. The disruption of intermolecular bond in cellulose molecule led to decreased size of particles (Santos et al. 2018).

The surface charge was examined through zeta potential analysis, and it is a valuable measure to determine the dispersion of cellulose. Larger value of zeta potential implies better dispersion in water, whereas smaller value means poor dispersion stability. According to Table 2, DPP has potential value of  $5.43 \pm 9.00$  mV, meanwhile all cellulose powder from pepper pericarp had negative charge point within the range of  $-17.60$ - $30.67$

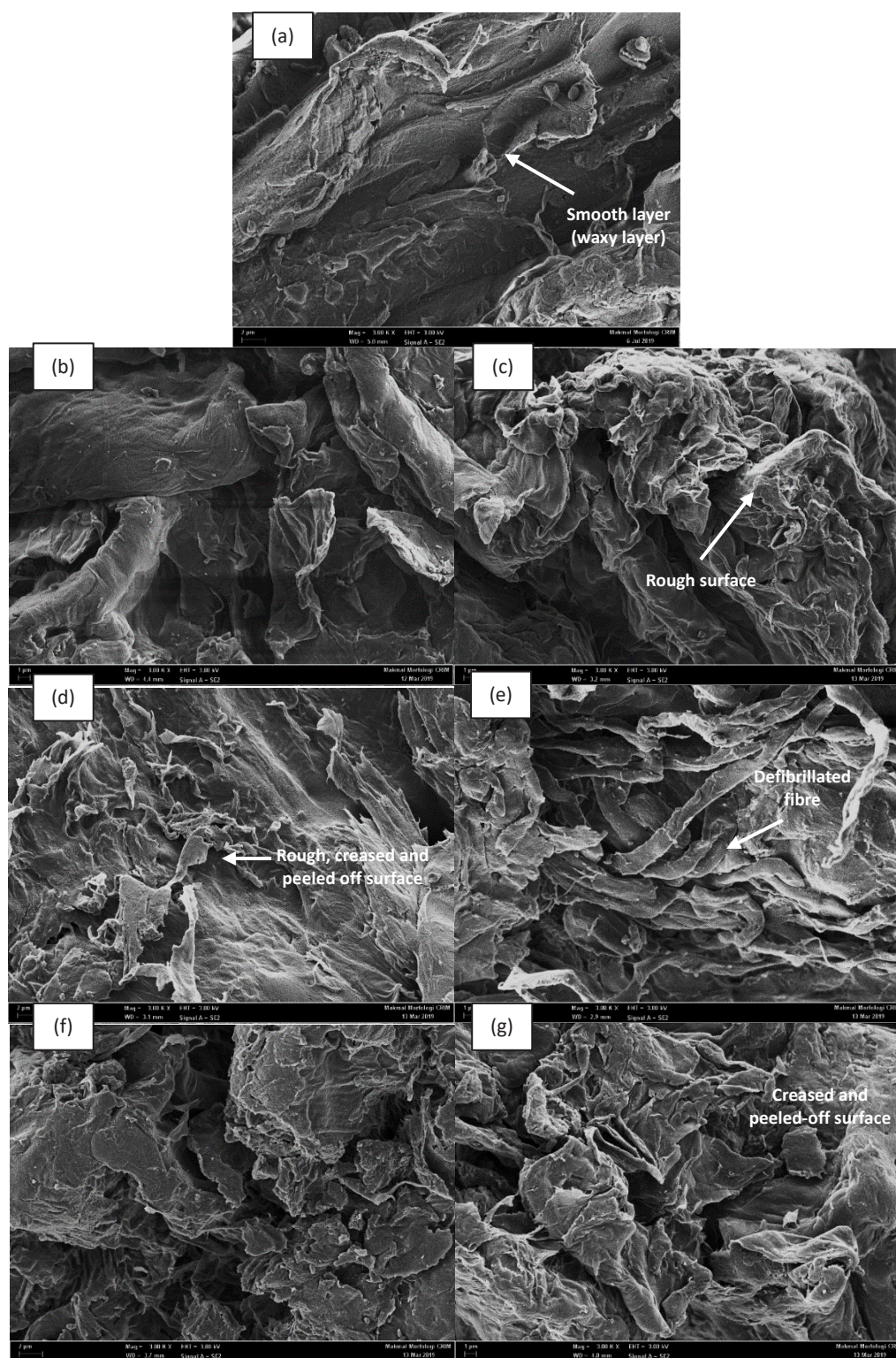


FIGURE 2. FESEM (a) images of DPP and CPP with 4, 5 and 6% NaOH at 3 and 4 of alkali cycle ((b) CPP4%.3C, (c) CPP4%.4C, (d) CPP5%.3C, (e) CPP5%.4C, (f) CPP6%.3C & (g) CPP6%.4C)

mV. A similar pattern was observed for cellulose from sugar beet leaves (Tenorio et al. 2017) and mangosteen rind (Winuprasith & Suphantharika 2013). According to Tenorio et al. (2017), a complete removal of non-cellulosic material should produce charge-free pure cellulose at all pH values. These observed negative charge in this study may be due to the presence of protein and pectin or COO- groups of raw material (Wallecan et al. 2015; Winuprasith & Suphantharika 2013).

Nevertheless, such condition may contribute to potential properties of the material especially as emulsifier (Tenorio et al. 2017). The highest magnitude of  $\zeta$ -potential recorded was  $-30.67 \pm 5.48$  mV for CPP4%.4C sample. The collected cellulose powder displayed a moderate stability as the magnitude of zeta potential is greater than the threshold for coagulation or flocculation in emulsion to occur which is  $-15$  mV (Zhou et al. 2012). Thus, exhibit the ability to produce a stable colloidal system.

#### COLOUR ANALYSIS

Figure 3 displays the physical appearance of fresh green pepper berries, dried pepper pericarp, and cellulose fibres from pepper pericarp. The DPP was dark

brown in colour and after subjected to alkalization and bleaching process, all CPP were completely white-like cotton. The findings were comparable with previous research conducted by Fareez et al. (2018), which observed pineapple waste turns from brown to white colour after alkali and bleaching treatment. The change in colour is due to ionisation of chromogen group which responsible to the original colour of lignocellulosic material together with the removal of lignin and tannins (Tibolla et al. 2018). Table 2 illustrates the whiteness index (WI) of DPP, and cellulose obtained after alkalization using different concentration of NaOH and number of alkali cycle. DPP has the lowest value of WI ( $46.04 \pm 0.30\%$ ), this value increased significantly after chemical treatment ( $p < 0.05$ ). The cellulose at CPP5%.3C has the highest whiteness index followed by CPP4%.4C and CPP4%.3C with value of  $82.53 \pm 0.61$ ,  $77.00 \pm 1.10$  and  $76.74 \pm 0.06\%$ , respectively. The increase whiteness index may indicate effective removal of non-cellulosic material. Even though the whiteness index fluctuates as the concentration of NaOH, and number of alkalization cycles increase, all cellulose samples have high whiteness index as compared to raw pepper pericarp.

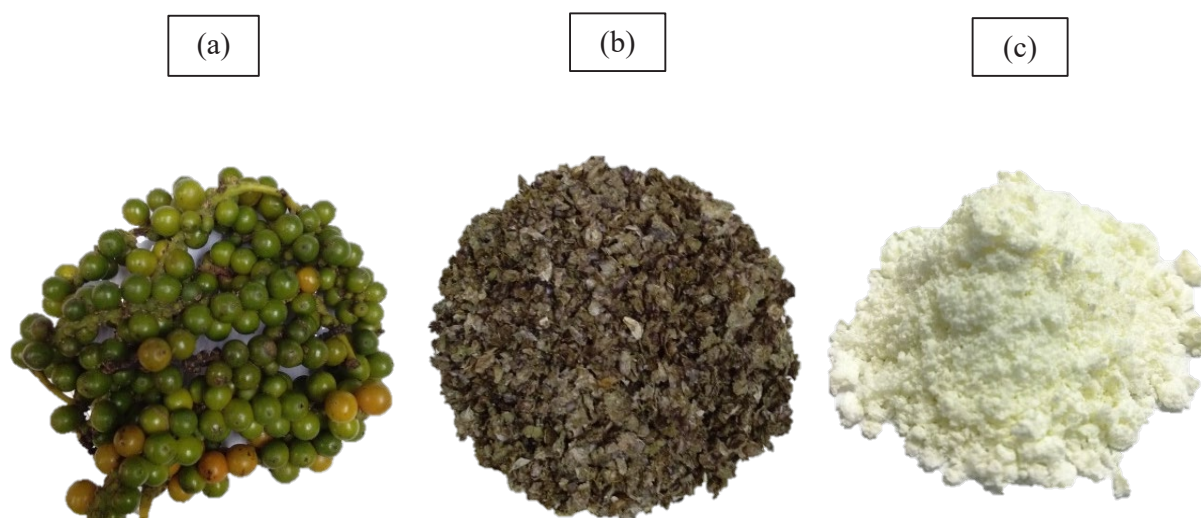


FIGURE 3. Colour changes on pepper pericarp after treatments. (a) fresh green pepper berries, (b) dried pepper pericarp & (c) cellulose powder after alkaline and bleaching treatment

#### FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY ANALYSIS

The comparison FTIR spectra of DPP and CPP after each stages of treatment are shown in Figure 4. The broad

absorption bands at  $3500\text{--}3200$  and  $2900\text{--}2890$   $\text{cm}^{-1}$  in all samples are due to hydroxyl groups (-OH groups) and C-H stretching of cellulose. The peak at  $1000$  and  $800$   $\text{cm}^{-1}$  in treated and untreated pepper pericarp are



attributes to C-H stretching vibration of C-O in cellulose structure (Fiore et al. 2014). These suggest that cellulose is still present in the structure after exposed to alkaline and bleaching treatment.

A noticeable difference was observed in the decreasing intensity of the vibration peak at  $1732\text{ cm}^{-1}$  in all alkali-treated pepper pericarp. This highly visible peak in the spectrum of dried pepper pericarp is associated to C=O stretching of hemicelluloses, or the ester carbonyl groups of lignin (Fiore et al. 2014). The downtrend intensity corresponds to the increase in NaOH concentration and number of alkaline cycles which had partially removed the non-cellulosic material. Similar changes were observed between untreated and alkali-treated pepper pericarp at  $1630\text{ cm}^{-1}$  which

attributed to C=C stretching of the lignin carboxyl groups and  $1245\text{ cm}^{-1}$  which refers to C-O-C stretching of aryl-alkyl ether linkage in lignin (Cai et al. 2015).

Mariño et al. (2018) reported similar findings where cellulose from orange waste still displayed peak in  $1730$  and  $1600\text{ cm}^{-1}$  after alkaline and bleaching treatment. Lignin was found a bit difficult to be completely remove with alkaline solution (Oushabi et al. 2017). Besides, the composition of pepper pericarp itself could possibly influenced the chemical changes of pepper pericarp cellulose. Pepper pericarp was discovered to contain a significant amount of oleoresin and other volatile oils such as piperine (Ojalere et al. 2018). The peak at  $1630\text{ cm}^{-1}$  found in untreated and treated pepper pericarp may be due to the presence of piperine which correspond to the -CO-N group (Rosa et al. 2010).

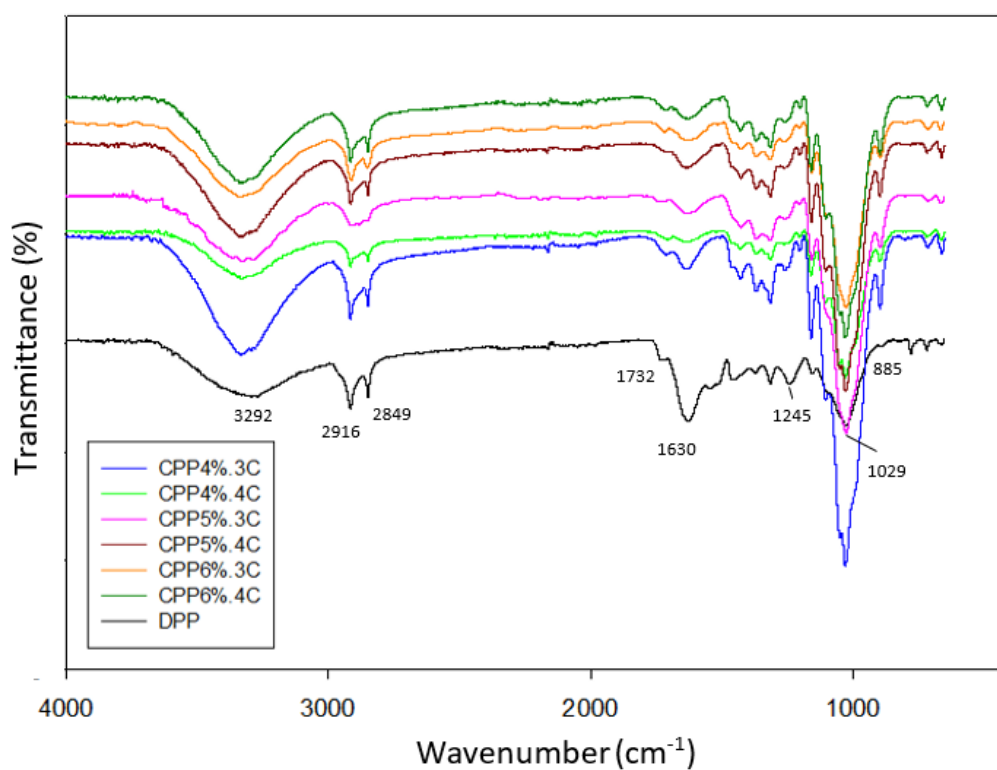


FIGURE 4. FTIR spectra recorded for DPP and CPP with 4, 5 and 6% NaOH at 3 and 4 of alkali cycle (CPP4%.3C, CPP4%.4C, CPP5%.3C, CPP5%.4C, CPP6%.3C & CPP6%.4C)

#### X-RAY DIFFRACTION (XRD) ANALYSIS

Figure 5(a) shows the x-ray diffraction patterns of untreated pepper pericarp and cellulose powder obtained. The cellulose data indicates three main visible crystalline peaks around  $2\theta = 16^\circ$ ,  $22^\circ$ , and  $34.5^\circ$  which

displayed a typical pattern of cellulose I (Rosa et al. 2010). The intensity of these peaks becomes stronger and narrower in all treated samples compare to untreated sample. This notified elimination of impurities and other non-cellulosic material from dried pepper pericarp. NaOH

solution penetrated into the fibre and dissolved the low molecular weight materials such as hemicelluloses, wax, and lignin at the surface of the fibre and revealing the cellulose (Santos et al. 2018).

The crystallinity index was recorded in Figure 5(b). The raw pepper pericarp has the lowest value of CrI (6.47%) as it is highly composed of amorphous region. After subjected to 4% NaOH with 3 cycles of alkaline treatment, the CrI value increase to 50.37% and continue to increase when the number of cycle increase (51.37%). The effective removal of amorphous region may contribute to the increment. It is widely known that cellulose mainly composed of crystalline and amorphous region of hemicellulose and lignin. The alkaline and bleaching treatment removed the

non-cellulosic material, leaving behind the fragment of crystalline structure of cellulose (Oushabi et al. 2017).

However, as the concentration of NaOH (5 & 6% NaOH) and number of alkaline cycles increase (3 & 4 times), the crystallinity index started to decrease. The strong alkali solution might have removed the amorphous region as well as dissolved some parts of crystalline structure. But, the calculated CrI values of cellulose obtained in this work were higher than other agricultural waste such as rice husk (59.0%) (Johar et al. 2012), pomelo albedo (57.5%) (Zain et al. 2014) and were closer to pure microcrystalline cellulose (61.9%) (Widiarto et al. 2017). The high crystallinity structure indicates an increase in strength of fibres, specifically in terms of stiffness and rigidity (Johar et al. 2012).

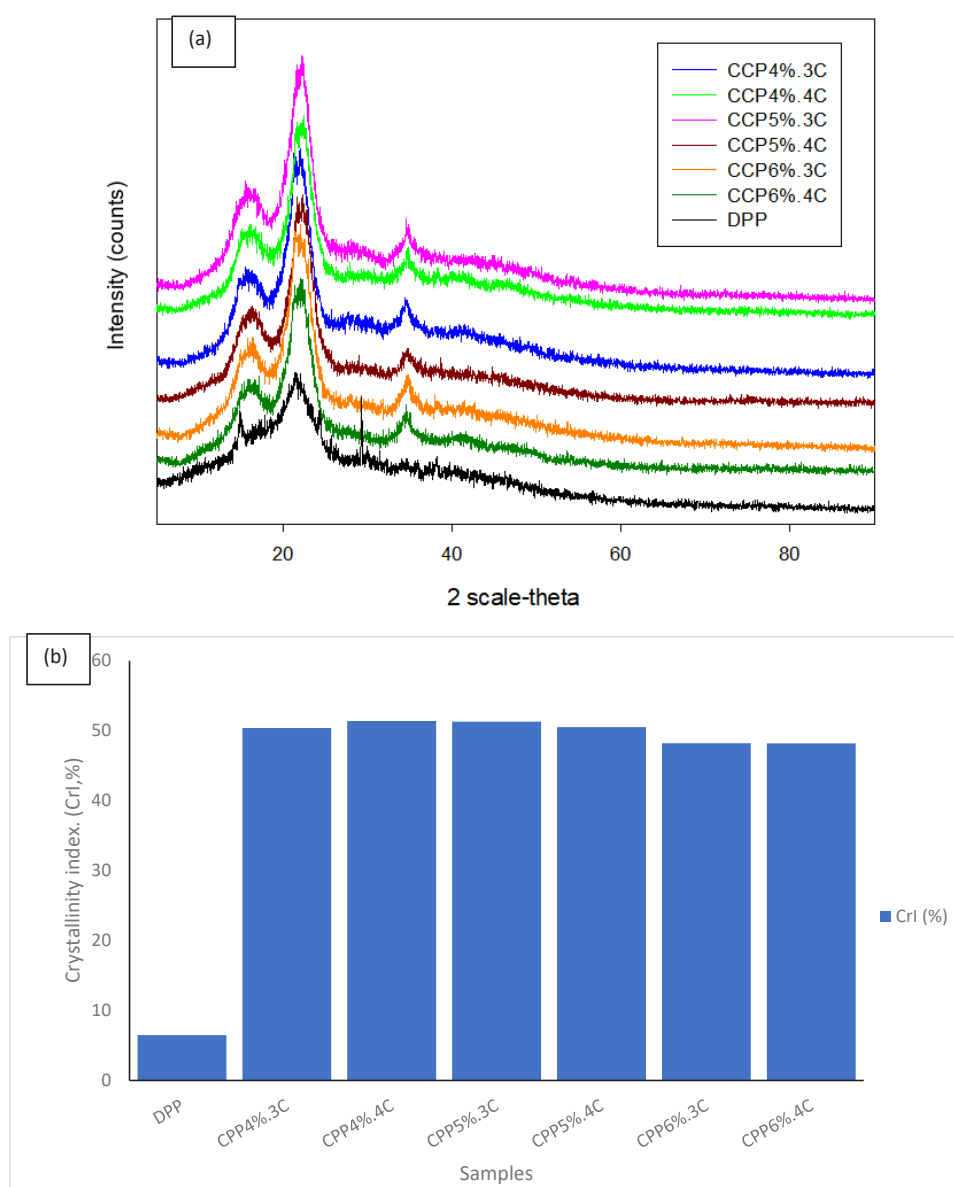


FIGURE 5. (a) X-ray diffraction patterns and (b) crystallinity index (CrI) of DPP and CPP with 4, 5 and 6% NaOH at 3 and 4 of alkali cycle (CPP4%.3C, CPP4%.4C, CPP5%.3C, CPP5%.4C, CPP6%.3C & CPP6%.4C)

## THERMOGRAVIMETRIC (TGA) ANALYSIS

Figure 6(a) shows the degradation of DPP and six cellulose powder (CPP4%.3C, CPP4%.4C, CPP5%.3C, CPP5%.4C, CPP6%.3C & CPP6%.4C) which occur at three major point. The first weight loss at below 100 °C is ascribed to evaporation of water, 250 to 380 °C was due to decomposition of hemicellulose and above 400 °C is because of cellulose and lignin degradation (Radakisnin et al. 2020; Rosa et al. 2010). DPP showed

earlier decomposition starting approximately at 235 °C, compared to cellulose powder which began to degrade at around 355 °C. The elimination of non-cellulose component through alkali and bleaching treatment as proven by FTIR and XRD result in this research, had increased the thermal stability of cellulose powder.

Based on Figure 6(b), cellulose samples of CPP4%.3C and CPP4%.4C have higher degradation temperature (334 °C) than the other cellulose pepper

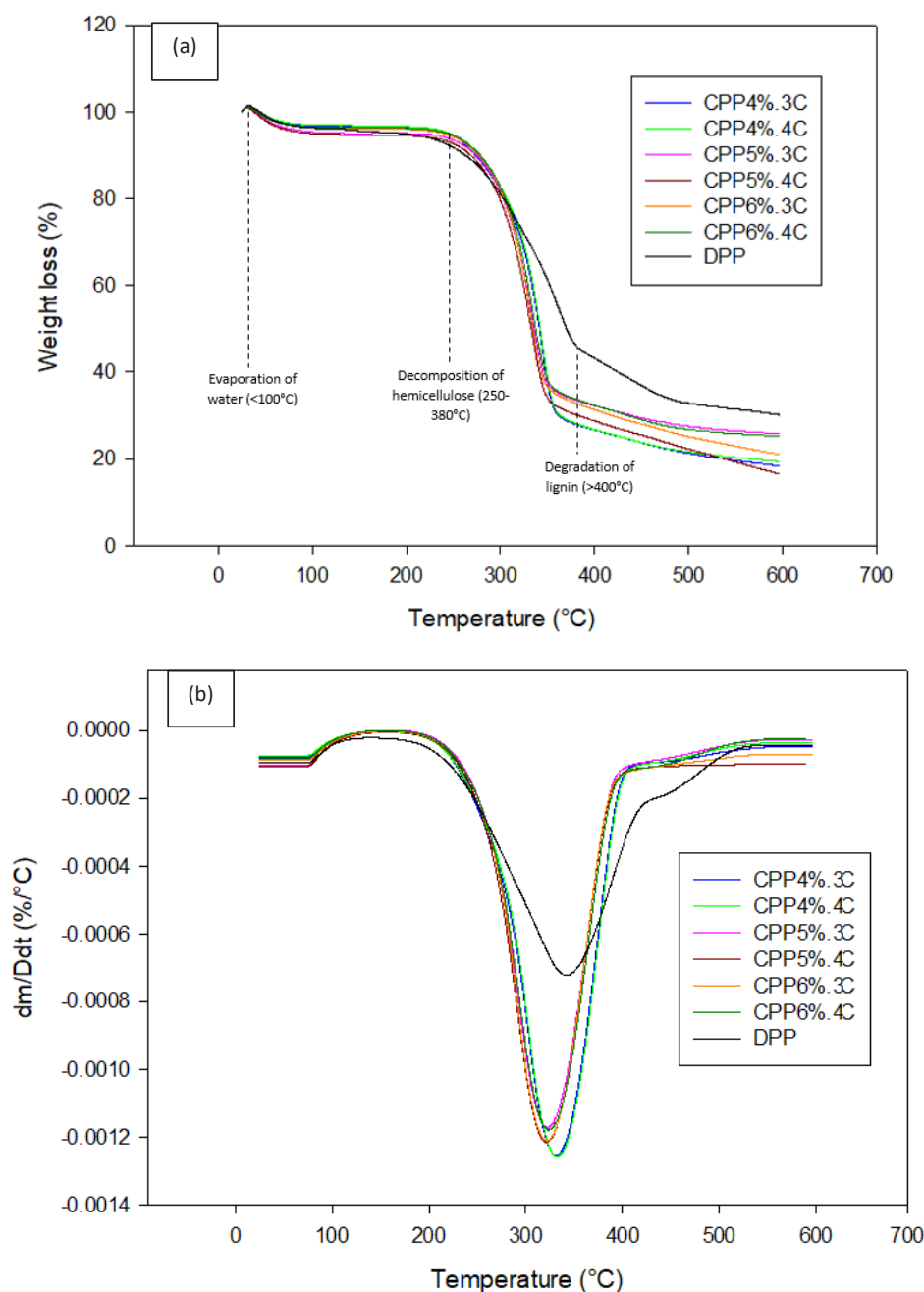


FIGURE 6. (a) Thermogravimetric curve and (b) differential residual mass (DTG) of DPP and CPP with 4, 5 and 6% NaOH at 3 and 4 of alkali cycle (CPP4%.3C, CPP4%.4C, CPP5%.3C, CPP5%.4C, CPP6%.3C & CPP6%.4C)

pericarp samples (322-320 °C). This indicates a considerable increase in thermostability and demonstrates an effective removal of lignin and hemicellulose at 4% concentration of NaOH. The thermal stability decreases at higher concentration but remain unchanged when the alkali treatment duration was lengthened. According to Ouajai and Shanks (2005), the decline in thermal stability after high alkaline solution treatment, suggesting that a poor order of cellulose structure. This happened when too much of NaOH load penetrates the crystalline region and dissolved the fibre. The loses of crystalline structure is believed to induce poor thermal stability properties (Rosa et al. 2010). However, Reddy et al. (2013) observed a slight change of improved thermal stability when longer alkali treatment (1, 4, 7 & 12 h) was used at 5% NaOH. In other studies, Yew et al. (2019) reported a small change of decomposition rate between 24 and 48 h NaOH treated samples. They discovered that the longer alkaline treatment, the more hemicellulose being removed, the better thermal stability.

#### CONCLUSION

Cellulose from pepper pericarp waste was successfully extracted with combination of alkaline and bleaching treatment. In this study, pepper pericarp was treated with 4, 5, 6% NaOH at different number of alkali cycle (3 & 4 times). It is important to carefully identify the optimum NaOH load and cycles of alkaline treatment for efficient extraction process of cellulose powder. Based on the results obtained in this study, pepper pericarp treated with 4% NaOH for 4 alkalization cycles were observed to extract cellulose at highest yield of cellulose, highest  $\alpha$ -cellulose content, zeta potential value and crystallinity index. The process used was able to produce white solid particle powder similar to pure cellulose. FTIR result confirmed the presence of cellulose and partial removal of hemicellulose and lignin. The extracted cellulose has small size particle, good thermal stability with observed charged particles which can be further explored in detail for various industrial application. The current findings reported that not only preparation condition affect the properties of end fibres, but the composition of raw material might also affect the changes of cellulose obtained.

#### ACKNOWLEDGEMENTS

The authors gratefully acknowledge the funding support from Universiti Kebangsaan Malaysia (UKM) under research grant GUP/2018/111.

#### REFERENCES

- Abdullah, N.A., Sainorudin, M.H., Rani, M.S.A., Mohammad, M., Abd Kadir, N.H. & Asim, N. 2021. Structure and thermal properties of microcrystalline cellulose extracted from coconut husk fiber. *Polimery* 66(3): 187-192.
- Aziz, N.S., Sofian-Seng, N.S., Mohd Razali, N.S., Lim, S.J. & Mustapha, W.A. 2019. A review on conventional and biotechnological approaches in white pepper production. *Journal of the Science of Food and Agriculture* 99(6): 2665-2676.
- Aziz, N.S., Sofian-Seng, N.S. & Mustapha, W.A.W. 2018. Functional properties of oleoresin extracted from white pepper (*Piper nigrum* L.) retting wastewater. *Sains Malaysiana* 47(9): 2009-2015.
- Cai, M., Takagi, H., Nakagaito, A.N., Katoh, M., Ueki, T., Waterhouse, G.I. & Li, Y. 2015. Influence of alkali treatment on internal microstructure and tensile properties of abaca fibers. *Industrial Crops and Products* 65: 27-35.
- Chen, Y., Stevens, M.A., Zhu, Y., Holmes, J. & Xu, H. 2013. Understanding of alkaline pretreatment parameters for corn stover enzymatic saccharification. *Biotechnology for Biofuels* 6: 8.
- Chien, J.P.U. & Mansel, E.L. 2017. *100 Years of Agricultural Development in Sarawak*. Kuching, Malaysia: Sarawak Agriculture Veteran Association. pp. 1-245.
- Ching, Y.C. & Ng, T.S. 2014. Effect of preparation conditions on cellulose from oil palm empty fruit bunch fiber. *BioResources* 9(4): 6373-6385.
- Devi, T.B., Ravi, Y. & Dawange, S.P. 2018. Processed products from black pepper. *Kerala Karshakan e-Journal* August: 9-14.
- Din, N.A.S., Lim, S.J., Maskat, M.Y. & Zaini, N.A.M. 2020. Bioconversion of coconut husk fibre through biorefinery process of alkaline pretreatment and enzymatic hydrolysis. *Biomass Conversion and Biorefinery* 11(3): 815-826.
- Entebang, H., Wong, S.K. & Mercer, Z.J.A. 2020. Development and performance of the pepper industry in Malaysia: A critical review. *International Journal of Business and Society* 21(3): 1402-1423.
- Fareez, I.M., Ibrahim, N.A., Wan Yaacob, W.M.H., Mamat Razali, N.A., Jasni, A.H. & Aziz, F.A. 2018. Characteristics of cellulose extracted from Jospine pineapple leaf fibre after alkali treatment followed by extensive bleaching. *Cellulose* 25(8): 4407-4421.
- Fatmawati, A., Gunawan, K.Y. & Hadiwijaya, F.A. 2017. Hydrolysis of alkaline pretreated banana peel. In *IOP Conference Series: Materials Science and Engineering*. IOP Publishing. 273(1): 012011.
- Fiore, V., Scalici, T. & Valenza, A. 2014. Characterization of a new natural fiber from *Arundo donax* L. as potential reinforcement of polymer composites. *Carbohydrate Polymer* 106: 77-83.
- German Association of Cellulose Chemists and Engineers. 1951. Bestimmung der Alphacellulose und de langeunloslichen Anteils von Zellstoffen. *Markblatt IV/29 Zellcheming*.

- Gomes, A., Matsuo, T., Goda, K. & Ohgi, J. 2007. Development and effect of alkali treatment on tensile properties of curaua fiber green composites. *Composites Part A: Applied Science and Manufacturing* 38(8): 1811-1820.
- Held, M.A., Jiang, N., Basu, D., Showalter, A.M. & Faik, A. 2015. Plant cell wall polysaccharides: Structure and biosynthesis. In *Polysaccharides: Bioactivity and Biotechnology*, edited by Ramawat, K.G. & Mérillon, J.M. New York: Springer Science+Business Media. pp. 3-54.
- International Pepper Community. 2018. *Pepper Statistical Yearbook*. <http://www.ipcnet.org/n/psy2018/html/swps.html>. Accessed on 18 February 2019.
- Johar, N., Ahmad, I. & Dufresne, A. 2012. Extraction, preparation and characterization of cellulose fibres and nanocrystals from rice husk. *Industrial Crops and Products* 37(1): 93-99.
- Kathirselvam, M., Kumaravel, A., Arthanarieswaran, V.P. & Saravanakumar, S.S. 2019. Characterization of cellulose fibers in *Thespesia populnea* barks: Influence of alkali treatment. *Carbohydrate Polymer* 217: 178-189.
- Kim, J.S., Lee, Y.Y. & Kim, T.H. 2016. A review on alkaline pre-treatment technology for bioconversion of lignocellulosic biomass. *BioResources* 199: 42-48.
- Kunusa, W.R., Isa, I., Laliyo, L.A.R. & Iyabu, H. 2018. FTIR, XRD and SEM analysis of microcrystalline cellulose (MCC) fibers from corncobs in alkaline treatment. In *Journal of Physics: Conference Series* 1028(1): 012199.
- Mariño, M.A., Rezende, C.A. & Tasic, L. 2018. A multistep mild process for preparation of nanocellulose from orange bagasse. *Cellulose* 25(10): 5739-5750.
- Mohd, N.H., Ismail, N.F.H., Zahari, J.I., Fathilah, W., Kargarzadeh, H., Ramli, S., Ahmad, I., Yarmo, M.A. & Othaman, R. 2016. Effect of aminosilane modification on nanocrystalline cellulose properties. *Journal of Nanomaterials* 2016: 4804271.
- Olalere, O.A., Abdurahman, H.N., Yunus, R.B.M., Alara, O.R., Ahmad, M.M., Zaki, Y.H. & Abdlrhman, H.S.M. 2018. Parameter study, antioxidant activities, morphological and functional characteristics in microwave extraction of medicinal oleoresins from black and white pepper. *Journal of Taibah University for Science* 12(6): 730-737.
- Ouajai, S. & Shanks, R.A. 2005. Composition, structure and thermal degradation of hemp cellulose after chemical treatments. *Polymer Degradation and Stability* 89(2): 327-335.
- Oushabi, A., Sair, S., Hassani, F.O., Abboud, Y., Tanane, O. & El Bouari, A. 2017. The effect of alkali treatment on mechanical, morphological and thermal properties of date palm fibers (DPFs): Study of the interface of DPF-polyurethane composite. *South African Journal of Chemistry* 23: 116-123.
- Radakisnin, R., Abdul Majid, M.S., Jamir, M.R.M., Jawaid, M., Sultan, M.T.H. & Mat Tahir, M.F. 2020. Structural, morphological and thermal properties of cellulose nanofibers from Napier fiber (*Pennisetum purpureum*). *Materials* 13(18): 4125.
- Raman, G. & Gaikar, V.G. 2002. Extraction of piperine from *Piper nigrum* (black pepper) by hydrotropic solubilization. *Industrial & Engineering Chemistry Research* 41(12): 2966-2976.
- Reddy, K.O., Maheswari, C.U., Shukla, M., Song, J.I. & Rajulu, A.V. 2013. Tensile and structural characterization of alkali treated Borassus fruit fine fibers. *Composites Part B: Engineering* 44(1): 433-438.
- Rosa, M.F., Medeiros, E.S., Malmonge, J.A., Gregorski, K.S., Wood, D.F., Mattoso, L.H.C., Glenn, G., Orts, S.H. & Imam, S.H. 2010. Cellulose nanowhiskers from coconut husk fibers: Effect of preparation conditions on their thermal and morphological behavior. *Carbohydrate Polymers* 81(1): 83-92.
- Rosnah, S. & Chan, S.C. 2014. Enzymatic rettings of green pepper berries for white pepper production. *International Food Research Journal* 21(1): 237-245.
- Santos, E.B.C., Moreno, C.G., Barros, J.J.P., Moura, D.A.D., Fim, F.D.C., Ries, A., Wellen, R.M. & Silva, L.B.D. 2018. Effect of alkaline and hot water treatments on the structure and morphology of Piassava fibers. *Journal of Materials Research* 21(2): e20170365.
- Segal, L.G.J.M.A., Creely, J.J., Martin Jr., A.E. & Conrad, C.M. 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Research Journal* 29(10): 786-794.
- Singanpong, R., Tochampa, W., Kongbangkerd, T. & Sodchit, C. 2014. Extraction and properties of cellulose from banana peels. *Suranaree Journal of Science & Technology* 21(3): 201-213.
- Sosiati, H., Pratiwi, H. & Wijayanti, D.A. 2015. The influence of alkali treatments on tensile strength and surface morphology of cellulose microfibrils. *Advanced Materials Research* 1123: 147-150.
- Sreekala, G.S., Meenakumari, K.S. & Vigi, S. 2019. Microbial isolate for the production of quality white pepper (*Piper nigrum* L.). *Journal of Tropical Agriculture* 57(2): 114-121.
- Tenorio, A.T., Gieteling, J., Nikiforidis, C.V., Boom, R.M. & Van der Goot, A.J. 2017. Interfacial properties of green leaf cellulosic particles. *Food Hydrocolloid* 71: 8-16.
- Tibolla, H., Pelissari, F.M., Martins, J.T., Vicente, A.A. & Menegalli, F.C. 2018. Cellulose nanofibers produced from banana peel by chemical and mechanical treatments: Characterization and cytotoxicity assessment. *Food Hydrocolloids* 75: 192-201.
- Tran, A.T., Cao, N.H., Le, P.T.K., Mai, P.T. & Nguyen, Q.D. 2020. Reusing alkaline solution in lignocellulose pre-treatment to save consumable chemicals without losing efficiency. *Chemical Engineering Transactions* 78: 307-312.
- Wallecan, J., McCrae, C., Debon, S.J.J., Dong, J. & Mazoyer, J. 2015. Emulsifying and stabilizing properties of functionalized orange pulp fibers. *Food Hydrocolloid* 47: 115-123.

- Widiarto, S., Yuwono, S.D., Rochliadi, A. & Arcana, I.M. 2017. Preparation and characterization of cellulose and nanocellulose from agro-industrial waste-cassava peel. In *IOP Conference Series: Materials Science and Engineering* 176(1): 012052.
- Winuprasith, T. & Suphantharika, M. 2013. Microfibrillated cellulose from mangosteen (*Garcinia mangostana* L.) rind: Preparation, characterization, and evaluation as an emulsion stabilizer. *Food Hydrocolloid* 32(2): 383-394.
- Yew, B.S., Muhamad, M., Mohamed, S.B. & Wee, F.H. 2019. Effect of alkaline treatment on structural characterisation, thermal degradation and water absorption ability of coir fibre polymer composites. *Sains Malaysiana* 48(3): 653-659.
- Zahari, M.J.I., Jahi, N.M., Mohd, N.H., Ahmad, I., Baharum, A., Lazim, A.M., Ramli, S. & Othaman, R. 2018. Enhanced performance of cellulose from palm oil empty fruit bunch (EFB) via acetylation and silylation. *Preprints 2018*: 2018070314.
- Zain, N.F.M., Yusop, S.M. & Ahmad, I. 2014. Preparation and characterization of cellulose and nanocellulose from pomelo (*Citrus grandis*) albedo. *Journal of Nutrition & Food Science* 5(1): 1000334.
- Zhou, Y.M., Fu, S.Y., Zheng, L.M. & Zhan, H.Y. 2012. Effect of nanocellulose isolation techniques on the formation of reinforced poly (vinyl alcohol) nanocomposite films. *eXPRESS Polymer Letters* 6(10): 794-804.

\*Corresponding author; email: soffalina@ukm.edu.my