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)

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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, α-cellulose content, particle size, zeta potential, morphology, whiteness index, crystallinity degree and thermal stability. The white powder cellulose after 4th cycle treatment with 4% NaOH appeared to have the highest yield (23.63%), α-cellulose (65.97%), crystallinity structure (51%) and better thermal stability at 334 °C. FTIR spectrum at band around 1732 cm⁻¹ 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 α-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

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, kesa 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 α-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%), α-selulosa yang tinggi (65.97%), struktur kehabluran (51%) dan kestabilan terma yang lebih baik pada 334 °C. Spektrum FTIR pada sekitar julur 1732 cm⁻¹ 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
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(p<0.05) mempengaruhi hasil, saiz zarah dan potensi zeta, sementara α-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 owning 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 (NaClO₂) and acetic acid glacial (CH₃COOH) 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):

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

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 (NaClO₂), 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.

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

CHEMICAL COMPOSITION
The α-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 α-cellulose content was calculated in (3).

\( \alpha\text{-cellulose (%) = } \frac{\text{Dry mass of } \alpha\text{-cellulose obtained (g)}}{\text{Initial weight of dry sample (g)}} \times 100 \)
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 ζ-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× 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 3000x 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, Whunter = 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 (cm⁻¹) was recorded within the wavelength range from 400 to 4000 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-Kα radiation source (λ= 0.1539 nm). Results were obtained in 20 scale ranging from 10 to 50°. The crystallinity index (CrI) was calculated according to Segal method (Segal et al. 1959): crystallinity index (CrI%) = [(I₀₀₂ - Iₐₐₚ) / I₀₀₂] × 100, where I₀₀₂ is the intensity value of crystalline cellulose (I₀₀₂, 20= 22.6°) and Iₐₐₚ is the intensity value that represents amorphous part of the sample (Iₐₐₚ, 20=18°).

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 °C to 600 °C with constant nitrogen flow rate of 10 °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 ± 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±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.

α-CELLULOSE COMPOSITION

The α-cellulose content of untreated and treated pepper pericarp was presents in Table 2. The DPP contained 47.88±3.59% of α-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 α-cellulose content measure was in CPP4%.4C powder, followed by CPP6%.3C and CPP5%.3C with values of 65.97±1.08, 62.77±0.73, and 61.64±1.33%, respectively.

The two-way ANOVA test showed that the concentration of NaOH has significant effect on α-cellulose content (p<0.05), meanwhile no significant effect on the number of alkalinization cycle towards α-cellulose content (p>0.05). Kunusa et al. (2018) reported that cellulose from corncob produce the highest content of α-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, α-cellulose content, particle size and ζ-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 (%) | α-cellulose (%) | Particle size, d (nm) | ζ-potential (mV) | Whiteness index (%) |
|------------|-----------|-----------------|-----------------------|------------------|---------------------|
| DPP        | 4.85 ± 0.04 | 47.88 ± 3.59    | 1184 ± 130.54         | 4.52 ± 1.29      | 16.36 ± 0.74        |
| CPP4%.3C   | 24.17 ± 0.25 | 60.85 ± 1.02    | 549.72 ± 42.49        | -17.60 ± 2.83    | 76.74 ± 0.06        |
| CPP4%.4C   | 23.63 ± 0.25 | 65.97 ± 1.08    | 420.39 ± 52.50        | -30.67 ± 5.48    | 77.00 ± 1.10        |
| CPP5%.3C   | 20.02 ± 0.43 | 62.77 ± 0.73    | 367.75 ± 55.73        | -22.97 ± 4.60    | 82.53 ± 0.61        |
| CPP5%.4C   | 19.30 ± 0.11 | 59.86 ± 2.87    | 344.10 ± 56.88        | -24.37 ± 7.69    | 66.91 ± 0.52        |
| CPP6%.3C   | 18.58 ± 0.51 | 61.64 ± 1.33    | 343.02 ± 16.18        | -27.94 ± 2.25    | 71.78 ± 0.19        |
| CPP6%.4C   | 16.35 ± 0.47 | 58.07 ± 3.35    | 239.29 ± 44.01        | -28.23 ± 9.02    | 72.63 ± 0.15        |

*: 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 µm. 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

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 the 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±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)
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 ζ-potential recorded was -30.67±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±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±0.61, 77.00±1.10 and 76.74±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.

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 3500-3200 and 2900-2890 cm⁻¹ in all samples are due to hydroxyl groups (-OH groups) and C-H stretching of cellulose. The peak at 1000 and 800 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 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 cm$^{-1}$ which attributed to C=C stretching of the lignin carboxyl groups and 1245 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 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 (Olalere et al. 2018). The peak at 1630 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).

![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)](image)

**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 2θ = 16°, 22°, and 34.5° 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 alkaline 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 points. 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
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 α-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.

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