Introduction
Agro-industrial activities have produced vast amount of waste each year. Bharathiraja et al. (2017) reported that roughly 5 billion metric tons of agro-industrial by-product such as rice bran and straw, sugarcane bagasse, fruits, and vegetable wastes were discarded annually. The pile up of these residues, does not only cause negative impact to the environment but also a loss of potential material. This is because in general, agro-industrial waste composed of lignocellulosic material such as cellulose, hemicellulose, lignin, and ashes (Kalia et al. 2011; Mussatto et al. 2012; Rosa et al. 2010). Most of these wastes have been...
researched to be used as renewable resource of cellulose materials as it is cheaper and the cellulose from plant’s wall is easier to be extracted than from wood source (Alemdar & Sain 2008; Jonooib et al. 2015).

Production of high purity cellulose commonly involved alkali and chlorite bleaching treatment (Bhandari et al. 2013). The purpose of alkali treatment is to solubilise hemicellulose and lignin (Johar et al. 2012). Nevertheless, a single alkalisation process is unable to remove all the remaining lignin and hemicellulose, hence bleaching treatment is often used. Other than enhancing the whiteness of cellulose fiber, bleaching helps in producing smaller size with better uniformity of cellulose (Johar et al. 2012; Rosa et al. 2010). Recently, the production of cellulose from various sources of agro waste materials has been extensively studied, which include rice husk (Fathi et al. 2018), onion and garlic skins (Reddy & Rhim 2018), pomelo albedo (Zain et al. 2014) and mango seed (Henrique et al. 2013). However, there is no study conducted in extracting the cellulose from pepper pericarp waste.

Pepper (Piper nigrum L.) is one of the most important spices in history. Malaysia is currently ranked as the 5th pepper producer in the world (Kamarulzaman et al. 2013). White pepper has a lighter color, milder flavor and pungency compared to black pepper (Sephton et al. 2013). White pepper has a lighter color, milder flavor and pungency compared to black pepper (Sephton et al. 2013). White pepper has a lighter color, milder flavor and pungency compared to black pepper (Sephton et al. 2013). White pepper has a lighter color, milder flavor and pungency compared to black pepper (Sephton et al. 2013). White pepper has a lighter color, milder flavor and pungency compared to black pepper (Sephton et al. 2013). White pepper has a lighter color, milder flavor and pungency compared to black pepper (Sephton et al. 2013).

Pepper (Piper nigrum L.) was collected from Herba Bagus farm in Kluang (Johor, Malaysia). Sodium hydroxide (99% purity, QREC Sdn Bhd), sodium chlorite (Sigma-Aldrich) and acetic acid glacial (Friendemann Schmidt) were used for the extraction of cellulose. All the reagent grade chemicals were used without further purification.

MATERIALS AND METHODS

Green pepper berries (Piper nigrum L.) was collected from Herba Bagus farm in Kluang (Johor, Malaysia). Sodium hydroxide (99% purity, QREC Sdn Bhd), sodium chlorite (Sigma-Aldrich) and acetic acid glacial (Friendemann Schmidt) were used for the extraction of cellulose. All the reagent grade chemicals were used without further purification.

RAW MATERIAL PREPARATION

The green pepper berries were cleaned and washed with water to remove impurities and dirt. The pepper was soaked in a conical flask containing water at ratio of 1:2 (pepper: distilled water, w/v). The flasks were placed in a water bath shaker for 8 days at 28 °C. The softened pepper pericarp was peeled manually and dried overnight in a convection oven (Protech, Malaysia) at 40 °C until constant weight was obtained. Dried sample was then grinded and sieved.

ALKALINE TREATMENT

The alkaline and bleaching process was conducted according to Johar et al. (2012) with some modification. The powdered pepper pericarp was transferred into a round bottom flask and treated with an alkali solution (4 wt. % NaOH). The treatment was carried out under reflux condition at 80-90 °C for 3 h. The solid was then filtered and washed with water. After washing, the alkaline treatment was repeated three more times.

BLEACHING TREATMENT

Following alkaline treatment, the bleaching process was performed by using 1.7% NaClO₂, acetic buffer and distilled water in reflux environment for 4 h at 80-90 °C. The mixture was filtered and washed with water. The bleaching treatment was carried out in four cycles (1C, 2C, 3C and 4C) to extract cellulose from pepper pericarp. Sample 1C undergo only one bleaching treatment while sample 2C, 3C, and 4C subjected to bleaching treatment for 2, 3, and 4 times, respectively.

CHARACTERISATION

COLOUR ANALYSIS

The colour of untreated, alkali-treated and bleached were determined by Minolta colorimeter (Chroma meter CR 400, Japan) with a Hunter Lab color system (L*, a*, and b*). The whiteness value was measured based on Color iMatch Color Calculations Guide (2012) formula, \( W_{\text{hunter}} = L - 3b \). The optical microscopy of pepper pericarp at different stages of treatment was observed by an optical stereo microscope (Leica EZ/4) and captured with 16-megapixel (MP) camera (Huawei Nova 3e, China).

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY ANALYSIS

The FTIR spectrum of cellulose was recorded by using PerkinElmer attenuated total reflection (ATR-FTIR) spectrometer. The results were measured in the wavelength range from 400-4000 cm⁻¹, with a resolution of 4 cm⁻¹ and four scans were averaged for each sample (Zain et al. 2014). Samples before and after each alkali and bleaching (1C, 2C, 3C and 4C) treatment were analysed to observed changes in the functional group.

THERMOGRAVIMETRIC ANALYSIS (TGA)

The thermal stability of each sample was measured by using Mettler Toledo thermogravimetric analyser (TGA/SDTA 85-F). All samples were heated from 25 to 600 °C under nitrogen gas flow of 10 °C min⁻¹ (Mohd et al. 2016).
**STATISTICAL ANALYSIS**

All experiments were performed in three replications. The data for colour analysis were performed in triplicate. The results were expressed in mean ± standard deviation. The data was analysed using analysis of variance test (ANOVA) and Duncan’s multiple range test (DMRT) by using IBM SPSS Statistic Software version 20. Means are significantly different when (p<0.05).

**RESULTS AND DISCUSSION**

Visualisation via optical microscopy of the pepper pericarp changes after each treatment are as displayed in Figures 1 and 2. The original brown colour of untreated pepper pericarp changes to lighter brown after alkaline treatment and started to fade after bleaching. These images indicate effective removal of lignin, hemicellulose and other non-cellulosic materials. Sample 4C which has been subjected to four cycle of bleaching process has whiter colour of cellulose as compared to another bleached sample (1C, 2C, & 3C). The number of cycles in bleaching treatment was observed to play an important role in the removal of lignin. Table 1 shows the whiteness index of untreated pepper pericarp, alkali treated and all bleached samples (1C, 2C, 3C & 4C). As the bleaching cycle increased, the whiteness of each sample significantly increased (p<0.05). The bleached samples illustrate improvements in whiteness index. Bleached sample with 4 cycle of treatment was measured to have the highest whiteness index as compared to untreated, alkali treated and other low bleached treated samples with the value of 77.00 ± 1.10, 16.36 ± 0.74, -4.30 ± 1.11, -8.93 ± 0.52, 46.05 ± 0.92, and 70.39 ± 0.94, respectively.

**FTIR CHARACTERISATION**

The FTIR spectrums of untreated pepper pericarp, alkali treated and bleached sample (1C, 2C, 3C & 4C) are presented in Figure 3. The absorption peak at 3400-3300 cm\(^{-1}\) and 2900-2890 cm\(^{-1}\) was observed in all samples, which indicate the presence of O-H groups and C-H groups, respectively, in cellulose fibres (Ngadi & Lani 2014; Yin et al. 2015). The peak around 1734 cm\(^{-1}\) was highly visible in raw pepper pericarp spectrum. It represents the C=O of the acetyl and ester groups in pectin, hemicelullose and lignin (Phanthong et al. 2015). Comparison between samples 1C, 2C, 3C, and 4C showed how the intensity of this peak decreased and as the bleaching treatment prolonged with each additional cycle, depicting larger amount of non-cellulosic materials in pepper pericarp are being removed. The disappearance of peak around 1509-1609 cm\(^{-1}\) (aromatic C=C in lignin) indicated successful removal of remaining lignin as it was only found in pepper pericarp and alkali treated sample (Mandal & Chakrabarty 2011). The region of absorbance peak at 1460 cm\(^{-1}\) corresponding to group of –CH\(_3\) and/or –C=CH\(_2\) stretching in aromatic group of lignin and hemicelullose (Jonoobi et al. 2009). This peak started to disappear with the increasing number of bleaching cycles which proven to remove most of the hemicelullose and lignin from the pepper pericarp. The absorption peak at 1155 and 1029 cm\(^{-1}\) which were observed in all samples refer to –COC group due to anti-symmetrical deformation in cellulose (Silva et al. 2008) and aromatic ring of surface cellulose fibre (Karimi et al. 2014). Whereas the band around 895 cm\(^{-1}\) was attributed to the glycosidic C-H rock vibration that indicates the typical structure of cellulose.

**THERMOGRAVIMETRIC ANALYSIS**

Figure 4 shows the thermogravimetric weight loss and DTG of samples before and after bleaching for 1, 2, 3, and 4 cycles. The results in Figure 4(a) exhibited the weight loss of all samples primarily take place between 25-600 °C. Untreated pepper pericarp and all bleached samples started to have first weight loss at a temperature below than 100 °C. This initial change is due to water evaporation which was highly influenced by the moisture content of the sample (Ilyas et al. 2018). The degradation of hemicelullose and lignin were observed at 240 and 295 °C (Balaji & Nagarajan 2017). Compared to untreated and bleached treated fibres, bleached sample shows higher thermal stability. Through bleaching, the onset of thermal decomposition shifts to higher temperature due to removal of hemicelullose and lignin.

Based on Figure 4(b), bleached pepper pericarp (4C) was observed to have slightly higher degradation temperature at 332 °C as compared to other bleached pepper pericarp (1C: 322 °C, 2C: 326 °C & 3C: 329 °C). Higher cycle numbers of bleaching treatment lead to an effective removal of lignin. The results obtained were in agreement with research conducted by Ng et al. (2014) that found cellulose obtained with 8 and 12 h of bleaching treatment had higher thermal stability than untreated sample.

Table 2 shows the weight loss of untreated pepper pericarp and bleached samples (1C, 2C, 3C & 4C). Higher cycle of bleaching treatment has lower weight loss as compared to other bleached samples. Lee et al. (2004) reported that the thermal decomposition of lignocelullosic components started with hemicelullose, then lignin, followed by depolymerisation of cellulose, active flaming combustion and char oxidation. Therefore, indicating high thermal stability of stronger bleached sample (low weight loss) as most of the hemicelullose and lignin has been effectively removed.
**TABLE 1.** Whiteness index of untreated pepper pericarp, alkali treated and bleached sample

| Samples           | Whiteness index |
|-------------------|-----------------|
| Untreated         | 16.36 ± 0.74a   |
| Alkali treated    | -4.30 ± 1.11b   |
| Bleached 1C       | -8.93 ± 0.52c   |
| Bleached 2C       | 46.05 ± 0.92d   |
| Bleached 3C       | 70.39 ± 0.94e   |
| Bleached 4C       | 77.00 ± 1.10f   |

*a-f*: Means with different alphabet are significantly different (p<0.05)

**TABLE 2.** The weight loss of fibres

| Samples                  | Weight loss (%) |
|--------------------------|-----------------|
|                          | 100 °C | 200 °C | 300 °C | 400 °C | 500 °C |
| Raw pepper pericarp      | 3.72   | 5.14   | 18.70  | 56.66  | 69.90  |
| Bleached sample - 1 cycle| 6.49   | 7.28   | 23.36  | 64.45  | 77.12  |
| Bleached sample - 2 cycle| 6.52   | 7.16   | 22.61  | 71.23  | 84.05  |
| Bleached sample - 3 cycle| 6.79   | 7.37   | 21.60  | 72.53  | 80.99  |
| Bleached sample - 4 cycle| 3.06   | 3.42   | 17.05  | 73.19  | 80.65  |

**FIGURE 1.** Photographs of (a) fresh green pepper berries and (b) untreated pepper pericarp
FIGURE 2. Optical microscopy of (i) alkali treated, (ii) 1C bleached treated, (iii) 2C bleached treated, (iv) 3C bleached treated, and (v) 4C bleached treated.

FIGURE 3. FTIR spectra recorded for (a) raw, (b) alkali treated, (c) 1C-bleached sample, (d) 2C-bleached sample, (e) 3C-bleached sample, & (f) 4C-bleached sample.
CONCLUSION
Pepper pericarp waste was found to be an excellent source for production of cellulose. Combination of alkali and bleaching treatment seems to remove lignin and hemicellulose components successfully. The effect of preparation condition in terms of bleaching cycle, on the behaviour of cellulose was evaluated. Higher bleaching cycles produced whiter cellulose, with removal of most lignin and hemicellulose band in FTIR spectra and stable thermal properties. The optimum condition of bleaching treatment creates favourable changes towards chemical structure and thermal stability of cellulose produced.

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REFERENCES
Alemdar, A. & Sain, M. 2008. Isolation and characterization of nanofibers from agricultural residues-wheat straw and soy hulls. Bioresource Technology 99(6): 1664-1671.
Balaji, A.N. & Nagarajan, K.J. 2017. Characterization of alkali treated and untreated new cellulosic fiber from Saharan aloe vera cactus leaves. Carbohydrate Polymers 174: 200-208.
Bhandari, N.L., Mormann, W., Michler, G.H. & Adhikari, R. 2013. Functionalisation of bamboo and sisal fibres cellulose in ionic liquids. Materials Research Innovations 17(4): 250-256.
Bharathiraja, S., Suriya, J., Krishnan, M., Manivhasagan, P. & Kim, S.K. 2017. Production of enzymes from agricultural wastes and their potential industrial applications. In Advances in Food and Nutrition Research, edited by Kim, S.K. & Toldrá, F. Massachusetts: Academic Press. pp. 80: 125-148.

Bunchol, A.J. 2011. Interview with Food Technologist Officer Malaysian Pepper Board, Sarawak. Color iQC and Color iMatch Color Calculations Guide. 2012. Version 8.0: 14.
Fathi, H.I., El-Shazly, A.H., Elkady, M.F. & Madih, K. 2018. Assessment of new technique for production cellulose nanocrystals from agricultural waste. Materials Science Forum 928(August 2018): 83-88.
Henrique, M.A., Silvério, H.A., Neto, W.P.F. & Pasquini, D. 2013. Valorization of an agro-industrial waste, mango seed, by the extraction and characterization of its cellulose nanocrystals. Journal of Environmental Management 121: 202-209.
Ilyas, R.A., Sapuan, S.M., Sanyang, M.L., Ishak, M.R. & Zainudin, E.S. 2018. Nanocrystalline cellulose as reinforcement for polymeric matrix nanocomposites and its potential applications: A review. Current Analytical Chemistry 14(3): 203-225.
Johar, N., Ahmad, I. & Dufresne, A. 2012. Extraction, preparation and characterization of cellulose fibers and nanocrystals from rice husk. Industrial Crops and Products 37(1): 93-99.
Jonoobi, M., Oladi, R., Davoudpour, Y., Oksman, K., Dufresne, A., Hamzeh, Y. & Davoodi, R. 2015. Different preparation methods and properties of nanostructured cellulose from various natural resources and residues: A review. Cellulose 22(2): 935-969.
Jonoobi, M., Harun, J., Mishra, M. & Oksman, K. 2009. Chemical composition, crystallinity and thermal degradation of bleached and unbleached kenaf bast (Hibiscus cannabinus) pulp and nanofibers. BioResources 4(2): 626-639.
Kalia, S., Dufresne, A., Cherian, B.M., Kauth, B.S., Avérous, L., Njuguna, J. & Nassiopoulos, E. 2011. Cellulose-based bio- and nanocomposites: A review. International Journal of Polymer Science 2011: Article ID. 837875.
Kamarulzaman, N.H., Husin, H., Mohaydin, M.G. & Enchi, J. 2013. Buyers’ preferences among pepper farmers in Sarawak. Journal of Agribusiness Marketing 6: 1-13.
Karimi, S., Tahir, P.M., Karimi, A., Dufresne, A. & Abdulkhani, A. 2014. Kenaf bast cellulosic fibers hierarchy: A comprehensive

FIGURE 4. (a) & (b) TGA and DTG thermogram for raw pepper pericarp (a) and bleached samples- 1C (b), 2C (c), 3C (d) and 4C (e)
approach from micro to nano. *Carbohydrate Polymers* 101: 878-885.

Lee, H.L., Chen, G.C. & Rowell, R.M. 2004. Thermal properties of wood reacted with a phosphorus pentoxide-amine system. *Journal of Applied Polymer Science* 91(4): 2465-2481.

Mandal, A. & Chakrabarty, D. 2011. Isolation of nanocellulose from waste sugarcane bagasse (SCB) and its characterization. *Carbohydrate Polymers* 86(3): 1291-1299.

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: Article ID. 4804271.

Mussatto, S.I., Ballesteros, L.F., Martins, S. & Teixeira, J.A. 2012. Use of agro-Industrial wastes in solid-state fermentation processes. In *Industrial Waste*, edited by Show, K.Y. Rijeka: Intech. pp. 121-140.

Ng, T.S., Ching, Y.C., Awanis, N., Ishenny, N. & Rahman, M.R. 2014. Effect of bleaching condition on thermal properties and UV transmittance of PVA/cellulose biocomposites. *Materials Research Innovations* 18(sup6): S6-400-S6-404.

Ngadi, N. & Lani, N.S. 2014. Extraction and characterization of cellulose from empty fruit bunch (EFB) fiber. *Jurnal Teknologi* 68(5): 35-39.

Phanthong, P., Ma, Y., Guan, G. & Abudula, A. 2015. Extraction of nanocellulose from raw apple stem. *Journal of the Japan Institute of Energy* 94(8): 787-793.

Reddy, J.P. & Rhim, J.W. 2018. Extraction and characterization of cellulose microfibers from agricultural wastes of onion and garlic. *Journal of Natural Fibers* 15(4): 465-473.

Rosa, M.F., Medeiros, E.S., Malmonte, J.A., Gregorksi, K.S., Wood, D.F., Mattoso, L.H.C., Glenn, G., Orts, W.J. & 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.

Sephion, P.S. 2011. Spatial arbitrage in Sarawak pepper prices. *Canadian Journal of Agricultural Economics/Revue canadienne d’agroeconomie* 59(3); 405-416.

Silva, M.C., Lopes, O.R., Colodette, J.L., Porto, A.O., Rieumont, J., Chaussy, D., Belgacem, M.N. & Silva, G.G. 2008. Characterization of three non-product materials from a bleached eucalyptus kraft pulp mill, in view of valorising them as a source of cellulose fibres. *Industrial Crops and Products* 27(3): 288-295.

Yin, O.S., Ahmad, I. & Amin, M.C.I.M. 2015. Effect of cellulose nanocrystals content and pH on swelling behaviour of gelatin based hydrogel. *Sains Malaysia* 44(6): 793-799.

Zain, N.F.M., Yusop, M.S. & Ahmad, I. 2014. Preparation and characterization of cellulose and nanocellulose from pomelo (*Citrus grandis*) albedo. *Journal of Nutrition & Food Sciences* 5(1): 334.