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Exploring the Role of Chemo-mechanical Technique on the Properties of Nanocellulose Isolated from Different Varieties of Rice Bran

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Nanocellulose (NC) was isolated from an underutilized agricultural residue, defatted rice bran (DRB), obtained from three major paddy varieties in Malaysia, MARDI Rice (MR): MR 220, MR 219, and MR 297. The DRB was subjected to alkali (NaOH) and bleaching treatments prior to the isolation of NC. The NC from DRB was produced through the acid hydrolysis technique using 55%wt sulphuric acid (H_2SO_4) with the assistance of ultrasonication. The morphological structure of NC was analyzed through transmission electron microscopy (TEM). Based on the observation, a needle-like structure was observed for NC 219 while NC 220 and NC 297 showed nano-web structures. All obtained NC dimensions ranged from 183 to 263 nm long and 4.70 to 4.97 nm wide. Fourier transform infrared (FTIR) spectroscopy revealed the presence of all important compositions for the cellulose of each variety, indicating that the chemical structure of DRB cellulose has not been altered by acid hydrolysis. Particle size distributions portrayed a unimodal graph for NC 220 and NC 219. This comparative study provides an insight into the possibility of using DRB as a potential source of NC. The promising characteristics of NC from MR 219 allowed its application as an emulsifier for stabilizing a solid particle-based emulsion.

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INTRODUCTION

In recent years, the production of novel nanomaterials from abundant and renewable biomass, such as agricultural by-products, has drawn interest in the nanotechnological subject. Several agricultural wastes, such as garlic straw (Kallel *et al.* 2016), onion waste (Reddy and Rhim 2018), walnut shell (Zheng *et al.* 2019), hemp hurd (Tyagi *et al.* 2021), *etc*., have been studied previously as a promising source for the production of the natural nanofibres. These types of fibres are of great interest due to their low cost and possibility of biodegradability, renewability, and ease in processing (Eichhorn *et al.* 2010; Joanitti and Silva 2013; Mohamad Haafiz *et al.* 2014).

Lignocellulosic biomass of a particular plant cell wall structure mainly consists of three kinds of polymers, *i.e.*, lignin, hemicellulose, and cellulose. Cellulose is a major component that stands out as one of the abundant and important natural biopolymer sources compared to the other compounds of interest. Cellulose is regarded as an eco-friendly material due to its natural origin, biodegradability, and being a high-molecular polymer. Hence, it has received increased attention for sophisticated application in the past few

years, especially when it is isolated as nanosize cellulose material from the cellulose fibre, using several methods (Morais *et al.* 2013; Liu *et al.* 2016). Such materials are called nanocellulose, in recognition of their unique nanoscale dimension that is generally less than 100 nm in diameter with several micrometers in length. In addition to its distinctive physicochemical properties, nanocellulose has a lower impact on the environment. It is also a low-density, lightweight material (1.6 g/cm^3) with an excellent strength property. Nanocellulose is also a transparent material and contains many reactive surface hydroxyl groups that can work with various surface properties (Abdul Khalil *et al.* 2012; Dufresne 2013).

Cellulose nanocrystals (CNC) and nanofibrillated cellulose (NFC) are among the types of nanocellulose. NFC is also known as cellulose nanofibril. These two materials can be majorly differentiated through their shape and dimensions. The CNC has a short-rod-like shape, while NFC has longer fibril shapes (Moon *et al.* 2011; Mohamad Haafiz *et al.* 2014; Abitbol *et al.* 2016). CNC is also different from NFC in terms of its crystallinity. In general, CNC has higher crystallinity (45 to 90%) compared to NFC. This can be explained by the complete removal of amorphous compositions (hemicellulose, lignin, *etc.*) after the chemical treatments, which can enhance the crystalline part of the CNC (Nagalakshmaiah *et al.* 2019). Fundamental features of nanocellulose, such as dimension, morphology, crystallinity, and surface chemistry, are influenced by the source of raw material and also the extraction process. It is critical to determine these key properties that affect the end-use of isolated nanocellulose (Liu *et al.* 2016). Novel applications of CNC can be found in the food industry as food stabilizers, functional food ingredients, and also biodegradable packaging (Mu *et al.* 2019), whereas in the medical field, CNC has structures that allow them to act as a drug delivery carrier for encapsulation purpose (Qing *et al.* 2016). In contrast, due to its special nanosize structures, NFC offers potential in the paper and coating industry (Mazhari Mousavi *et al.* 2017), as a wound dressing material (Sun *et al.* 2017), in nanopaper (Henriksson *et al.* 2007), and aerogels (Kettunen *et al.* 2011).

A great number of methods can be used to produce CNC and NFC. They are divided into two steps, pretreatment and extraction. Pretreatment methods that were performed on the biomass have a goal of facilitating the conversion of cellulose into nanocellulose by increasing the efficiency of biomass conversion process and overcoming the recalcitrance. Meanwhile, the extraction step plays an important role in the removal of disordered amorphous regions and leaves out the highly crystalline regions. The strong acid hydrolysis method is a typical method used to isolate nanocellulose from cellulose fibres. Among the common strong acids used are sulphuric acid (Ghazy *et al.* 2016) and hydrochloric acid (Yu *et al.* 2013). Other possible acids that have been utilized in nanocellulose fabrication are formic acid (Liu *et al.* 2016), nitric acid (Gismatulina *et al.* 2015), or the mixture of acids (Niu *et al.* 2017).

Rice can be considered the largest produced cereal crop in the world. The processing of paddy into rice is also one of the sources that contribute to the production of lignocellulosic biomass. Around 35.5% proportions of the paddy milling process are represented by the rice by-products, including 23.5% rice husk, 8% of rice bran, 3% of crushed rice, and 1% of *temukut* rice (MAFI 2017). In Malaysia, around 217 kilometric tonnes (MT) of rice bran were produced between 2014 and 2017 (MAFI 2018), which indicates its abundance. Rice bran currently serves as an animal feed (Casas *et al.* 2018) and low-cost attractive feedstock for bioethanol production (Tiwari *et al.* 2015) and biodiesel (Mohd Jaafar and Safiullah 2018). The production of nanocellulose from rice bran has been reported previously (Angkuratipakorn *et al.* 2017; Arun *et al.* 2020).

However, to date, limited information is available on the lignocellulosic compositions of different varieties of rice bran used and the effect of acid hydrolysis on the physicochemical properties, crystallinity, and the thermal behaviour of the isolated nanocellulose from varieties of rice bran remains unknown. Hence, this study aimed to explore and compare the properties of nanocellulose extracted from rice bran of different paddy varieties in Malaysia using chemo-mechanical methods. The properties of nanocellulose produced, such as chemical compositions, yield, morphology, particle size, crystallinity, and thermal stability, were investigated.

EXPERIMENTAL

Materials

Rice bran was obtained from three different paddy varieties noted as: Malaysian Agricultural Research and Development Institute (MARDI) Rice (MR): MR 219, MR 297, and MR 220. All varieties were collected from a local seed company in Pendang, Kedah, Malaysia. MR 219 and MR 220 were planted in paddy fields located in Kedah, Malaysia (Northern region/main season (August-February)), while MR 297 was planted in Selangor, Malaysia (Central region/off-season (March-July)). The chemical reagents used for pretreatments, including sodium hydroxide (NaOH), sodium chlorite (NaClO$_2$), and acetic acid (CH$_3$COOH), were supplied by Sigma Aldrich (Darmstadt, Germany). The sulphuric acid (H$_2$SO$_4$) that was used to isolate nanocellulose was procured from Merck (Selangor, Malaysia).

Methods

Sample preparations

Rice bran of each variety was prepared by milling 40 kg of the paddy seeds in a 50-kg capacity rice mill located at the Malaysian Agricultural Research and Development Institute (MARDI) station, Bukit Raya, Kedah, Malaysia. To obtain uniform particle size, the samples were screened through a 500-µm sieve and immediately stabilized using an autoclave machine (Sterilizzazione Usata; MeCan, Guangdong, China) at 120 °C for 20 min. The stabilization step is important to inactivate the lipase activity for a longer shelf-life and improves the functional properties of the rice bran (Irakli *et al.* 2021). The samples were oven-dried at 60 °C until 5% moisture content was reached. Defatted rice bran (DRB) was produced by using a soxhlet apparatus (Pyrex, Arizona, USA) following the method described by Abdul Khalil *et al.* (2016). An extraction thimble containing 5 g of stabilized rice bran was inserted in a soxhlet extraction unit and 250 mL of n-hexane was used as an extraction solvent. The extraction process was conducted at 75 °C for 90 min, and the resulting residue in the thimble was collected and dried in an oven at 40 °C overnight. The product of this extraction is called defatted rice bran (DRB). Afterwards it was kept at -18 °C in a chest freezer (249-L capacity) (Toshiba, Tokyo, Japan) until further use.

Chemical composition of DRB

The lignocellulosic compositions of the DRB of each variety were identified as described in the methods reported by the Technical Association of Pulp and Paper Industry (TAPPI). Briefly, the hemicellulose and cellulose content was analyzed following TAPPI T9 m-54 (1998) and TAPPI T201 su-70 (2004) standards, respectively. The acid-insoluble

lignin content was determined through a scaled-down process using concentrated sulphuric acid (H_2SO_4) (72%) according to the TAPPI T222 om-11 (2011) method.

Pre-treatment of lignocellulosic materials

The removal of lignin from DRB was performed based on the method proposed by Wise and Karl (1962). Meanwhile, to remove the hemicellulose, a method suggested by Angkuratipakorn *et al.* (2017) was used. Prior to the bleaching treatment, traces of oil were removed from the DRB by repeated washing and filtration using ethanol and warm water. The bleaching process was initiated by the addition of 1.5 g of sodium chlorite, 160 mL of distilled water, and 10 drops of acetic acid to 5 g of DRB for 1 h at 70 °C. The addition of 1.5 g of sodium chlorite and 10 drops of acetic acid was repeated four times (1.5 g × 4 h). The mixture was allowed to cool in an ice bath, before being centrifuged and washed using cold distilled water and acetone several times. Alkali treatment was conducted following the bleaching procedure by treating the washed DRB with a 4 wt% sodium hydroxide (NaOH) solution at reflux temperature (80 °C) for 4 h. The mixture was centrifuged at 3000 rpm for 15 min and washed repeatedly with distilled water.

Acid hydrolysis

The isolation of nanocellulose was performed by conducting hydrolysis on the pre-treated sample using preheated 55 wt% sulfuric acid solution (1:20) at 45 °C for 1 h under vigorous stirring, as suggested by Angkuratipakorn *et al.* (2017). A 10-fold dilution of cold distilled water was added to quench the reaction and centrifuged at 3000 rpm for 15 min for solvent removal. Water was added to the precipitate before the dialysis step. This was performed using a dialysis membrane (Sigma-Aldrich, Darmstadt, Germany) against distilled water for 3 to 4 days until neutral pH was achieved. Ultrasonication treatment was performed using a UP200H ultrasonicator (Hielscher, Teltow, Germany) with a 14-mm probe. The resulting suspension was collected and freeze-dried for 48 h and denoted as nanocellulose (NC). The sample was kept in a desiccator for further use. Nanocellulose produced from the three paddy varieties were denoted as NC 219, NC 297, and NC 220 throughout the study.

Yield of nanocellulose

The yield of nanocellulose produced was calculated based on Eq. 1,

$$\text{Nanocellulose yield (\%)} = \frac{\text{dry weight of nanocellulose (g)}}{\text{dry weight of cellulose (g)}} \times 100 \qquad (1)$$

Transmission electron microscopy

Transmission electron microscopy (TEM) (Zeiss Libra 120; Carl Zeiss Microscopy, Oberkochen, Germany) was used to examine the dimensions of the nanocellulose isolated from the rice bran. As reported by Jiang and Hsieh (2015), a drop of 0.1 wt% of nanocellulose suspension was placed onto a copper grid, and filter paper was used to remove the excess water after 3 min. The specimen was negatively stained using 2 wt% uranyl acetate solution for 1 min, blotted with filter paper and was allowed to dry at ambient temperature for 15 min prior to the analysis at the accelerating voltage of 120 kV. Measurements were taken only from the nanocellulose with clear distinguishable ends.

Dynamic light scattering

The average hydrodynamic diameter of the nanocellulose produced (0.1% wt) was determined by dynamic light scattering (DLS) using a Zetasizer AT (Malvern Panalytical Ltd, Malvern, Worcestershire, UK) instrument. The measurement was performed within the 2 to 3000 nm range. The analysis was performed in triplicate.

Fourier transform infrared spectroscopy (FTIR)

The FTIR analysis was performed using a Nicolet iS50 FTIR spectrometer (Thermo Fisher, Waltham, MA, USA). The sample was finely ground and mixed with potassium bromide, KBr, in a ratio of 1:100 (w/w) before being compressed into pellet form. The spectral analysis used for the FTIR analysis was recorded in a transmittance mode within the wavenumber range of 400 to 4000 cm^{-1}.

X-ray diffraction (XRD)

The crystallinity of the nanocellulose produced was observed using an Empyrean Alpha 1 X-ray diffractometer (Malvern Panalytical Ltd, Malvern, UK). The diffraction intensities were tested at $2\theta = 10$ to 50 °C with a scan rate of 4 °C/min. The crystallinity index (CrI, %) was determined according to Eq. 2 as stated by Arun *et al.* (2020),

$$CrI\ (\%) = \frac{I_{cry} - I_{am}}{I_{cry}} \times 100 \tag{2}$$

where I_{cry} is the crystalline region at a 2θ angle around 22° and I_{am} is amorphous material taken as the lowest intensity at a diffraction angle around $2\theta = 18°$.

Thermogravimetric analysis (TGA)

The TGA measurements were performed by using a thermogravimetric analyzer (Mettler-Toledo AG, Schwerzenbach, Switzerland). The thermal measurements were taken by heating a mass of 5 mg sample from 30 to 800 ℃ at a heating rate of 10 ℃/min under a nitrogen atmosphere with a gas flow of 10 mL/min.

Statistical analysis

A one-way analysis of variance (ANOVA) of SPSS software (SPSS version 17.0, SPSS Inc., Chicago, IL, USA) was used to analyze the results. The level of significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition of Rice Bran and Yield of Nanocellulose

Table 1 shows the chemical compositions and nanocellulose yield for all varieties; MR 219, MR 297, and MR 220. It shows that 70 to 80% of the rice bran composition was mainly lignocellulosic components, of which 16 to 28% were cellulose proportions. These results were supported by Gupte and Madamwar (1997), who stated that rice bran contains 34.0% cellulose, 28.2% hemicellulose, and 24.8% lignin. The data obtained in this study were also comparable with other by-products of paddy, like rice husk (Wang *et al.* 2016) and rice straw (Shawky *et al.* 2011). From Table 1, a higher cellulose and lignin content were observed in the DRB from MR 297 variety compared to the other two varieties. Meanwhile, the percentage of hemicellulose recorded by MR 219 was lower than MR 297

and MR 220. Variations in the chemical compositions of rice bran in this study were explained by the difference in climate, type of paddy, planting season, harvesting method, and geographical conditions (Agblevor *et al.* 2003; Chandrasekhar *et al.* 2003). Unlike the other two varieties, MR 297 was collected from the Central region of Malaysia during the off-season period (March-July). The Southwest Monsoon (May-September) in Malaysia, that occurs during this season has resulted in less rainfall compared to the Northeast Monsoon (October-March), which can be one of the explanations for this difference in the lignocellulosic composition of the paddy varieties.

Table 1 also shows that the nanocellulose was least isolated from the cellulose fibres of MR 220 variety, while MR 297 and MR 219 yielded a comparable amount of nanocellulose. These values were higher compared to the amount of nanocellulose reported using a similar method of hydrolysis (sulphuric acid) in the study conducted by Flauzino Neto *et al.* (2013) and Liu *et al.* (2016), where 20% and 34.5% of nanocellulose were reported, respectively. The different amounts of nanocellulose yielded might be due to the variations of amorphous and crystalline regions in each variety (Chen 2014). This has been influenced by the difference in lignocellulosic compositions of each variety of rice bran where higher cellulose content has more crystalline regions that greatly contributed to the highest yield of nanocellulose from MR 297 variety as shown in Table 1.

Table 1. Chemical Composition of Rice Bran and Yield of Nanocellulose from MR 219, MR 297, and MR 220

Sample	Cellulose* (%)	Hemicellulose* (%)	Lignin^p (%)	Others (Acid Soluble Lignin, Ash, and Silica)	Yield of Nanocellulose (%)
MR 219	21.31	21.66	23.72	32.30	56.66
MR 297	28.79	26.29	25.62	20.50	58.30
MR 220	16.01	31.58	24.31	28.10	47.28
* Based on 5 g of DRB; ^{p} Based on 1 g of DRB					

TEM and Particle Size Distributions

Figure 1 shows the TEM micrographs of nanocellulose resulted from the hydrolysis process using sulphuric acid. Highly diluted samples of hydrolyzed suspension were used to identify the length and diameter of the fibres. Figure 1(a) reveals the efficiency of acid hydrolysis treatment towards NC 219 by the formation of rod-like, short, and tiny fibres. Similar structures of nanocellulose were observed in rice husk (Johar *et al.* 2012), cotton linter (Morais *et al.* 2013), and corn cob (Liu *et al.* 2016) that were also hydrolyzed using sulphuric acid. The length and diameter of the nanocellulose from different varieties are summarized in Table 2. Indeed, it is verified from Table 2 that NC 219 has the shortest length among all the varieties, while its diameter showed a significant difference as compared to NC 297. Under controlled conditions, the prolonged acid attack is expected to cleave the amorphous region other than partly destroying the crystalline zones of the fibrils causing the reduction of the fibres' size from micron to the nanometer scale (Ghazy *et al.* 2016). Pretreatment and hydrolysis conditions were reported to influence the nanocellulose structure and dimensions (Ibrahim *et al.* 2015; Niu *et al.* 2017). The aspect ratio (*L/D*) can be used to determine the types of resulting nanocellulose as either cellulose nanocrystals (CNC) (width: 3 to 10 nm with *L/D* > 5) or cellulose nanofibers (NFC) (width: 5 to 30 nm - *L/D* > 50) (Mariano *et al.* 2014). It is interesting to note that NC 219 and NC 220 were classified as CNC while NC 297 was considered as NFC.
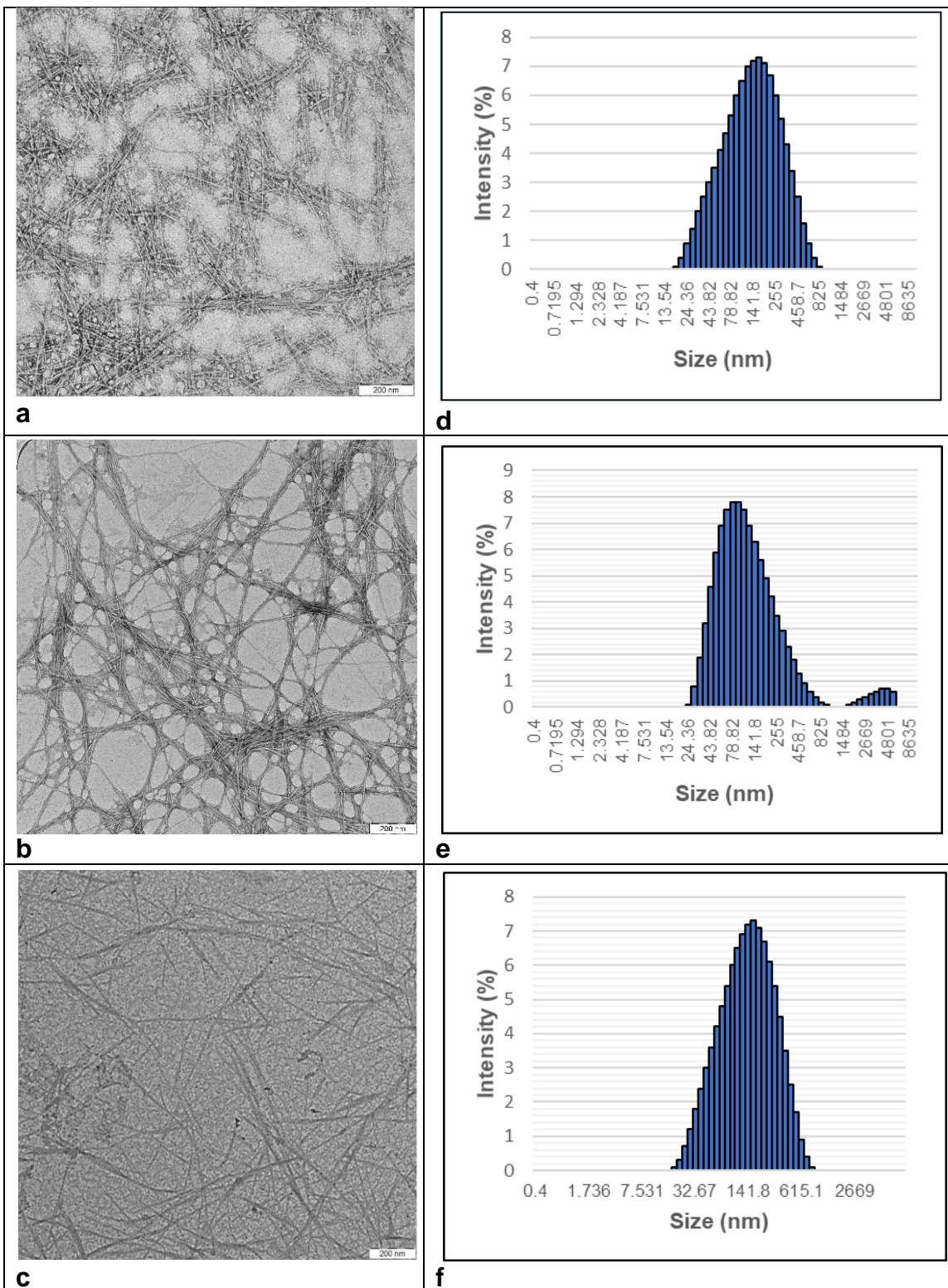
Fig. 1. TEM images of (a) NC 219, (b) NC 297, and (c) NC 220 corresponding to their particle size distributions; (d) NC 219, (e) NC 297, and (f) NC 220

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According to Salas *et al.* (2014), the type and shape of nanocellulose can influence its properties (stability, rheology, and optical characteristics), which largely contribute to its applications.

Table 2. Dimension, Aspect Ratio, and Polydispersity Index of the Isolated Nanocellulose

Sample	Length (nm)	Diameter (nm)	Aspect Ratio (L/D)	Polydispersity Index
NC 219	183 ± 22^b	4.70 ± 0.3^a	39	0.389 ± 0.04^a
NC 297	263 ± 75^a	4.89 ± 0.3^b	54	0.403 ± 0.07^a
NC 220	204 ± 38^b	4.97 ± 0.6^a	41	0.386 ± 0.08^a
All values are expressed as mean value (n = 3) ± SD, and different alphabets in the same column denote significant differences (P < 0.05)				

The intensity distributions particle size of three different varieties of rice bran is also shown in Fig. 1 (d), (e), and (f). Nanocellulose suspension of NC 220 and NC 219 exhibited a single peak with an average particle size value of 97.85 ± 3.16 nm and 100.00 ± 0.88 nm, respectively. Although MR 297 demonstrated the smallest average particle size (83.86 ± 4 nm), a formation of bimodal distribution with peaks between 24 to 955 nm and 1718 to 5560 nm was observed, representing two groups of particle size that were majorly present in this sample. Different results shown by TEM analysis and DLS method regarding the particle size of all varieties were mainly contributed by rod-like and nano-web structures of the CNC and NFC produced. According to Frone *et al.* (2011), the measurement method of DLS considers that all particles are spherical and the orientation of the fibres in suspension affects the size values. These were in agreement with several studies that reported in most cases a measurement obtained by DLS method was higher compared to the dimensions measured using the microscopy method (Morais *et al.* 2013; Shankar and Rhim 2016). In addition to the particle size distributions, the homogeneity of the nanocellulose isolated from all varieties was also supported by their polydispersity index (PDI) values, as shown in Table 2. It is stated that a lower PDI value would lead to a more uniform system, as defined in ISO 22412 (2017) standards. Smaller dimensions of nanocellulose with high and uniform homogeneity for NC 219 and NC 220 offer suitable applications as an emulsifier for the stabilization of solid particle-based emulsion (Xiao *et al.* 2016; Ngwabebhoh *et al.* 2018). On the contrary, the features of NFC shown by NC 297 contribute to its promising properties as rheology modifiers (Sun *et al.* 2016), and material in foams (Wang *et al.* 2019), coatings (Mazhari Mousavi *et al.* 2017) and films (Li *et al.* 2019).

FTIR Spectroscopy Analysis

The FTIR spectra recorded for untreated DRB and nanocellulose of each variety are depicted in Fig. 2. Different chemical treatments that were introduced to produce nanocellulose from untreated DRB were expected to cause changes in their chemical functionality. According to Aprilia *et al.* (2018), the functional group of cellulose could be determined from several spectra at 3247 cm^{-1} (C-H), 1369 cm^{-1} (C-H), 1055 cm^{-1}, and 898 cm^{-1} (C-H). Nanocellulose of all varieties showed similar spectra signifying the similarities of chemical compositions present in each variety. Comparable results were obtained by Musa *et al.* (2017) and Moreno *et al.* (2018). The wide absorption spectrum observed in the region of 3300 to 3500 cm^{-1} for all samples was characterized for the free O-H

stretching intramolecular hydrogen bonds of hydroxyl groups in the molecules of cellulose. This peak became narrower as the nanocellulose was extracted from the untreated DRB, indicating the isolation of more cellulose components after all the chemo-mechanical treatments were subjected. The occurrence of the spectrum between 2850 and 2970 cm^{-1} and the peak at 1430 cm^{-1} showed the characteristics of C-H stretching vibration and -CH$_2$ groups bending, respectively (Evans *et al.* 2019), while the peak around 1640 cm^{-1} in all spectra was associated with the adsorbed water (Danial *et al.* 2020). The spectrum at 1730 cm^{-1} was attributed to the C-O stretching vibration of the acetyl and uronic ester groups contain in hemicellulose or ester linkage of carboxylic groups existing in ferulic and p-coumaric acids in lignin (Mou *et al.* 2014). Complete removal of these peaks in spectra of nanocellulose of all varieties signifies a complete removal of hemicellulose and lignin during the alkaline treatment, which was similar to the study reported by Moreno *et al.* (2018).
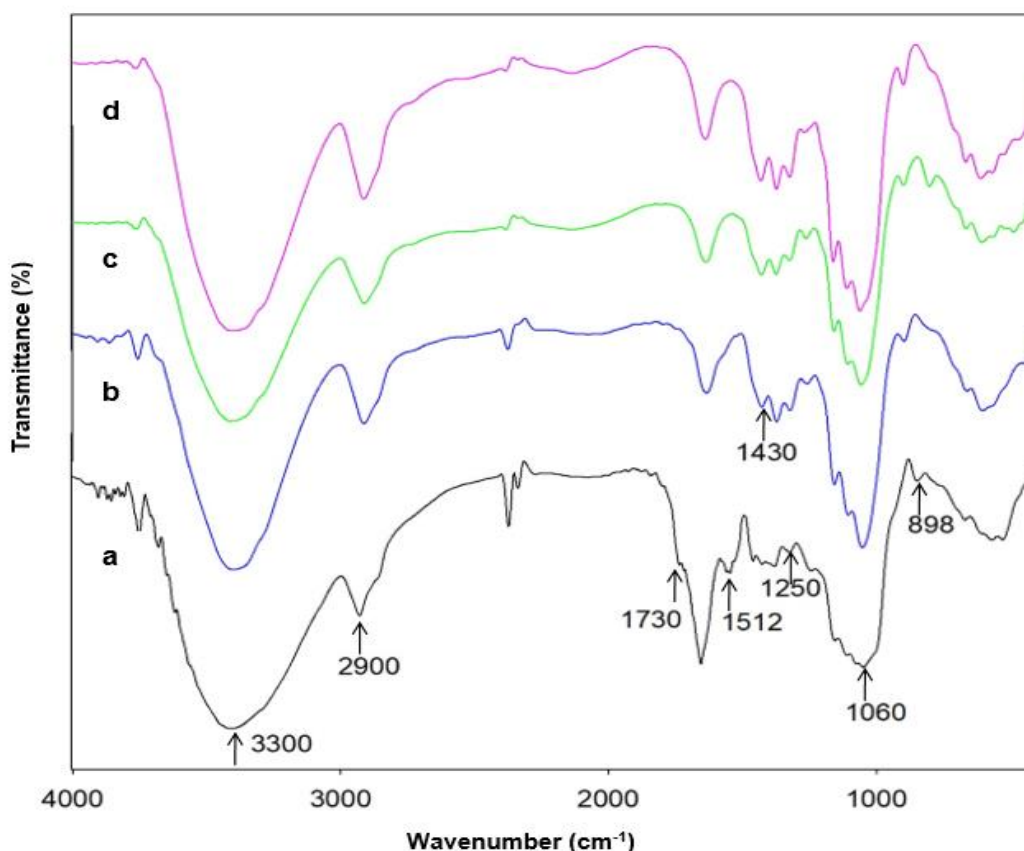

Fig. 2. FTIR spectra of (a) untreated DRB, (b) NC 219, (c) NC 297, and (d) NC 220

Peaks at 1512 and 1250 cm^{-1} were contributed by the aromatic ring and stretching of C-O bond out of plane caused by the presence of aryl groups in lignin (Ghazy *et al.* 2016). The intensity reduction of these peaks from untreated DRB to nanocellulose confirmed the removal of lignin during the bleaching process. Furthermore, the peak at 1060 cm^{-1} that showed higher intensity in nanocellulose compared to untreated DRB was ascribed to the C-O stretching and the C-H rocking vibrations consisted in the cellulose fibres (Kaur *et al.* 2018). Another spectrum was observed at 898 cm^{-1} corresponding to the symmetric C-O-C stretching of β-glycosidic linkages between glucose units in cellulose

(Kian *et al.* 2018). Both peaks gradually increased in the isolated nanocellulose for all varieties, confirming the success in isolating nanocellulose from untreated DRB. Complete isolation of nanocellulose from the non-cellulosic components is important to ensure its quality for further applications.

XRD Measurements

Cellulose is widely known to contain both a crystalline and amorphous part (Chen 2014). Figure 3 shows the X-ray diffraction patterns of the nanocellulose from different varieties. The diffractograms of all varieties showed more intense peaks at 22.3° compared to the untreated DRB, signifying the increase in crystallinity of the nanocellulose due to the complete removal of hemicellulose and lignin. Meanwhile, a peak at 16° that was observed in all varieties corresponds to the amorphous regions of the samples. Both aforementioned peaks at 22.3° and 16° that reflect on the crystallographic planes of (200) and (110) respectively, suggested cellulose I structure due to the absence of doublet peaks at 22.3° (Mohamad Haafiz *et al.* 2014; Danial *et al.* 2020).
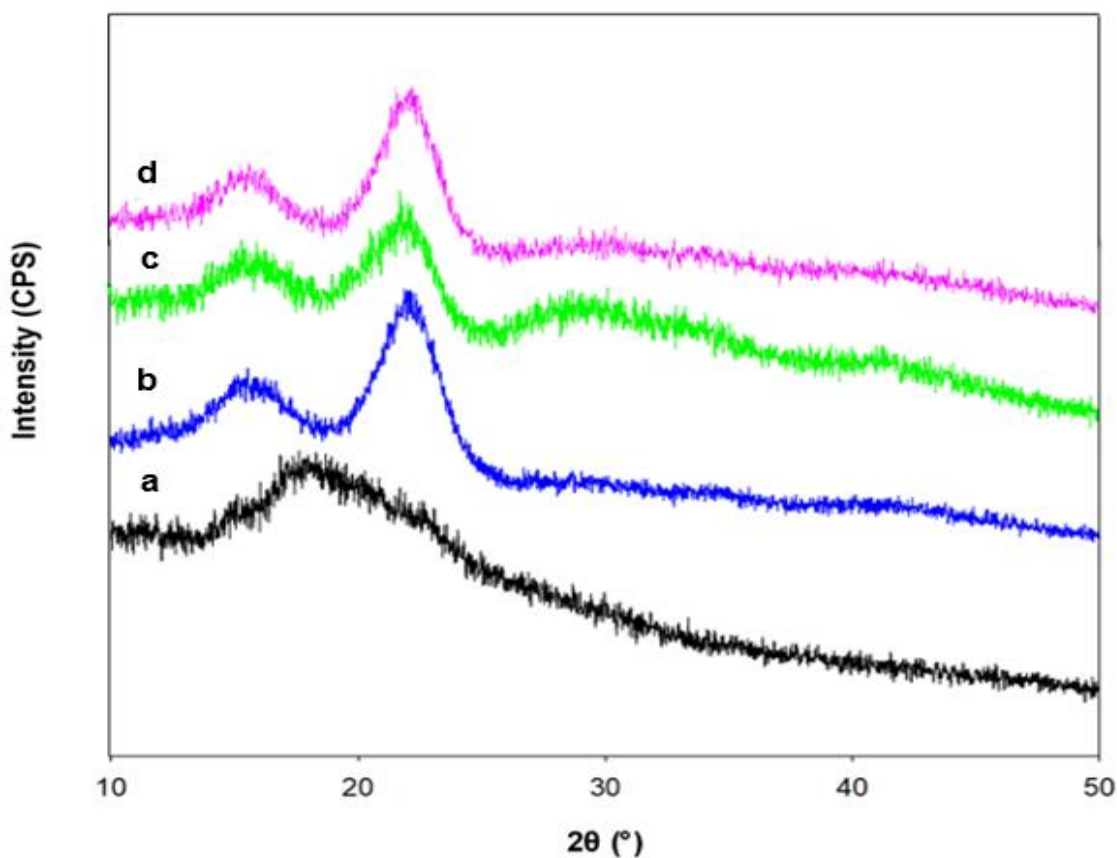

Fig. 3. X-ray diffractogram of (a) untreated DRB, (b) NC 219, (c) NC 297, and (d) NC 220

An increase in the crystallinity of all nanocellulose samples compared to the untreated DRB was observed in Table 3. NC 219 showed the highest crystallinity with 50.1%, which was demonstrated by slightly sharper diffraction at 22.3° compared to other samples as illustrated in Fig. 3. This is attributed to the success in eliminating amorphous regions in cellulose, led by the hydrolytic cleavage of glycosidic bonds, finally causing a rearrangement of the crystalline order and releasing individual crystallites (Spagnol *et al.* 2012; Santmartí and Lee 2018).

Table 3. Crystallinity of Nanocellulose from All Varieties

Sample	Crystallinity (%)
Untreated DRB	29.06
NC 219	50.10
NC 297	36.67
NC 220	47.10

Nanocellulose produced from rice bran in this study demonstrated a higher crystallinity value compared to the findings reported by Arun *et al.* (2020), who recorded a crystallinity index of 45.20% after the chemical treatment. Lu *et al.* (2018) isolated nanofibers from bamboo chips using chemical and mechanical refining with sulphuric acid and reported about 52.70% of crystallinity. However, Agustin *et al.* (2014) reported a higher crystallinity index (76.1%) when nanocellulose was isolated from rice straw, while a comparable crystallinity index with a value of 56.1% was recorded by Razali *et al.* (2021). According to Park *et al.* (2010) and Nagalakshmaiah *et al.* (2019), the variations in the crystallinity of nanocellulose can be explained by the differences in the measurement method and raw materials used, respectively. According to Rosa *et al.* (2012), the increment in crystallinity can improve the mechanical properties of the biocomposite by increasing its rigidity and contribute to a higher tensile strength of the fibres. It is noteworthy that the increase in the crystallinity index of NC 219 and NC 220, in addition to the smaller dimensions observed in the TEM analysis, contributed to the excellent properties of CNC structures of these two varieties compared to the NFC structure shown by NC 297, which has lower crystallinity index as reported by Mu *et al.* (2019).

Thermogravimetric Analysis

The thermal stability for nanocellulose from all varieties that were investigated under a nitrogen atmosphere is shown by thermogravimetric analysis (TGA) and derivative thermogram (DTG) curves in Fig. 4. All TGA curves displayed weight loss in the range of 30 to 100 °C due to the evaporation of moisture (Radakisnin *et al.* 2020). In untreated DRB, the presence of hemicellulose, lignin, and pectin together with cellulose was observed based on the multiple stage decompositions process that occurred at around 200 °C (hemicellulose and some of the major lignin) and 300 °C (cellulose) (Rosli *et al.* 2013; Kaur *et al.* 2018). Similar TGA curves were observed in the untreated walnut shell (Zheng *et al.* 2019) and sugarcane bagasse (Evans *et al.* 2019). Meanwhile, weight loss observed at a temperature between 415 and 436 °C resulted from the presence of lignin and hemicellulose in the untreated DRB, as reported by Shahi *et al.* (2020).

In contrast, the TGA curves of nanocellulose from all varieties revealed a one-stage decomposition process, indicating complete removal of non-cellulosic materials during the chemical and hydrolysis steps. This explains the increment of the onset temperature (T_{on}) and maximum degradation temperature (T_{max}) of the nanocellulose samples (NC 219, NC 297, and NC 220) compared to the untreated DRB, as shown in Table 4. According to Rosli *et al.* (2013), the improvement in thermal stability could be the effect of increasing crystallinity, which enhances the heat resistance of the nanocellulose produced. Additionally, it is noteworthy that a lower $T_{10\%}$, T_{on}, and T_{max} were observed for NC 219 compared to NC 220 and NC 297. This could be well understood as a reason for the smaller fibre dimensions of NC 219 as illustrated previously by the TEM image. Small dimensions of nanocellulose fibre indeed contribute to the increase in the surface areas that were exposed by heat, leading to partial disruptions of the crystal structure of cellulose (Liu *et*

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al. 2007). This allows the materials to decompose at a lower temperature compared to other samples, which had been reported earlier by Liu *et al.* (2016).
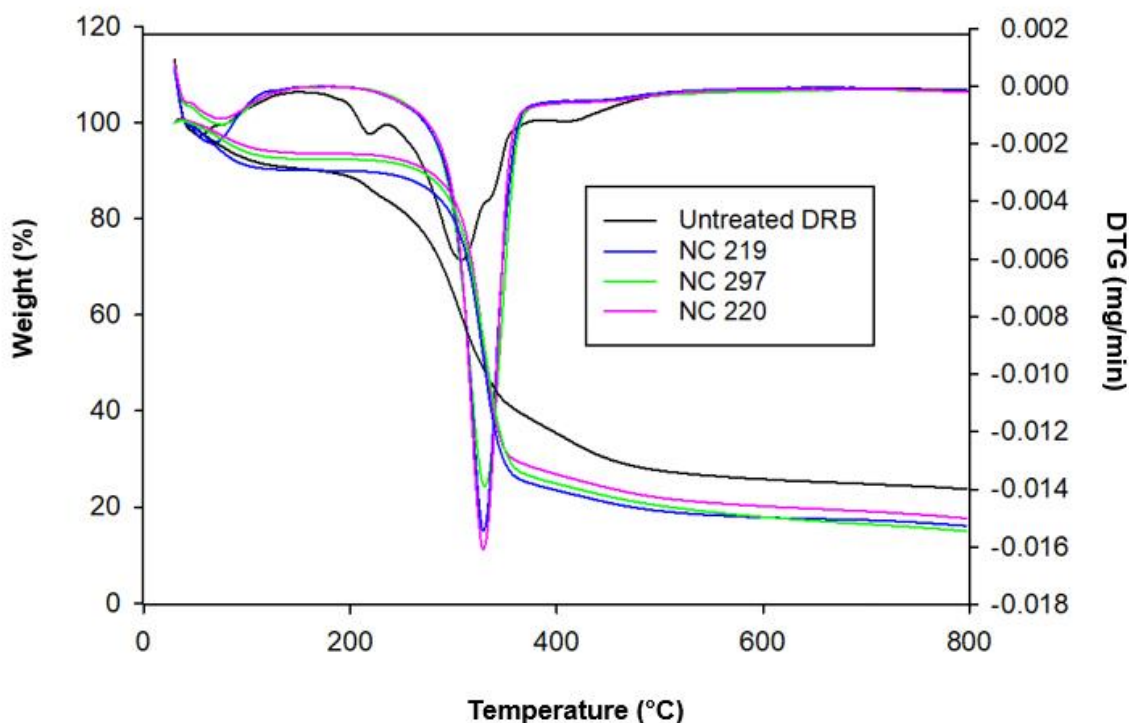

Fig. 4. TGA and DTG curves of untreated DRB, NC 219, NC 297, and NC 220

The higher char residue recorded for untreated DRB at 800 °C corresponds to the presence of lignin that might not be completely burned due to a slow degradation rate (Johar *et al.* 2012; Evans *et al.* 2019). A reduction in residual amounts of all nanocellulose samples confirms the effectiveness of pretreatment and hydrolysis steps in removing the non-cellulosic materials from the isolated nanofibers. Additionally, it is worth noting that NC 297 has the lowest char residue followed by NC 219 and NC 220.

Table 4. Thermal Properties of Untreated DRB, NC 219, NC 297, and NC 220

Samples	Degradation Temperature (°C) $T_{10\%}$	Onset Temperature (°C) T_{on}	DTG Peak Temperature (°C) T_{max}	Residual Weight % at 800 °C
Untreated DRB	171	203	312	23.77
NC 219	195	306	328	16.08
NC 297	265	307	329	14.97
NC 220	273	310	328	17.63

A slight increment of residual mass in NC 219 and NC 220 could have resulted from two factors: 1) the presence of a flame retardant material, such as sulphate groups, on the surface of nanocellulose that act as a barrier in protecting both mass and energy from the burning materials to the attached polymeric chains (Mohamad Haafiz *et al.* 2013); or 2) a smaller particle size that contributes to the exposure of a large number of free end chains that cause decompositions at a lower temperature (Fortunati *et al.* 2016). The

findings in this study suggested that the thermal stability of nanocellulose from all varieties can be assumed to have high thermal stability, which is consistent with several nanocellulose from various agricultural sources (Kaur *et al.* 2018; Evans *et al.* 2019; Nie *et al.* 2019).

CONCLUSIONS

1. Nanosize cellulose was successfully isolated from three varieties of defatted rice bran (DRB) using a combination of sulphuric acid hydrolysis and ultrasonic treatment. The DRB from MR 297 showed the highest cellulose composition (28.79%) compared to the other two varieties indicating its potential to be an excellent source of nanocellulose. This was also demonstrated by its high nanocellulose yield.

2. Small aspect ratio (*L/D*) found in NC 219 (39) and NC 220 (41) variety were observed through TEM confirmed their cellulose nanocrystal (CNC) structure, whereas NC 297 exhibited a nanofibrillated cellulose (NFC) structure. Morphological investigation and infrared spectroscopy analyses confirmed the isolation of nanocellulose from the lignocellulosic materials.

3. All varieties showed equivalent thermal stability, whereas a lower polydispersity index (PDI) value indicated their uniform nanocellulose suspension.

4. A higher percentage of crystallinity index was shown by NC 219 and NC 220 in comparison to NC 297, verifying the removal of a large portion of amorphous region and isolating the individual crystallites.

5. The overall results showed that DRB has a promising potential to exploit the solid particle-based emulsions and is also a suitable material to be used in foam, coating, and film.

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