## Feasibility Study and Structural Analysis of Cellulose Isolated from Rice Husk: Microwave Irradiation, Optimization, and Treatment Process Scheme

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The goal of this study was to pretreat rice husk (RH) using a microwaveassisted pretreatment process coupled with chlorite delignification and alkaline treatment to facilitate the isolation of cellulose. The isolated cellulose was characterized and subjected to structural analysis and a thermal stability test to ascertain the efficiency of the isolation from a visual perspective. The optimum condition for the microwave-assisted pretreatment of RH was determined by response surface methodology (RSM). The effects of three independent variables—microwave power. irradiation time, and solvent ratio-were investigated based on the maximum content of the RH being pretreated. At the optimum parameters of microwave power of 400 w, a 10-min duration, and a solvent ratio of 80.0% v/v, the pretreatment efficiency of RH was 10.0%. Compared with the conventional Soxhlet technique, the microwave pretreatment was superior. The X-ray powder diffraction (PXRD) result for the isolated cellulose showed that cellulose was highly crystalline (Crl = 65.0%). Fourier transform infrared spectroscopy (FT-IR) verified that most of the lignin and hemicelluloses were removed from the isolated cellulose after the chemical treatment. Furthermore, the TGA study revealed that the thermal stability of RH cellulose was higher than the original RH.

Keywords: Rice husk; Cellulose; Microwave-assisted pretreatment; RSM; Pretreatment mechanism; Structural analysis

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## INTRODUCTION

With increasing environmental awareness, lignocellulosic materials and industrial solid wastes with high cellulose content have gained considerable attention (Mondragon *et al.* 2014). Lignocellulosic fibers have many environmental advantages due to their abundance, low price, renewable nature, and low energy consumption in production. The main components of lignocellulosic fibers with complex structures are hemicellulose, cellulose, and lignin (Reddy and Yang 2005). These waste materials pose a serious environmental issue if not utilized, and thus efficient utilization of these agricultural by-products would critically reduce the environmental impact while also generating substantial benefit (Maheswari *et al.* 2012).

Cellulose is the most abundant renewable natural material in the biosphere, and it is extensively dispersed among higher plants, in some marine animals, and to a small extent in fungi, algae, and bacteria (Habibi *et al.* 2010). It is a polysaccharide made of D-glucose connected *via*  $\beta$ -1,4-glycosidic bonds. Due to its intrinsic nature, cellulose has been used to produce a wide spectrum of chemicals, including cellulosic ethanol and hydrocarbons; it is also used as a starting material for the production of polymers. Cellulose derivatives

have been well-received globally, which can be attributed to its biodegradability, plenitude, and minimum environmental effect (Das *et al.* 2014).

Rice husk (RH) is a waste product generated from the rice milling industry. The composition of RH is 53.0% cellulose, 20.0% lignin, 4.60% hemicellulose, and 13.9% silica/ash. RH is normally burnt as a crude source of energy (Munaf and Zein 1997; Jansen *et al.* 2004), though it has also been used as bio-fertilizer, absorbent material, and pest control agent (Battegazzore *et al.* 2014). This biomass source could be explored more extensively in view of its promising composition and advantageous features such as low density, renewability, and reasonable strength and stiffness (Shukla *et al.* 2013).

Microwave power has been explored as a potential pretreatment method to improve different lignocellulosic by-products (Monteil-Rivera *et al.* 2012). Microwave irradiation is preferred to conventional heating due to its impressive acceleration of various processes. The effective internal heating generated by microwave power is faster than conventional heating, thereby overcoming slow and ineffective energy transfer associated with the conventional systems (Kappe 2008). Due to their electromagnetic nature, microwaves hold magnetic and electric fields that are perpendicular to each other. The electric field leads to heating through two concurrent mechanisms, specifically, dipolar rotation and ionic conduction. Dipolar rotation occurs *via* the alignment of a molecular electric field having a dipole moment, which can be permanent or even induced by the electric field in both the solid samples and the solvent. This oscillation causes collisions with nearby molecules, liberating thermal energy into the sample medium. Unlike conventional techniques, microwaves heat the sample simultaneously and uniformly. In cellulose isolation processes, the advantage of the microwave method is the disruption of weak hydrogen bonds, which is promoted by dipole rotation (Kaufmann and Christen 2002).

Today, opportunities to expand the usage of natural resources have attracted increasing attention due to their availability, biodegradability, non-toxicity, and low cost. There are numerous studies concerned with the extraction of different products using microwave-assisted extraction, such as extraction of piperine from coarsely powdered black pepper (*Piper nigrum*) (Raman and Gaikar 2002), extraction of lignin from triticale straw (Monteil-Rivera et al. 2012), extraction of methylmercury from aquatic sediments (Vazquez et al. 1997), and extraction of terpenes from caraway seeds (Chemat et al. 2005). Also there are studies about the micro and nanocrystalline cellulose isolation using microwave (Kos et al. 2014; Kushnir et al. 2015; Kusumattaqiin and Chonkaew 2015). The word "isolation" will be used here to denote procedures in which a raw material is essentially solubilized or broken down, leaving behind the sought component, such as the cellulose. This is the first time microwave as a convenient heating method was used to pretreat the RH. The process was optimized using CCRD in RSM, and the effect of variables governing the microwave efficiency was studied for the first time. Results were compared with the conventional method based on Soxhlet extraction, which is a very common technique to pre-treat lignocellulosic biomass. Therefore, this study can be significant and add value for further research as to use the fast, simple, and cost-effective technique for pre-treatment and isolation of a desired product.

This study provides an illustration of the experimental technique of microwaveassisted pretreatment of RH using response surface methodology (RSM) to investigate the influence of microwave power, irradiation time, and solvent ratio on the pretreatment efficiency. In addition to regression analysis, cellulose was prepared *via* chemical methods, then characterization and structural studies were carried out.

## EXPERIMENTAL

### Materials

Rice husk was collected from Sekinchan, Selangor, Malaysia, and supplied by the Ng Trading Company (Selangor, Malaysia). All chemical reagents were of analytical grade and used without purification, including ethanol (99.7%, R & M Chemicals, Essex, UK); sodium chlorite (NaClO<sub>2</sub>, 80.0%, Fluka, St. Louis, MO, USA); acetic acid glacial (CH<sub>3</sub>COOH, 100%, Merck, Darmstadt, Germany); NaOH (99.0%, Merck), H<sub>2</sub>O<sub>2</sub> (30%, Merck), HNO<sub>3</sub> (70.0%, Sigma-Aldrich, St. Louis, MO, USA), and HCl (37.0%, Sigma-Aldrich). The solutions were freshly prepared using distilled water. All glassware was washed with a solution of HNO<sub>3</sub>/HCl (3:1, v/v) and then rinsed with distilled water.

## Chemical composition of rice husk

The chemical composition of the rice husk was measured according to the standard method of Association of Official Analytical Chemists (AOAC) (Moongngarm and Saetung 2010). Table 1 shows the measured composition of RH and some potential lignocellulosic biomass, which were used in the isolation of cellulose. The chemical compositions of the crop residues are different; RH is one of the most available lignocellulosic biomasses and has higher cellulose content (53.2  $\pm$  0.4 wt.%) compared with other byproducts. Thus, it is a good source for cellulose isolation.

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Silica/Ash (%)	Reference
Rice husk	53.2 ± 0.4	$4.6 \pm 0.6$	19.7 ± 0.3	13.9± 0.6	Current study
Rice straw	$36.0 \pm 0.9$	17.0 ± 0.5	$23.0 \pm 0.6$	-	(Zhu <i>et al.</i> 2006)
Sugarcane bagasse	41.0 ± 0.1	30.1 ± 0.7	21.2 ± 0.2	0.7± 0.3	(Yoon <i>et al.</i> 2012)
Wheat straw	$48.8 \pm 0.30$	27.70 ± 0.12	8.17 ± 0.9	6.68± 0.01	(Saha <i>et al.</i> 2005)
Pineapple leaf	36.3 ± 3.8	22.9 ± 2.0	27.53 ± 0.11	2.85± 0.24	(Dos Santos <i>et al.</i> 2013)

## Table 1. Chemical Composition of Lignocellulosic Biomass

## **Microwave-Assisted Pretreatment**

The rice husk (RH) sample was washed 4 to 5 times with warm water to remove all dirt and aqueous soluble compounds before being dried in an oven at 60 °C for 24 h. The dry, clean material was ground to 60-mesh particle size. RH (5 g) was placed in closed capped bottles together with 100 mL of a solvent containing ethanol/water in different solvent ratios; the microwave power and irradiation time according to the RSM design. Various microwave irradiations (20 runs) with cooling at 30-s intervals between them were used to pretreat the RH; the suspension was then filtered, washed, and dried in an oven at 70 °C for 24 h. The dewaxed powder was delignified with 1.4% NaClO<sub>2</sub> (175 mL) at pH 4.50, adjusted with acetic acid at 70 °C for 5 h. The delignified powder was filtered from the light yellow suspension and washed with distilled water until the filtrate became neutral. It was then dried at 60 °C for 24 h. Silica and hemicellulose in the delignified powder were removed with 100 mL of 5.0% NaOH at room temperature for 24 h, and the powder was then heated to 90 °C for another 2 h. The base treated sample was further bleached at 55 °C for 3 h with 100 mL of 5.0% H<sub>2</sub>O<sub>2</sub>, with the pH adjusted to 11 by NaOH. The white cellulose powder was filtered from the suspension and washed with an adequate amount of distilled water until the filtrate became neutral. A schematic illustration of cellulose isolation is shown in Fig. 1.



Fig. 1. Schematic illustration of cellulose extraction from RH

## **Experimental Design**

A central composite rotatable design (CCRD) was applied to obtain the optimum condition for the microwave-assisted pretreatment of RH. The optimization studies were carried out by investigating three independent variables (k) including microwave power, irradiation time, and solvent ratio. The variables were examined at five levels, namely the factorial points levels (+1 and -1), the replication point level (0) that was situated in the cubic design center, and the axial points that were placed at the outer edge of the cubic design relating to the  $\alpha$  values (±1. 68) for each factors. The independent factors are expressed in Eq. 1,

$$x_j = \frac{\tilde{X}_j - \tilde{X}_{j0}}{i_j} \tag{1}$$

where  $x_j$  is attributed to the coded value of the factor,  $\tilde{X}_j$  and  $\tilde{X}_{j0}$  are the natural value of the factor and the basic level, respectively,  $i_j$  is the interval of variation and j = 1, 2, or 3 (the number of the factor).

The ranges and levels of the variables studied are shown in Table 2. The percentage of pretreatment (Y) was considered as the response of the experimental design. The response could be explained as second-order polynomial equation, according to Eq. 2,

$$Y = \beta_0 + \sum_{i=1}^2 \beta_i x_i + \sum_{i=1}^2 \beta_{ii} x_i^2 + \sum_{i=1}^2 \sum_{j=2}^3 \beta_{ij} x_i x_j + \varepsilon$$
(2)

where *Y* is the predicted response,  $\beta_0$  is the constant term,  $\beta_i$  is the coefficient of the linear effects,  $x_i$  is the variable,  $\beta_{ii}$  is the squared effect,  $\beta_{ij}$  is the coefficient of the interaction effects, and  $\varepsilon$  is the residual attributed to the experiments.

The design of the experiment was carried out with Design of Expert software (version 7.0.0, StatEase, Minneapolis, USA). The variables of the design, which are coded, and actual values were evaluated by the software based on the values of k and  $\alpha$ . Table 3

summarizes the values of each variable used for the experimental design of RH pretreatment.

Symbol	Indonendent Verichlee		Co	els		
	independent variables	-α	-1	0	1	α
A	Microwave power (w)	331.82	400	500	600	668.18
В	Irradiation time (min)	3.30	5	7.5	10	11.70
С	Solvent ratio (v/v)	53.18	60	70	80	86.82

	Table 2.	The	Coded	Independent	Factors	Applied in	n the	RSM	Design
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**Table 3.** Experimental and Predicted Values of RH Pretreatment Based on

 Central Composite Rotatable Design (CCRD)

_	Irradiation Time	Microwave	Solvent Ratio	RH Pretreatment (%)		
Run	(min)	Power (w)	(v/v)	Exp.	Pre.	
1	5	600	80	8.45	8.63	
2	7.5	500	70	7.81	8.13	
3	7.5	500	70	8.106	8.13	
4	7.5	500	53.18	7.81	7.95	
5	11.7	500	70	8.97	8.80	
6	7.5	500	70	8.01	8.13	
7	7.5	500	70	8.35	8.13	
8	7.5	500	70	8.48	8.13	
9	10	400	60	9.79	9.61	
10	5	400	80	9.53	9.37	
11	7.5	668.18	70	7.91	7.80	
12	7.5	331.82	70	8.21	8.46	
13	3.3	500	70	7.82	7.45	
14	7.5	500	70	7.82	8.13	
15	7.5	500	86.82	8.21	8.30	
16	5	600	60	8.50	8.48	
17	10	600	60	9.41	9.57	
18	7.5	300	70	8.51	8.52	
19	9	500	70	8.67	8.37	
20	6	500	70	7.61	7.89	

#### **Characterization Methods and Instruments**

The pretreatment of RH was performed using a household microwave oven (Samsung, ME711K, Korea). Scanning electron microscopy (SEM) was used to study the morphology of agricultural biomass and isolated cellulose. SEM was carried out with a Quanta<sup>™</sup> 450 FEG, Oxford instruments (USA). Fourier transform infrared spectroscopy (FT-IR) in the range between 400 to 4000 cm<sup>-1</sup> was utilized to investigate the structures of the agricultural residue before and after cellulose isolation. The FT-IR spectra were monitored using a Spectrum 400 FT-IR/FT-FIR spectrometer (Perkin Elmer, Waltham, MA, USA); Thermal gravimetric analysis (TGA) was used to study the thermal behavior of samples. The TGA was acquired with a Pyris Diamond TG/DTA (PerkinElmer). Powder

X-ray diffraction (PXRD) with Cu K $\alpha$  radiation was applied to determine the crystallinity of cellulose. PXRD was carried out using a D8 Advance X-ray diffractometer (Bruker AXS, Billerica, MA, USA). Crystallinity index (*CrI*) of the RH and RH cellulose samples was measured using Eq. 3 (Lu and Hsieh 2012),

$$CrI(\%) = \frac{I_{200} - I_{am}}{I_{200}} \times 100$$
(3)

where  $I_{200}$  is the maximum intensity of the crystalline portion in RH at  $2\theta = 22.7^{\circ}$  and  $I_{am}$  is the minimum intensity related to the amorphous region of RH, which is between 200 and 110 at  $2\theta = \sim 18.6^{\circ}$ .

## **RESULTS AND DISCUSSION**

### Model Fitting and Analysis of the Variance (ANOVA)

Table 4 presents the results of the 2FI model for the pretreatment of RH based on ANOVA. The Prob > F of the model was much smaller than 0.05, which shows that the terms of the model such as A, B, C, AB, AC, and BC were significant and influenced the pretreatment of RH. The linear term of irradiation time had the most significant effect with an F-value of 32.12. Microwave power had an F-value of 10.33, but the solvent ratio showed no significant effect.

The  $R^2$  values in Table 4 imply that the model had good estimation capability of more than 91.81% of the variability in the responses. The  $R^2$  values in the regression model were comparatively high, with agreement between predicted and experimental. Furthermore, the adjusted  $R^2$  of 0.8740 was in reasonable agreement with the predicted  $R^2$ , further confirming the predictability of the model. Based on ANOVA, the coefficient of variation (CV) at the low value of 2.85 verified the reliability, reproducibility, and accuracy of the model.

To ensure the accuracy and reproducibility of experiments, repetition experiments were performed. All 20 runs were carried out in triplicate, and standard deviations were found to be less than 0.9%. The obtained consistent results indicated that the experiment was significant with the model based on the  $R^2$  value. The lack of fit F-value of 0.63 confirmed that it was not significant attributing to pure error. There was a 73.18% chance to have the lack of fit F-value at this level because of the presence of noise for pretreatment of RH. A non-significant lack of fit was observed, which is desired for the fitting of the model.

The application of the RSM based on the measured variables demonstrated an empirical relationship between the independent variables and response, which can be expressed by Eq. 4,

$$Pretreatment\% = 8.08 - 0.20A + 0.40B + 0.10C - 0.96AB - 1.14AC - 1.10BC + 0.11B^{2}$$
(4)

where A, B, and C are the independent variables of microwave power (watts), irradiation time (min), and solvent ratio (%vol.), respectively.

		etreatment of I	eatment of RH			
Source	Sum of Squares	<sup>a</sup> DF	Mean Square	<i>F</i> -value	<i>P</i> -value Prob> <i>F</i>	
Model	8.51	7	1.22	20.82	<0.0001	
A-Microwave power	0.60	1	0.60	10.33	0.0068	
<b>B-Irradiation time</b>	1.88	1	1.88	32.12	< 0.0001	
C-Solvent ratio	0.12	1	0.12	2.05	0.1758	
AB	2.71	1	2.71	46.35	< 0.0001	
AC	3.78	1	3.78	64.75	< 0.0001	
BC	3.57	1	3.57	61.10	< 0.0001	
B <sup>2</sup>	0.17	1	0.17	2.96	0.1092	
Residual	0.76	13	0.058	-	-	
Lack of fit	0.38	8	0.048	0.63	0.7318	
Pure error	0.38	5	0.075	-	-	
	Pre	treatment of	RH			
Standard deviation				0.24		
pCA			2.85			
$R^2$			0.9181			
Adjusted R <sup>2</sup>			0.8740			
Predicted R <sup>2</sup>			0.7434			
Adequate Pre		14.554				

#### Table 4. Analysis of Variance for the Pretreatment of RH

<sup>a</sup> Degree of Freedom

<sup>b</sup> Coefficient of Variation

#### **Three Dimensional Plots of Response Surface**

Figure 2 contains 3-D surface plots of interactions between microwave power, irradiation time, and solvent ratio. Microwave power and irradiation time had the most significant effect on the pretreatment of RH. Figure 2a indicates the influence of microwave power and irradiation time on the pretreatment efficiency. When both the microwave power and irradiation time were increased, the pretreatment was enhanced due to the microwave heating mechanism, as previously discussed (Monteil-Rivera *et al.* 2012).

The effect of time and solvent ratio is shown in Fig. 2b. As the solvent ratio increased, the pretreatment efficiency also increased. This result reflects that the chosen solvent has a high dielectric constant and strongly absorbs microwave irradiation, though the selectivity of the isolation and the medium interaction with microwave irradiation could be adjusted by manipulating the solvent mixture (Kaufmann and Christen 2002).

As previously mentioned, the percentage of pretreatment increased with an increase in the irradiation time, as this allowed greater microwave interaction with the solvent. This phenomenon was more obvious for polar solvents, whereby localized heating promoted by the increasing time caused the cell walls of RH to expand and fracture. As a result, oils, waxes, and pigment were released into the solvent (Kaufmann and Christen 2002). The maximum irradiation time of 10.0 min corresponded to a pretreatment efficiency of 9.79% when the irradiation time decreased to 3.3 min (Fig. 2b and Table 3). The pretreatment yield declined to 7.82%, which implied that shorter irradiation time might not be sufficient for the microwaves and solvent to interact fully during RH pretreatment.



**Fig. 2.** Response surface 3-D plot showing the effect of irradiation time and microwave power (a) or solvent ratio and irradiation time (b) on RH pretreatment

#### **Optimization and Validation of the Model**

Having elucidated the effect of variables consisting of microwave power, irradiation time and solvent ratio, the experiment was verified under the optimized conditions of microwave power of 400 w, irradiation time of 10.0 min, and solvent ratio of 80.0%. These conditions gave a 10.0% pretreatment of RH, signifying the validity of the model as the measured value is close to the predicted value of 9.89% (Table 5). Table 5 shows the verification of the model with six sets of validation parameters. The experimental values are comparable with predicted values, confirming the efficiency and the significance of the model.

	Independent Variables		Pret	Pretreatment of RH %		
	Microwave power (w)	Irradiation time (min)	Solvent ratio (v/v)	<sup>a</sup> Exp.	<sup>b</sup> Pre.	°RSE(%)
	550	7.5	70	8.12	7.97	1.88
	450	7.5	70	8.32	8.18	1.71
Validation	300	7.5	70	8.70	8.48	2.60
Set	500	9.0	70	8.93	8.36	1.55
	500	8.0	70	8.11	8.16	0.61
	500	7.5	75	8.20	8.13	0.86
Optimum Condition	400	10	80	10	9.89	1.10

## **Table 5.** Experimental and Predicted Values for the Model in Optimum Conditions

<sup>a</sup>Experimental

<sup>b</sup>Predicted

°Relative Standard Error

# Comparison of the Microwave-Assisted Pretreatment with Conventional Method and the Proposed Mechanism

A conventional Soxhlet method is an extraction technique that is often used for the pre-treatment of lignocellulosic biomass. It is an effective reference for comparing other

conventional and new methods. Among the techniques used for pre-treatment, Soxhlet extraction has been used for a long time. This assertion is supported by the fact that the Soxhlet method has been a standard technique for more than one century and, at present it is the main reference to which the performance of other methods is compared. Conventional Soxhlet extraction was originally used for the determination of fat in milk (Soxhlet 1879), the sample was placed in a thimble-holder, and during operation gradually filled with condensed fresh solvent from a distillation flask. When the liquid reached the overflow level, a siphon aspirated the solute of the thimble-holder and unloaded it back into the distillation flask, carrying the extracted analytes into the bulk liquid. This operation was repeated until complete extraction is achieved. This performance makes Soxhlet a hybrid continuous-discontinuous technique.

The microwave pretreatment in this study was found to be clearly superior to Soxhlet pretreatment (Table 6). Microwave pretreatment requires lower volumes of organic solvents than the Soxhlet method; moreover, the total sample processing time was reduced to no more than 10.0 min with the ability to perform simultaneous extraction in up to 12.0 samples (Vazquez *et al.* 1997). In comparison, Soxhlet extraction is performed with only one sample at a time and is time consuming, involving manual techniques and high volume of solvent for extraction. The most significant drawbacks of Soxhlet extraction, as compared to the other conventional techniques for solid sample preparation are, long extraction time and large consumption of solvent, which is not only expensive to be disposed but itself could have caused additional environmental problems. Samples are usually extracted at the boiling point of the solvent involved for a long period of time and which may incur thermal decomposition of a compounds. Moreover, conventional Soxhlet device is unable to provide agitation to accelerate the process. Due to the large amount of solvent used, an evaporation step after the extraction is mandatory. In addition, the technique is restricted to solvent selectivity (Luque de Castro and Garcia-Ayuso 1998).

The differences in the techniques are due to their respective mechanisms. Figure 3 indicates the microwave mechanism for RH pretreatment. During irradiation, RH cells were thermally stressed. As the temperature of the cells increased, the cell walls ruptured, which facilitated rapid extraction without induction.



Fig. 3. Schematic illustration of microwave irradiation mechanism.

Table 6 shows that the best microwave pretreatment was performed at 400 W for 10.0 min. Based on these results, it appears that a 10.0 min treatment time was sufficient for dissolution and transport of material from the cells, leading to greater internal surface area, which was available for absorption of radiation.

Thus, the proposed mechanism can be described in two steps. First, extractives diffuse from the cell wall, which occurs for both microwave and conventional method. The second step includes a fast transudation of extracted matter into the solution *via* RH cell wall rupture by implosion, which might happen through dehydration (Chemat *et al.* 2005). The longer the duration of radiation, the higher values of rate constant and the smaller half times were obtained. Based on previous work, when exposure time is raised, the temperature may rise to the boiling point of the solvent, allowing equilibrium extraction to be achieved rapidly (Chen and Spiro 1995). Table 6 shows the efficiency and optimum conditions for RH pretreatments using microwave and Soxhlet methods.

Method	Time (min)	Solvent Volume (mL)	*Number of sample	Yield (%)
Microwave	10	100	12	10
Soxhlet	420	450	1	7

#### Table 6. The Optimum Condition Derived by the Microwave and Soxhlet

\*The possible number of extraction sample at the same time

**Fig. 4.** SEM images of RH surface with different magnification (a, b), after alkali treatment (c) and RH cellulose (d)

## Characterization of the Isolated Cellulose

Scanning electron microscopy (SEM)

Figure 4 shows SEM images of untreated RH at two different magnifications and the isolated cellulose. The smooth surface of untreated rice husk fiber is shown in Figs. 4a, and b. The micrographs of RH indicate that hemicellulose and lignin were deposited on the RH surface (Maheswari et al. 2012). After alkali treatment (Fig. 4c), the fiber surface became rougher. This might indicate the partial removal of outer non-cellulosic layer composed of materials such as hemicelluloses, lignin, pectin, wax, and other impurities contained in the rice husk. Both wax and pectin are known to surround the surface of natural fibers as a protective layer. The effect of the subsequent bleaching treatment was evident from the comparison of micrographs in Fig. 4d. It is observed that the RH fiber bundles were separated into individual fibers which their diameters are lower than diameter of original RH (~160 µm). The decreased diameter showed that after the potent chemical treatment, all components bound to the fibril structure were removed and thus, individual fibers were separated (Johar et al. 2012). The size and morphological structure of fibers might be changed because of lignin and hemicellulose removal from the original RH. The final yield of isolated RH cellulose using the microwave pre-treatment was 50.9% which was higher than isolated RH cellulose using the Soxhlet pre-treatment (44.7%).

#### Fourier transform infrared spectra (FT-IR)

Figure 5 shows the FT-IR spectra of RH, bleached RH, and RH cellulose. In the IR spectrum of RH, the broad absorption peak placed at 3325 cm<sup>-1</sup> is related to the stretching of -OH groups (located at 2941 cm<sup>-1</sup>). This is attributed to the C-H stretching vibrations. Meanwhile, the peak located at 1735 cm<sup>-1</sup> is attributed to the acetyl and ester groups in the hemicellulose of RH (Johar *et al.* 2012). Compared with the spectrum of RH cellulose, the lack of this peak implied that hemicelluloses were extensively removed.



Fig. 5. FT-IR spectra of RH, bleached RH, and RH cellulose

The spectrum for RH shows a weak peak at 1516 cm<sup>-1</sup> that is related to C= C stretching of the aromatic ring in lignin, but it was not detected in the spectrum of RH after

being treated with NaClO<sub>2</sub> (bleached RH). The absence of this band suggests that the highest possible amount of lignin was removed. This is consistent with the effective isolation method that was used, in which most of the lignin was removed, as shown by the disappearance of the lignin peak at 1510 cm<sup>-1</sup> (Rosa *et al.* 2012). However, the peak located at 1047 cm<sup>-1</sup> represented C-O stretching vibrations in cellulose, hemicellulose, and lignin in RH. The shape of this peak changed in RH cellulose and appeared as a doublet (Rosa *et al.* 2012). The presence of peaks at 463 to 560 cm<sup>-1</sup> in RH is related to the Si-O-Si stretching in silica. Its disappearance in RH cellulose indicates that silica might have also been removed from RH (Shukla *et al.* 2013).

#### Powder X-ray diffraction analysis (PXRD)

The PXRD patterns of RH and isolated cellulose are shown in Fig. 6. The isolated cellulose had four peaks at 15.5°, 16.5°, 22.7°, and 34.8°, which were characteristic of cellulose crystal assignments of the  $(1\overline{10})$ , (110), (200), and (004) planes, respectively. Comparing the PXRD patterns of RH and RH cellulose, the crystallinity degree of RH cellulose (CrI = 65.0%) was higher than RH (CrI = 50%). The former result was attributed to the removal of amorphous compounds such as lignin and hemicellulose during the RH treatments (Battegazzore *et al.* 2014). Furthermore, cellulose has a crystalline structure due to the hydrogen bond interactions and Van der Waals forces between adjacent macromolecules (Zhang and Lynd 2004). The PXRD pattern of RH cellulose confirmed the presence of high crystalline cellulose.



Fig. 6. The PXRD patterns of RH and RH cellulose

#### *Thermogravimetric analysis (TGA)*

The thermal stability of the RH and RH cellulose, and their degradation temperatures, are depicted in Fig. 7. The samples were heated from 30.0 °C to 900.0 °C under a nitrogen atmosphere at 20 °C min<sup>-1</sup>. TGA of RH (Fig. 7a) showed three stages of degradation. The first stage, recorded below 100 °C, corresponds to the moisture evolution of the water absorbed. The second, which occurs above 200 °C until approximately 285 °C, resulted mainly from the thermal decomposition of hemicelluloses and some portion of lignin. The last stage in the high temperature range (285 to 360 °C) was associated with

degradation of cellulose and lignin. Within this temperature range, almost all the cellulose component was decomposed. However, lignin was most difficult to decompose due to the presence of phenyl groups in it and thus its decomposition was extended to the entire temperature range.

The TGA thermogram showed that RH cellulose had a higher thermal stability compared with the original RH. The initial degradation temperatures of RH and RH cellulose were around 219 °C and 226 °C, respectively. The initial degradation temperature of the RH cellulose (226 °C) was appreciably increased compared to that of the original RH (219 °C) due to the low initial decomposition temperatures of hemicelluloses, lignin, and pectin. Therefore the higher initial temperature of thermal decomposition of the RH cellulose is related to the removal of hemicelluloses, lignin, and pectins by purification processes (Dos Santos et al. 2013). Figure 7a shows that the RH cellulose degraded in two steps, the first step was related to the removal of absorbed moisture and the second step confirming the removal of lignin and hemicellulose in the isolated RH cellulose. The final residue at 500 °C of RH (28.30%) was higher than that of the isolated cellulose (13.75%) attributed by the presence of higher amount of ash in RH. In the DTG curve of RH cellulose (Fig. 7b), there was no shoulder peak close to the cellulose peak, which is attributed to hemicellulose. The main decomposition peak in the untreated RH was much wider than in RH cellulose because of the degradation of lignin and hemicelluloses. The maximum content of RH cellulose degradation occurred at 357 °C (Rosa et al. 2012).



Fig. 7. The TGA (a) and DTA (b) thermograms of RH and RH cellulose

## CONCLUSIONS

- 1. This study illustrated the efficiency of microwave pre-treatment of RH using response surface methodology (RSM) followed by chemical isolation of cellulose. The combined method was confirmed by experimental work based on three independent variables including microwave power, irradiation time, and solvent ratio.
- 2. The microwave-assisted pre-treatment was compared to the conventional methods such as Soxhlet and the results further showcase the superiority of the microwave technique for RH pre-treatment than the Soxhlet method.
- 3. The optimum conditions for RH pre-treatment were microwave power of 400 W, irradiation time of 10.0 min, and solvent ratio of 80.0% v/v for the pre-treatment yield of 10.0%.
- 4. Compositional and structural analyses reflected the efficiency of cellulose isolation subsequently carried out using acidic and basic treatment as the yield of the isolated cellulose was 50.9%.
- 5. SEM images demonstrated the effectiveness of bleaching treatment via the separation of RH fiber into individual fiber, while FT-IR spectra confirmed the efficiency of the chemical treatment in removing lignin and hemicellulose from RH.
- 6. PXRD analysis showed the higher crystallinity of the isolated cellulose with CrI of 65.0%, and the TGA thermogram revealed higher thermal stability of cellulose compared to the untreated RH.

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