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Optimization of microwave pre-treatment conditions for maximum lignin recovery from rice husk using central composite design (CCD) by response surface methodology (RSM)

Research article

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Abstract

Response surface methodology based on the central composite design (CCD) was applied to investigate the optimum conditions for lignin recovery from rice husk using microwave pre-treatment. Three operating variables namely microwave irradiation time (min), solid loading (%) and microwave power (Watt), with a total of 20 experiment conditions were conducted to optimize the interaction effects of these variables. The results reported that the second-order model was sufficient for all the independent variables on the response with R^2 = 0.9861. Response Surface Methodology predicted a maximum lignin recovery of 34.9076% at optimum conditions for microwave irradiation time, solid loading and microwave power were 16.57 min, 9.66%, and 664 Watt respectively. An experiment was run at the optimal condition and lignin recovery of 33.1667% was obtained. The predicted result was thus experimentally verified. The obtained lignin was characterized by Fourier Transform Infrared Spectroscopy (FTIR), Hydrogen Nuclear Magnetic Resonance (^{1}H NMR) and Carbon Nuclear Magnetic Resonance (^{13}C NMR). The lignin produced from microwave pretreatment showed the presence of varieties of functional groups and potentially used for future applications.

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Public Interest Statement

Lignin is a well-known polyphenolic compound which contain largely of aromatic monomers and has the potential to produce varieties of value-added products in different types of industry. Generally, a huge amount of lignin is produced every year from paper and pulp industry but only 1-2% of lignin is considered for value added products and the rest of it can be considered as non-valuable source. The conventional ways to dispose rice husk are by burning or by leaving it at the field. These routine activities would create the environmental pollution, as well as the production of dangerous gases to the surrounding. Therefore, this study aims to find the alternative to utilize the rice husk. Moreover, it is also possible to highlight the rice husk as a main source of lignin, since rice has become the second most cultivated crop in the world. Pretreatment of lignin is done in order to isolate all the fractions of lignocellulosic biomass and obtain high purity of unaltered structures. This study reported the potential lignin recovery from rice husk by using the greener microwave treatment and the conditions were optimized using Central Composite Design (CCD) of Response Surface Methodology (RSM) to obtain the optimum yield of lignin.

Introduction

Lignin, the most important plant-derived aromatic macromolecule, is currently considered by experts due to its availability, which may result in a major and profitable assembling of lignin-based chemical compounds. Lignin is the second most abundant natural polymer after cellulose and comprises around 10-30 % of the dry weight of lignocellulose (de Gonzalo *et al.*, 2016). Different from the polysaccharide of cellulose and hemicellulose, lignin is constituted by three monolignols: coniferyl alcohol, p-coumaryl alcohol, and sinapyl alcohol connected by various ether and carbon-carbon bonds. The presence of these monolignols highly depend on the plant species and tissue. Every year, around 100 million tons of lignin are being produced in chemical pulping as by-products, most of them are burnt to generate energy and to recover inorganic chemicals (Prado *et al.*, 2016). However, only a small amount of lignin is utilized efficiently in the industrial process. Lignin is the abundant renewable aromatic biopolymer which is widely used for biofuel (Bi *et al.*, 2015; Cheng and Brewer, 2017), phenolic resin (Pinheiro *et al.*, 2017; Tachon *et al.*, 2016) antioxidant (Piña *et al.*, 2015; Tachon *et al.*, 2016) , dye dispersant (Ding *et al.*, 2016; He *et al.*, 2016) and chemicals (Fernandez-Rodriguez *et al.*, 2017; Holmelid *et al.*, 2017).

Currently, the main industrial methods used for extracting lignin from woody or nonwoody lignocellulosic feedstocks are the organosolv (Watkins *et al.*, 2015; Wildschut *et al.*, 2013), kraft (Petersson, 2014; Wallberg *et al.*, 2006), ionic liquid (Oghbaie *et al.*, 2014; Pinkert *et al.*, 2011) and etc. Most of these methods require high temperature and pressure as well as high production cost which involves harsh acidic conditions. Removal of the solvents from the system is necessary using distillation or evaporation techniques and these two approaches posed difficulties in the recovery process when it involves solvents with high boiling points.

In an effort to improve the lignin recovery from lignocellulosic material, microwave treatment becomes the alternative process due to the vibration of polar molecules, extensive collision and the movement of ions which lead to the dissolution of products. It utilizes thermal and non-thermal effects generated by microwaves in aqueous conditions (Zhou *et al.*, 2012). The effective separation technique involves the breaking of the compact network structure of lignocellulosic biomass while preventing its cellulose, hemicellulose, and lignin from the degradation process prior to its utilization for the biorefinery concept. Microwave heating is preferred instead of conventional heating as it is able to heat the target object directly by applying an electromagnetic field to dielectric molecules (Singh *et al.*, 2013). Microwave radiation is also reported to change the supermolecular structure of lignocellulosic material to improve the reactivity process and to increase the homogeneity (Gong *et al.*, 2010).

At present, the fundamental techniques for the extraction of lignin includes alkaline crystalline method, acid precipitation method, and the ultrafiltration method. Recently, a common technique used to recover lignin is the acid precipitation method. In the current research framework, we use this method to recover lignin from paddy husk after undergoing microwave pre-treatment. The aim of the paper is to investigate the influence of microwave irradiation time, percentage of solid loading and microwave power on the recovery of lignin from rice husk.

The interactions between multiple parameters of the experimental data cannot be explained by single-variable optimization method due to misinterpretation of analysis (Chen *et al.*, 2012). The conventional approach involves many experiments, which makes the process time-consuming and inefficient to determine the combined effects of parameters involved in all the experimental conditions. Therefore, in this study, central composite design by response surface methodology is applied to investigate the optimum conditions of microwave pre-treatment that would affect the recovery of acid-insoluble lignin from rice husk. Response surface methodology is quicker than conventional methods as it describes both the influence of different factors and the effect between their interactions (Saha *et al.*, 2017). For optimization, three main factors namely, microwave irradiation time, percentage of solid loading and microwave power were selected to investigate the effect of these three factors towards lignin recovery. The lignin recovered from rice husk is characterized using Fourier Transform Infrared Spectroscopy (FTIR) and Nuclear Magnetic Resonance (NMR).

Materials and methods

Raw materials

Raw rice husk was collected from the Malaysian Agricultural Research and Development Institute (MARDI) of Kedah. It was washed to remove unwanted dirt, soil, dust, and insects prior to oven-dried at 70 ºC overnight. The drying rice husk was ground into fine powder by Biotrade Resources Sdn. Bhd. Standard steel sifters were used to sieve it to 600 µm particle size. The

dried rice husk was then stored in a closed container at room temperature until further use. The moisture content of the provided rice husk was 8.16%.

Microwave treatment

Microwave pretreatment was carried out in a general-purpose laboratory microwave oven (Panasonic Corporation, model NN-ST34HM/W) with an operating frequency of 2450 MHz, internal volume of 25L and maximum output power of 800 W. The optimization studies were carried out by investigating three factors which were microwave irradiation time (5-30 min), solid loading (5- 25 %) and microwave power (80- 800 Watt) while the output was lignin recovery. The beaker was positioned at the centre of a rotating circular glass plate in the microwave oven. At the end of the desired reaction time, the slurry was rapidly cooled down to the room temperature and the liquor was separated by vacuum filtration to neutral pH with distilled water. The neutral solid obtained was then dried at 60°C for 24 hours. Dried solid was later proceeded for the lignin preparation study. The samples were performed in triplicate.

Preparation of Klason Lignin

After the microwave pretreatment step, 3 g of rice husk was mixed with 180 mL of acetone in 250 mL Erlenmeyer flask. After incubating it at 85°C for 2 hours, the mixture was filtered and washed several times with distilled water until there was no more precipitate left. The solid residue was dried in the furnace at 105°C for 24 hours until the weight became constant (Di Blasi *et al.*, 1999; Lin *et al.*, 2010). The dried residue was later referred as extractives-free biomass. Prior to the isolation process, 2 g of the extractives-free biomass were treated with 60 mL of 98% (v/v) of H_2SO_4 and left to stand overnight at room temperature without stirring to completely hydrolyzed and solubilized the carbohydrate from rice husk biomass. The obtained precipitate was then incubated in a water bath at 100 °C for 1h and later cooled down for 10 minutes. The black liquor was isolated from the solid residue by vacuum filtration and washed with distilled water for five times to eliminate soluble carbohydrate and the remaining sulphuric acid until a clear supernatant was produced prior to the process of drying in the furnace at 105°C for 24h. The dried lignin was crushed and ground into a fine powder using mortar and pestle. The final weight of dried precipitate was recorded as the value of lignin in rice husk (Di Blasi *et al.*, 1999; Lin *et al.*, 2010). The amount of lignin was determined gravimetrically using equation 1:

$$\% \text{ Lignin} = (\text{Wt. of drying residue (g)} / \text{initial oven-dried sample wt.}) \times 100 \quad (1)$$

One Factor at a Time experiments

The conventional OFAT approach was used to select the significant parameters and the initial test range of the three variables, i.e. microwave irradiation time, percentage of solid loading (% w/v) and microwave irradiation power (Watt). The effect of the parameters was checked by

varying one parameter at a time and keeping the other parameters and process conditions constant. The parameter levels at which maximum lignin recovery were chosen as the centre point values to enhance the pretreatment process by RSM.

Central Composite Design (CCD) and statistical analysis

Experimental design using Central Composite Design (CCD) was performed to generate the design of expert (DOE), provide the statistical analysis, optimize the independent variables and create a suitable model that fits with the respective variables (Jaliliannosrati *et al.*, 2013). The three levels of parameters were performed according to a preliminary experiment in the one-factor-at-a-time (OFAT) method. The experimental design comprised a total of 20 experiment trials, one run was carried out at the centre point values and the remaining runs were conducted by the combinations of high (+) and low (-) levels of all variables (**Table 2**). The Design-Expert 7.0 (Stat-Ease Inc., Minneapolis, USA) software was used for statistical regression analysis, analysis of variance (ANOVA), and 3D graphics model graphs to determine the fitness of the model, the significance of the variables studied and the interactions between the variables towards lignin recovery.

Table 1 Experimental design matrix consisting of 20 sets along with the results of Central Composite Design (CCD) during microwave pretreatment

Run	Variables			Response
	Microwave irradiation time	**Solid loading (%)**	**Microwave power (Watt)**	**Lignin recovery (%)**
1	5	5	600	16.00
2	25	5	600	17.67
3	5	15	600	25.00
4	25	15	600	24.50
5	5	5	700	23.83
6	25	5	700	27.00
7	5	15	700	18.00
8	25	15	700	20.00
9	0	10	650	18.00
10	31.82	10	650	25.00
11	15	1.59	650	18.33
12	15	18.41	650	19.00
13	15	10	565	26.00
14	15	10	734	30.83
15	15	10	650	34.50
16	15	10	650	34.80
17	15	10	650	33.90

18	15	10	650	36.30
19	15	10	650	33.80
20	15	10	650	34.60

Table 2 The actual and coded independent variables for optimization using Central Composite Design

Symbol	Variables	Levels		
		(-1)	(0)	(+1)
A	Microwave irradiation time (min)	5	15	25
B	Solid loading (%)	5	10	15
C	Microwave power (Watt)	600	650	700

Structural characterization of recovered lignin

Fourier Transform Infrared Spectroscopy analysis

The functional groups in lignin fractions after the optimum conditions of microwave pretreatment were identified using a Nicolet Is5FT-IR Spectrometer (Thermo Fisher Scientific Inc., USA) to equip the use of Attenuated Total Reflectance mode with a diamond crystal in the region wavenumber range from 4000-500 cm-1. The spectra of the samples were analyzed using OMNIC 8.2 software (Thermo Scientific., USA) to determine the peak position. All the dominant peaks obtained were identified after correcting the raw spectra for zero baselines.

Nuclear Magnetic Resonance Spectroscopy analysis

The ^{1}H NMR and ^{13}C NMR spectra were performed using a Bruker, 400-MHz at 300K. The lignin sample was dissolved in Dimethyl sulfoxide (DMSO) solvent. Spectral analysis of NMR was performed using Bruker software.

Results and discussion

Optimization of lignin recovery parameters using OFAT

Preliminary studies of the initial screening of parameters were performed to determine their effects on the lignin recovery. Three main parameters were studied namely; microwave irradiation time (5,10,15,20,25 min), solid loading (5,10,15,20%) w/v and microwave irradiation power (80, 350, 650,800 Watt) and their effects to lignin extraction were discussed further in the sections below.

Effect of irradiation time

The influence of different times of treatment on the recovery of extractives, cellulose, hemicellulose, and lignin was presented in **Figure 1.** Extension of the reaction time from 5 minutes to 15 minutes leads to an increase of lignin recovery but the recovery decreased when

the time reaction is prolonged until 25 minutes. The activation energy required by the contact between organic acid and lignin were not enough to excite dipoles when the microwave irradiation was too low. When the irradiation period increased, the rate of collisions among molecules also increased, and the hydrogen bond split due to the action of the AC electromagnetic field. Once the time was too long, the hydrogen bonds re-associated due to the large amount of intramolecular heat energy, consequently affecting pretreatment efficiency (Gong *et al.*, 2010). This finding indicates that the time of microwave treatment could partially disrupt the lignin structure and expose a more accessible surface area of cellulose (Ma *et al.*, 2009). 10.0 min microwave treatment was sufficient for dissolution and transport of material from the cells, that leads to the greater internal surface area, which was available for absorption of radiation as reported by Khandanlou *et al.* (2016).

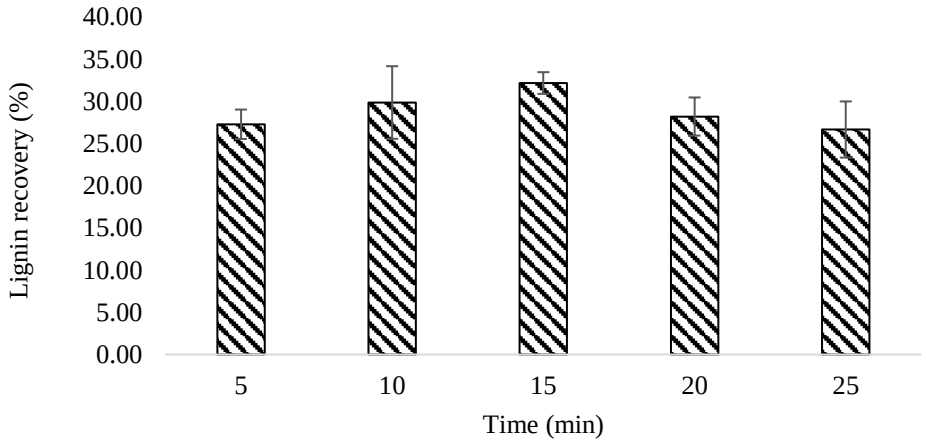

Fig 1 Effect of lignin recovery in various microwave time treatment

Effect of solid loading

The composition of extractives, cellulose, hemicellulose, and lignin at different solid loading percentages were depicted in **Figure 2**. The lignin recovery from 5% solid loading increased 1.148 fold when increase to 10% which is equivalent to 32.33 %. However, the increment of solid loading to 15% resulted in a decreased recovery and possessed no significant improvement. It might be due to the different energy collisions with different solid loading concentrations (Ethaib *et al.*, 2016). Microwave pretreatment in lignocellulosic biomass involves both thermal and non-thermal effects on the components which results in the explosion reaction of the biomass particles for disruption of silica waxy surface and the breaking down of the bonds of the lignin-hemicellulose matrix (Akhtar *et al.*, 2017). From the summarization of the results presented, this indicates that 10% of solid loading is sufficient to recover high amount of lignin.

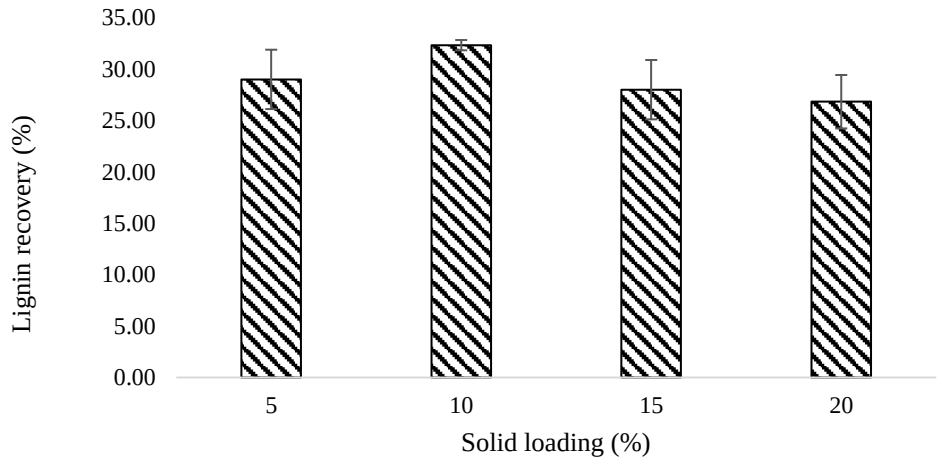

Fig 2 Effects of lignin recovery in various percentages of solid loading

Effect of microwave power irradiation

The effect of microwave power irradiation on the lignin recovery was shown in **Figure 3**. It was observed that lignin yield significantly increased when the higher microwave power was applied on the rice husk. The lignin recovery increased 1.345 fold when the power is increased from 80 watts to 350 Watts. The yield continuously increased to 1.236 fold which was equivalent to 37.50% when the power was set at 650 Watts. This was probably caused by specific and non-thermal microwave effects during the treatment. The increment might be due to the disruption of the ultrastructure of rice husk under microwave irradiation conditions (Lai *et al.*, 2014).

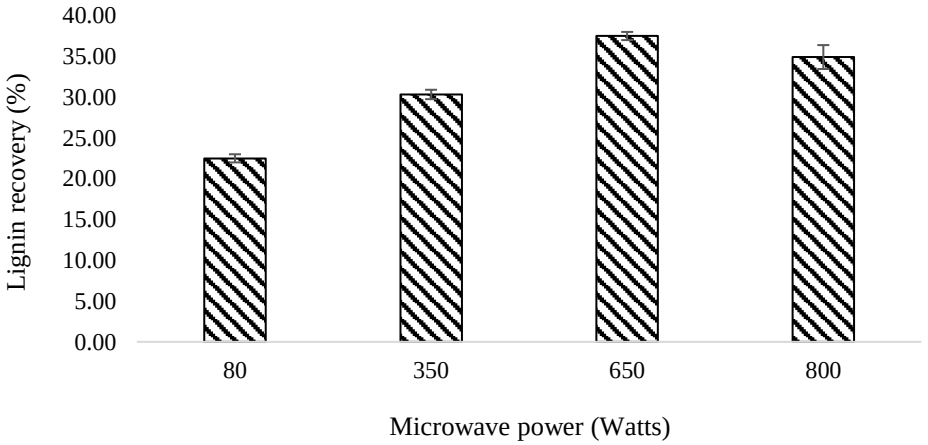

Fig 3 Effects of lignin recovery in various microwave power irradiation

Central Composite Design by Response Surface Methodology (RSM)

Based on the 20 experimental results produced in this study, the best model of the process order is the Quadratic process order. The response surface model predicted the relationships between the variables and the lignin yield response by a second-order polynomial equation:

$Y = 34.67 + 1.33\ A + 0.30\ B + 1.01\ C - 0.42\ AB + 0.50\ AC - 3.58\ BC - 4.79\ A^2 - 5.79B^2 - 2.34\ C^2$ (1) Where Y is the predicted response (lignin recovery), A, B, and C represent microwave

irradiation time, percentage of solid loading and microwave power respectively. The positive sign and negative sign in front of the terms refer to synergistic effect and antagonistic effect respectively that would indicate the influence of independent variables towards lignin recovery (Jaliliannosrati *et al.*, 2013). Our results showed that the 18th treatment run produced the maximum yield of lignin recovery (36.30%) while the minimum yield (16%) was observed in the first run which indicated that the different conditions of treatment strongly influenced the lignin recovery from lignobiomass. The yield was significantly better than those obtained by Xie *et al.* (2015) who performed the microwave treatment for bamboo, Chinese tallow tree, switchgrass, and pinewood using the microwave. The reported yield was below 25% at 550 Watts. This finding was in line with the reported recovery of lignin yield and delignification degree increased along with the increase of the microwave intensity. It was supported by the capability of mixtures to absorb in a controlled and fast manner when high power was applied (S. Zhou *et al.*, 2012). The original model given by Design-Expert 7 includes the interaction terms of AB, AC, and BC; however, these interactions were interpreted based on the results obtained in **Table 1** by using ANOVA analysis.

Statistical analysis

The ANOVA results of the quadratic model for recovery of lignin was summarized in **Table 3**. All data in **Table 1** was further interpreted to investigate the relationship and influence of the different variables towards response (Y).

Table 3 The analysis of variance (ANOVA) on lignin recovery

Source	Sum of squares	df	Mean square	F value	p-Value Prob > F
Model	914.19	9	101.58	78.92	<0.0001[a]
A-time	24.02	1	24.02	18.66	0.0015
B-solid loading	1.25	1	1.25	0.97	0.3482
C-microwave power	13.91	1	13.91	10.81	0.0082
AB	1.39	1	1.39	1.08	0.3225
AC	2	1	2	1.55	0.241
BC	102.67	1	102.67	79.77	<0.0001[a]
A^2	330.01	1	330.01	256.39	<0.0001[a]
B^2	482.73	1	482.73	375.04	<0.0001[a]
C^2	78.94	1	78.94	61.33	<0.0001[a]
Residual	12.87	10	1.29		
Lack of Fit	8.82	5	1.76	2.17	0.207[b]
Pure Error	4.05	5	0.81		
Cor total	927.06	19			

[a] Significant, [b] not significant, df: Degree of Freedom

As shown in **Table 3**, the model F value of 78.92 implies that the quadratic model was significant. Each term in the model was also tested for its significance. The model of the variables was considered to be significant if their p-value is less than 0.05. It was also observed that the linear term of the microwave irradiation time (A) and the microwave power (C) showed a significant effect on the lignin recovery with an F-value of 18.66 and 10.81 respectively. However, solid loading (B) seems to not bring any significant effects on the lignin recovery with an F-value of 0.97. The interactions between the variables (AB, AC) do not affect the lignin recovery but the interaction between variables BC exhibits a significant effect with a p-value less than 0.05. The quadratic terms of microwave irradiation time (A^2), solid loading (B^2) and microwave power (C^2) have large significant effects on the lignin recovery with an F-value of 256.39, 375.04 and 61.33, respectively. From the overall observations, the irradiation time (A) was the most significant variable for the production of bio vanillin due to its higher F value (18.66) and lower p-value (0.0015). The p-value <0.0001 means that there is only 0.01% chance that the model F-value this large, is the product of noise in the experiment. The lack of fit F-value of 2.17 implied that it is not significant relative to the pure error. There is a 20.70% chance that a 'Lack of Fit F-value' this large could occur due to noise.

The interaction between the process parameters was analysed clearly by demonstrating it using a three-dimensional surface (**Figure 4a**, **Figure 4b**, and **Figure 4c**). **Figure 4a** shows the 3D response surface plot for the influence of microwave irradiation time and solid loading on the recovery of lignin. With the increment of microwave irradiation time and solid loading, the lignin yield significantly increased until at one point and after these points, the lignin recovery was decreased. Similarly, lignin recovery increases with an increment in the microwave irradiation time and the decrement of the microwave power (**Figure 4b**). However, the effect of these two variables were insignificant and the evidence can be seen from the high p-value through the ANOVA analysis which indicated the insignificant value. **Figure 4c** shows the effect of microwave irradiation power and solid loading towards lignin recovery. The decreased in microwave irradiation power and the increased in the percentage of solid loading gave a significant effect on the lignin recovery. It can also be proven from the low p-value of this interaction parameter from the ANOVA analysis which indicated the significant effect on the lignin yield.

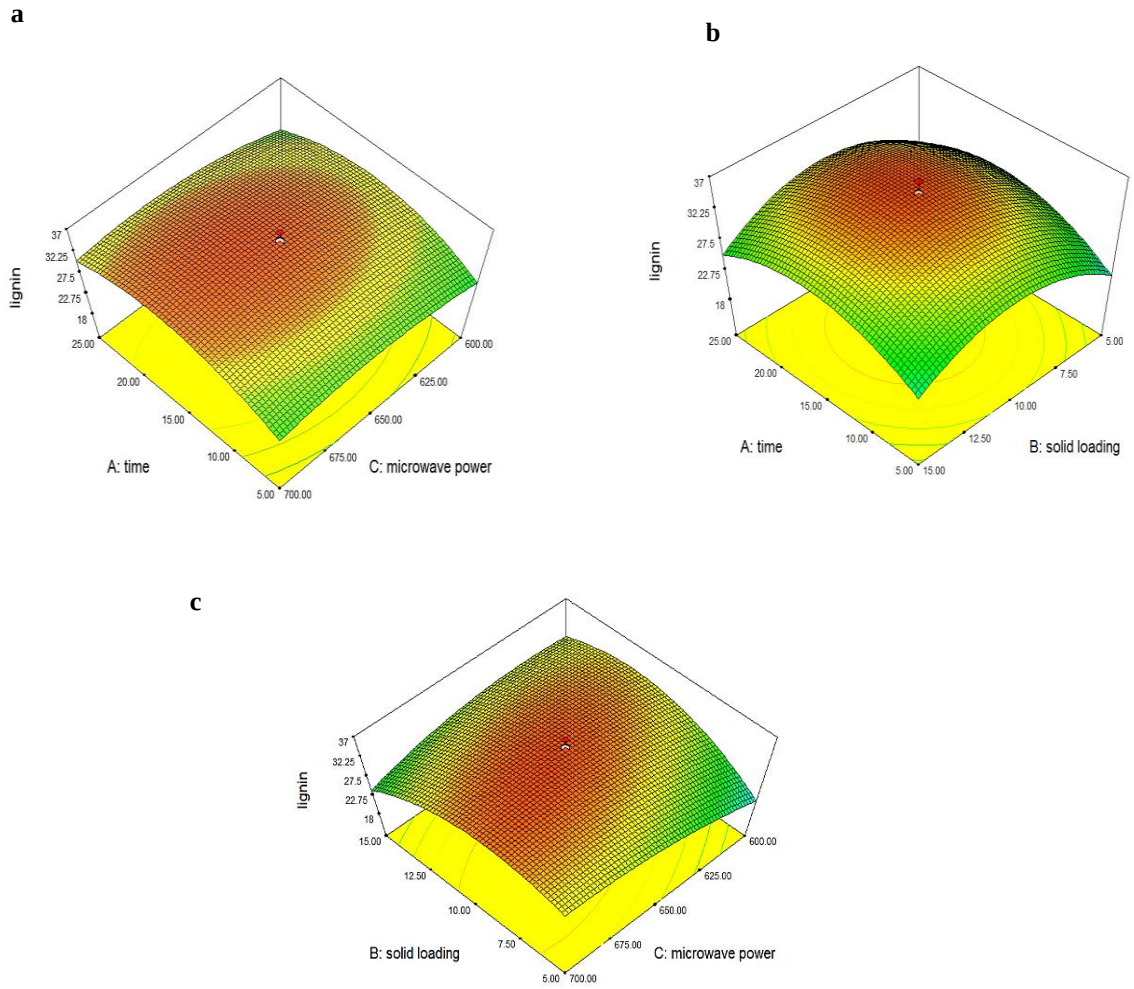

Fig 4. Response surface plot of lignin recovery: a. interaction between microwave irradiation time and solid loading at a fixed microwave power; b. interaction between irradiation time and microwave power at a fixed solid loading; c. interaction between solid loading and microwave power at a fixed time

In order to test the fitness of the model, the regression equation and the determination coefficient (R^2) were analyzed. The value of the determination coefficient (R^2= 0.9861) indicated that the sample variation of 98.61 % for lignin recovery was attributed to the independent variables and only 1.39% of the total variation could not be explained by the model. Moreover, the value of the adjusted determination coefficient (Adj R^2= 0.9736) was very high to support the high significance of the model (**Table 4**). 'Adeq precision' is defined as the ratio between the signal to noise and this value must be greater than 4 which had been successfully achieved in this study (23.748) (Farid *et al.*, 2014; Saha *et al.*, 2018). The ratio of the standard error of the estimate to the mean value of the response was represented by the coefficient of variation value (CV) and a factor to show the reproducibility of the model. The value of CV lower than 10% proved that the model was reproducible and highly reliable (Chen *et al.*, 2012; Saha *et al.*, 2018). The calculated value in this study was equal to 4.39% (**Table 4**).

Table 4 Model statistics related to the developed quadratic response surface model

SD	1.13	R-squared	0.9861
Mean	25.85	Adj R-squared	0.9736
C.V %	4.39	Pred R-squared	0.9213
Press	72.94	Adeq precision	23.748

The correlation between experimental and predicted values of the lignin recovery were shown in **Figure 5(a).** A higher value of correlation coefficient for the lignin recovery response (Y) showed a reasonable correlation between the independent variables studied. Equation 1 was used for the predicted yield and lignin recovery from the experimental data. **Figure 5(b)** plotted the studentized residuals versus predicted conversion. The plots showed that the residuals points had randomly scattered around the plotting area. The normal probability plot of the residuals for lignin recovery was shown in **Figure 5(c).** If the data points of the plot fall closer to the straight line, then the data are normally distributed (Antony *et al.*, 2003). It was shown that the data points of lignin recovery were fairly close to the straight line and it indicated that the experiments came from a normally distributed population (Gottipati & Mishra, 2010).

Results obtained in the present study were then compared with earlier reports on lignin recovery by using microwave pretreatment. In another work related to the lignin recovery, the maximum yield of lignin from triticale straw at 1600 Watt for 30 minutes in the presence of 0.64% of H_2SO_4 which acted as a catalyst was reported to be 18.2% which was lower than our finding by using water without any catalyst (Monteil-Rivera *et al.*, 2012). The lignin recovery increased with the increment in microwave power and the decrement in the percentage of solid loading. This might be caused by the specific and non-thermal microwave effects, which remained controversial. The mixtures of the reaction were able to absorb microwaves in a controlled and fast manner when high power was applied and it is in line with the previous study by S. Zhou *et al.* (2012).

Microwave process has a unique mechanism leading to a direct interaction between the polar part of rice husk and electromagnetic field. During the treatment, the silicified structure of rice husk was disrupted, the lignin-hemicellulose complex was cleft, and both the silica and lignin were partially removed (Zhu *et al.*, 2015). The energy used during the microwave process was also more efficient and faster compared to those of conventional heating.

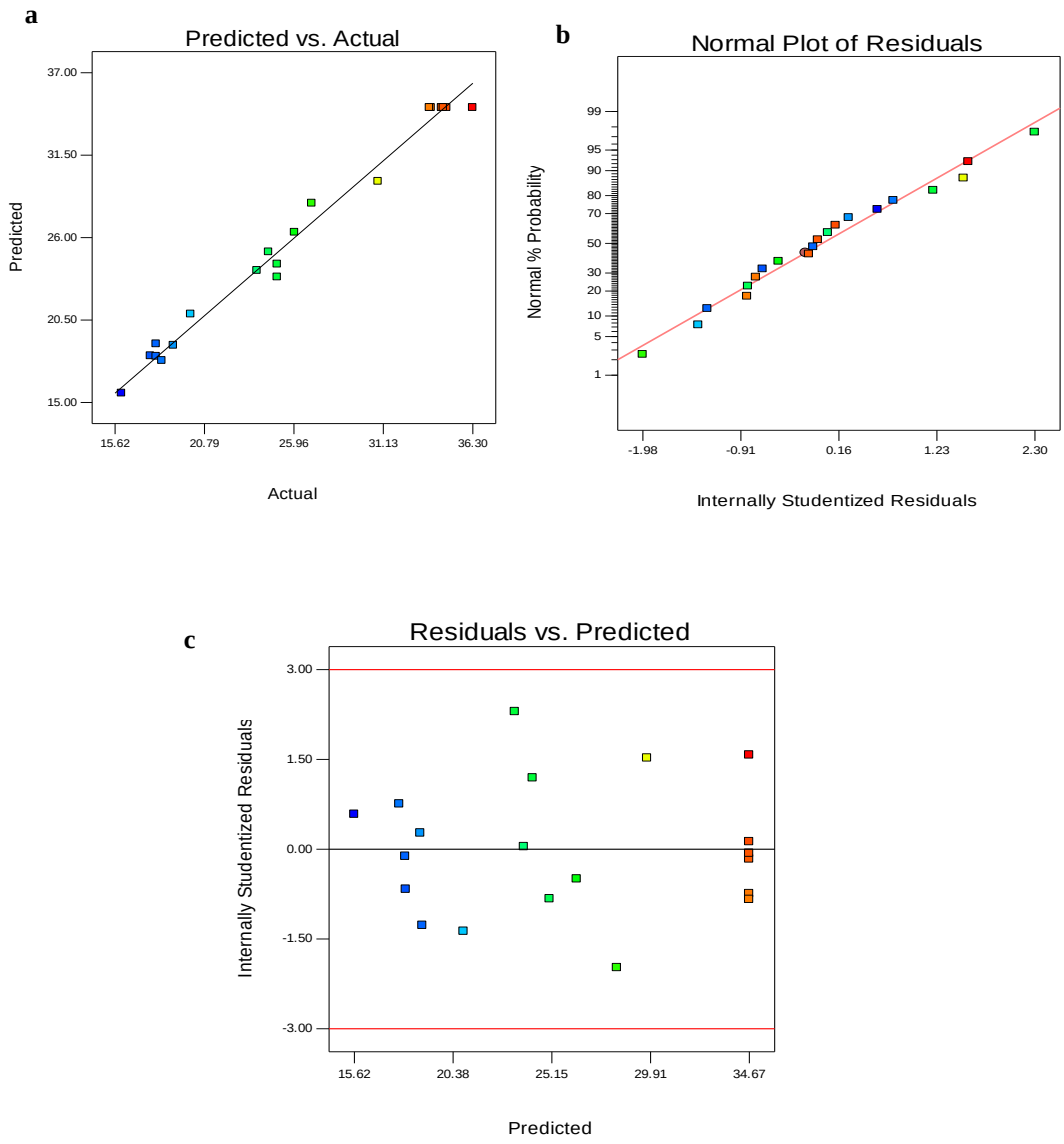

Fig 5 (a) Predicted values versus experimental values of lignin recovery. (b) Normal probability and studentized residual plot. (c) Residual versus predicted plot for lignin recovery

The predicted optimum conditions for the microwave pretreatment were at 16.57 minutes for reaction time, 9.67 % of solid loading and 664.21 Watts, resulting in 34.9076 % of lignin recovery. To confirm the predicted response, theoretical optimum experiments were carried out in triplicates and the lignin recovery obtained was 33.1667 %. This value indicated that the optimized conditions generated using Design-Expert software were sufficiently accurate because it was very close to the predicted response from the model. Therefore, through an optimization study, it was found that the experimental values for the lignin recovery from microwave pretreatment agrees to the predicted value.

Lignin characterization

Fourier-transform infrared spectroscopy (FTIR) analysis

Fourier-transform infrared spectroscopy (FTIR) result was shown in **Figure 6.** Normally, the FTIR spectra regarding lignin have similar frequencies and number of groups, even though the band intensities of each lignin relied on the lignin resources (Mohtar *et al.*, 2015). The broadband between 3600 – 3000 cm^{-1} is due to the strong hydroxyl groups, 0-H stretching in aliphatic and phenolic structures (Mohtar *et al.*, 2015). The couple of peaks between 2920 cm^{-1} and 2850 cm^{-1} were assigned to the stretching vibration of C-H bonds of CH_2 in cellulose and CH_3 of the propyl side chain (Morales *et al.*, 2018). However, the specific characteristic band between 2100 – 2360 cm^{-1} referred to the typical Si-H bond (Ma'ruf *et al.*, 2017). The band at 1629- 1651 cm^{-1} was indicated with a C=O stretching vibration in unconjugated carbonyl of lignin (de Carvalho Oliveira *et al.*, 2018). The peaks at 1508 cm^{-1} to 1512cm^{-1} and 1589 cm^{-1} to 1591cm^{-1} were related to the aromatic ring in guaiacyl and syringyl units (da Rosa *et al.*, 2017). Overall, the bands which happened to showcase more characteristic of lignin, were those of match to guaiacyl or syringyl units. A wavenumber at 1417 cm^{-1} to 1421 cm^{-1} indicated the C-H stretching in syringyl and guaiacyl units whereas 1267 cm^{-1} indicated the C-O bonds in guaiacyl units (da Rosa *et al.*, 2017). The particular peak at a wavelength of 1170–1164 cm^{-1} was certainly not observed in the spectra of lignins. The absence of this peak indicated the absence of sulfur in the lignin and became the significant characteristic since sulfur within the lignin was considered as contaminant and will further disrupt their use (Gonçalves *et al.*, 2016). A band spectra appeared around 1030 cm^{-1}, which served as an indicator of C–H in-plane deformation in guaiacyl (Prado *et al.*, 2013). In addition, the band at 840cm^{-1} indicated the peak for C-H bending (She *et al.*, 2012).

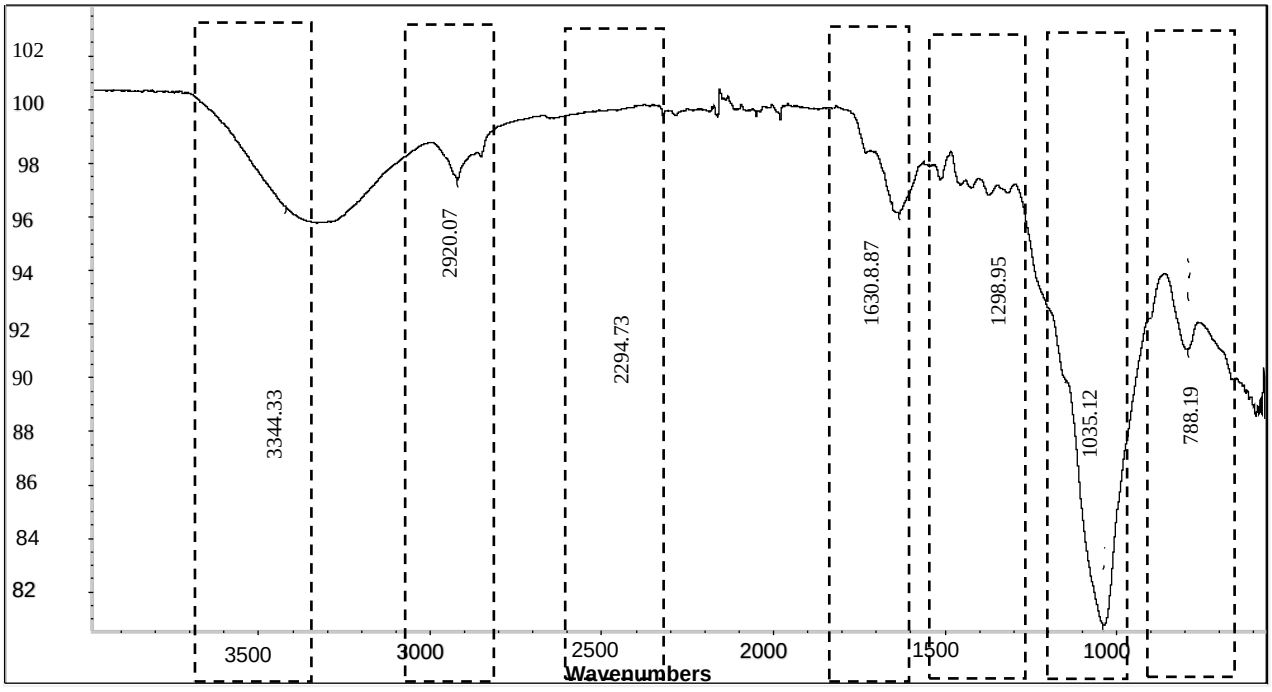

Fig 6 Fourier Transform Infrared Spectroscopy (FTIR) of lignin

Nuclear magnetic resonance (NMR) analysis

The ^{13}C NMR and ^{1}H NMR analysis were performed to study the structure of the isolated lignin. ^{13}C NMR and ^{1}H NMR spectrum of lignin preparation after microwave treatment at optimum conditions were shown in **Figure 7.** The signals at 10.00 – 8.00 ppm were attributed to the carboxyl and aldehyde group (Wang *et al.*, 2019). The peaks between 8.1 ppm to 6.4 ppm indicated the aromatics protons in guaiacyl and syringyl units in lignin (Rashid *et al.*, 2018). The signals between 6.8 ppm and 6.2 ppm were detected in klason lignin samples which represented syringylpropane and guaiacyl-propane units. The signals at 3.91 – 3.49 ppm were contributed by the protons in methoxyl groups (Wang *et al.*, 2019). The sharp peak at 2.5 ppm and 3.3 ppm were detected as a result from the solvent (Dimethylsulfoxide, DMSO) used to prepare the samples (associated with protons from water present in DMSO) (Cybulska *et al.*, 2012; Prado *et al.*, 2016). Signals between 2.0 ppm and 0.8 ppm were contributed by the protons in the aliphatic side chains of lignin (Cybulska *et al.*, 2012).

In ^{13}C NMR spectra, the region between 160.0 ppm to 100.0 ppm was reported as a fixed reference range to show the presence of guaiacyl (G), syringyl (S), and p-hydroxyphenyl (H) units (Latif *et al.*, 2019) (**Figure 8**). The previous literature also mentioned the particular signals for polysaccharides were in the range of 70 and 110 ppm. But, these signals were almost absent from associated polysaccharides in the lignin fractions. Peaks between 160 ppm and 100 ppm were attributed to aromatic carbon of syringyl units and guaiacyl units (Kim *et al.*, 2011). Specifically, the peak at 150 ppm 155 pm indicated the C3 and C4 of guaiacyl etherified or C3 and C5 of syringyl β-*O*-4 while 130 ppm to 137 ppm referred to C1 and C4 of Guaiacyl or syringyl ring structure. At 120 ppm, the signal referred to C6 in guaiacyl units and C5 of guaiacyl ring was referred at 116 ppm to 117 ppm (Dávila *et al.*, 2019). The absence of signals between 90 and 102 ppm indicated a low concentration of residual sugars in this lignin (El Hage *et al.*, 2009) (Wang *et al.*, 2019). The signals between 61.3 ppm to 58.0 ppm referred to the presence of β-O-4 linkages (Wen *et al.*, 2014). The particular peak at 55 ppm had been attributed to methoxyl carbons side chain of guaiacyl and syringyl unit which served as the monomer of softwood lignin (L. Zhou *et al.*, 2017). The strongest signal at 39.54 ppm was assigned to the dimethylsulfoxide, DMSO solvent used to prepare samples (Prado *et al.*, 2016). ^{13}C NMR analysis together with ^{1}H NMR, analysis had clearly shown the presence of guaiacyl unit in klason lignin extracted, which was parallel to the FTIR analysis.

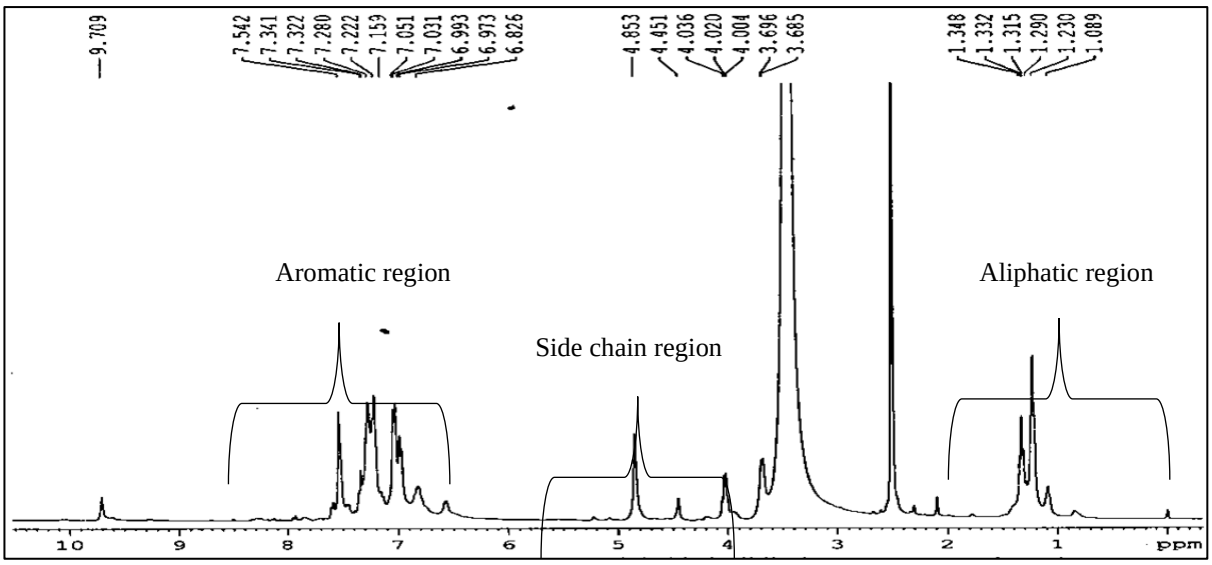

Fig 7 ^{1}H NMR of lignin spectra

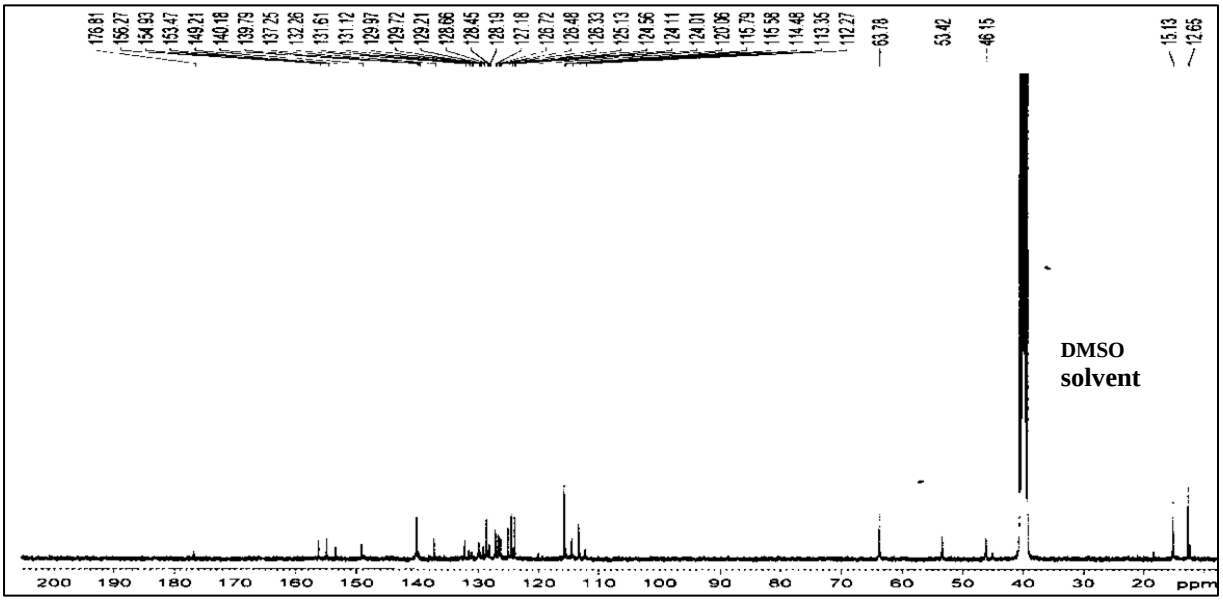

Fig 8 ^{13}C NMR spectra of the lignin

Conclusion

In the present study, the Central composite design application based on Response surface methodology was exploited to investigate the effect of microwave irradiation time, percentage of solid loading and microwave power towards the lignin recovery. Statistical analysis in this study revealed that only microwave irradiation time and microwave power give significant effects on the lignin recovery while solid loading was found to be an insignificant parameter. The maximum lignin yield was achieved at 16.57 min, solid loading of 9.66 % and microwave power of 664.23 Watt resulting in predicted maximum recovery lignin of 34.9076 %. The predicted value was validated by experimental values which are 33.1667 %. This showed that the predicted value of lignin recovery almost matched the experimental values. High yield of lignin at optimal conditions indirectly proved that the microwave irradiation condition is effective for lignin recovery within a lesser period of time.

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Conflict of Interest: On behalf of all authors, the corresponding author state that there is no interest conflict.

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