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Enhancing Reducing Sugar Yield from Fruit Peel Waste via Optimized Microwave Pretreatment

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ABSTRACT

Fruit peel waste (FPW) is a renewable and cheaper source of fermentable sugars for biofuel production. In this research, three types of FPW which are banana peel, orange peel, and pineapple peel were investigated for reducing sugar generation using microwave pretreatment. Pineapple peel waste was found to produce the highest yield of total reducing sugar compared to banana peel and orange peel, with the value of 10.58 g/L. The optimization condition for microwave pretreatment was studied by using the Central Composite Design under Response Surface Methodology. Microwave power (180 – 450 watt), time (3 – 7 minutes) and biomass loading (5 -15 % w/v) were the parameters used to optimize the reducing sugar yield from pineapple peel. The optimum condition for microwave pretreatment was found at biomass loading of pineapple peel of 15% (w/v), power of 450 watt with 5 minutes of treatment time with the concentration of 12.52 g/L. It was apparent that the structure of hemicellulose, cellulose, and lignin of the pineapple peel was affected by the microwave pretreatment condition based on Fourier Transform Infrared spectroscopy (FTIR) and Scanning Electron Microscope (SEM) analysis. FTIR showed alteration in a functional group while the SEM micrographs showed a marked change on the pretreated sample when compared with the untreated sample. In conclusion, microwave pretreatment is a promising approach for reducing sugar production from the agricultural residue such as FPW.

Keywords: Lignocellulosic biomass, Microwave pretreatment, Reducing sugars, Pineapple peel waste, Response Surface Methodology

1. INTRODUCTION

The expansion of the economy and rapid population growth in Malaysia have driven agricultural activities, particularly in fruit cultivation. These fruit crops serve as raw materials for a wide range of food and industrial products. However, the high consumption and processing of fruits generate substantial quantities of agro-industrial waste, contributing to environmental degradation. Consequently, significant research efforts have been directed towards the valorization of fruit waste through various bioconversion and pretreatment strategies (Wan-Mohtar et al., 2023; Sulaiman et al., 2022). One promising approach involves the extraction and production of reducing sugars from fruit waste, which can serve as precursors for bio-based chemicals and biofuels (Fanyin – Martin *et al*., 2023; Sim *et al.*, 2024). Reducing sugars are generally defined as any sugar with an aldehyde or ketone group in the basic solution. It can be produced via enzymatic hydrolysis of polysaccharides. However, the existence of lignin (aromatic polymer) in lignocelluloses, and multiple bonding between polysaccharides and lignin have complicated the process of converting lignocellulose into

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sugar. Lignocellulose conversion to reducing sugars requires high processing temperatures (>250 °C) and is prone to degradation reactions. Due to its properties, such as polydispersity, molar masses and hyperbranched structures, cellulose's hydrolysis became difficult (Gathergood et al., 2018).

During pretreatment, the degree of polymerization of cellulose decreases, and the solubility of cellulose (fractions) in water for efficient hydrolysis will increase. Hence, removing lignin and decreasing the crystallinity of cellulose is very important before hydrolysis (Ojo 2023). Due to the nature of biomass, pretreatment is an essential step in converting biomass to bio-based products. Of all the existing pretreatment technologies, microwave has been considered one of the most promising methods for efficient and rapid processing of biomass with both thermal and non-thermal effects. Microwave radiation has been chosen as an effective way to pretreat various types of biomasses, including agricultural residues, woody biomass, grass, energy plants, and industrial residuals, since it can directly interact with the material, thereby accelerating chemical, physical, and biological reactions (Gallego-García et al., 2023). Therefore, the goal of this research is to optimize fruit peel pretreatment processes using microwave for maximizing reducing sugar yield.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Banana peel (BP), orange peel (OP) and pineapple peel (PP) were collected from a fruit stall in Kangar, Perlis. The peels were cleaned and cut into small pieces, about 2-3 inch. The small pieces were sun dried for 2 days followed by oven-drying for 48 hours with temperature 70° C (Harahap et al., 2019). After that, dried peels were ground into fine powder followed by sieving using a sieve shaker (RETSCH AS 200, Germany) through 30 mesh size to obtain a particle size of 1–3 mm. The sieved materials were stored in airtight bags at room temperature (Tiwari et al., 2017) until further use.

2.2 Microwave Pretreatment of Fruit Peel Waste

Microwave pretreatments were performed using a domestic microwave (Electrolux EMS21400W, Sweden) with an operating frequency of 2450 MHz and output power range of 80 to 600 W. Sample pretreatment was done using 10% (w/v) biomass loading and distilled water. Ten g of fruit peel powder (BP, OP and PP) was thoroughly mixed with 100 ml of distilled water and heated in the microwave at 450 W for 5 minutes (Tongkham et al., 2017). Each experiment was done in triplicates and repeated to ensure data consistency. The pretreated samples were then separated by filter paper. The solid residues were washed with distilled water and dried in an oven (Memmert UF 110, Germany) at 60℃ for 24 hours (Jin et al., 2020) prior to enzymatic hydrolysis. Fruit peel powder which produced the highest reducing sugar concentration was selected for the next optimization work and characterized using SEM and FTIR analysis.

2.3 Optimization of Microwave Pretreatment of Fruit Peel Waste

Optimization of the pretreatment conditions was carried out using Response Surface Methodology (RSM) with a Central Composite Design (CCD) to determine the optimal conditions for maximum reducing sugar production. The fruit peel that yielded the highest reducing sugar concentration in the preliminary experiments was selected as the substrate. The optimization involved three independent variables: microwave power (X_1), reaction time (X_2), and biomass loading (X_3). The parameter levels (−1, 0, and +1) represented the low, moderate, and high settings of each variable, as shown in Table 1. A total of 15 experimental runs were generated using Design Expert software version 11.1.2 (Stat-Ease Inc., Minneapolis, MN, USA), with reducing sugar concentration as the response variable (Table 2). The ranges of the parameters; microwave power (180–450 W), reaction time (3–7 min), and biomass loading (5–15% w/v) were selected based on previous studies (Ethaib et al., 2020; Jomnonkhaow et al., 2024; Pooja et al., 2018; Tongkham et al., 2017). Analysis of

variance (ANOVA) was conducted to assess the model's adequacy and determine the significance of each factor using the p-value and F-value. Three-dimensional response surface and contour plots were generated from the regression models to visualize the effects and interactions among the variables.

Table 1: Experimental Levels of the Parameters

Parameter	Symbol	Parameter level		
		-1	0	1
Microwave Power (W)	X_1	180	300	450
Time (min)	X_2	3	5	7
Biomass Loading % (w/v)	X_3	5	10	15

Table 2: Optimized Conditions for Microwave Pretreatment

Run	Parameters		
	Power (Watt)	Time (min)	Biomass Loading (% w/v)
1	300	5	10
2	180	5	15
3	180	3	10
4	300	5	10
5	450	5	15
6	300	3	15
7	180	7	10
8	300	5	10
9	450	7	10
10	450	3	10
11	300	3	5
12	450	5	5
13	300	7	15
14	180	5	5
15	300	7	5

2.4 Enzymatic hydrolysis

Enzymatic hydrolysis was conducted in an incubator shaker at 50°C, 150 rpm for 48 hours using 0.1 M sodium acetate buffer solution (Pocan et al., 2018). First, 200 mg of treated fruit peel waste (FPW) were mixed with 20 ml of 0.1M sodium acetate buffer with pH 5 and 1 g of cellulase enzyme with an activity equivalent to 7.4 FPU/ml. After that, the samples were immersed in boiling water for 5 min to stop the enzymatic hydrolysis. Then, the samples were centrifuged at 5000 rpm for 3 minutes and the supernatants were tested for sugar analysis using DNS method (Pocan et al., 2018).

2.5 Sample Analysis

2.5.1 Determination of Reducing Sugar Concentration

The reducing sugar concentrations were determined using the DNS method. The supernatant obtained from the enzymatic hydrolysis were diluted with distilled water and DNS reagent with a ratio of 1:1. The test tubes were placed in the water bath at 100°C for 5 min and later cooled in ice water to room temperature. Absorbance of the samples were measured at 540 nm using UV-Vis spectroscopy (Agilent Cary 60, USA) (Jin *et al*., 2020).

2.5.2 Determination of Surface Morphology

The surface morphology of native and selected pretreated fruit peels was analyzed by using Scanning Electron Microscope (SEM) (JEOL JSM-IT300LV, Japan). The sample was placed on the conductive tape and coated with thin conductive platinum film. The image of the samples was observed at magnification of 100X (Jamsai *et al*., 2019).

2.5.3 Determination of Functional Groups

Fourier transform infra-red spectroscopy (FTIR) (Perkin-Elmer Model 1600, USA) was used to determine the functional groups of native and selected pretreated fruit peels due to changes in functional group after pretreatment. One mg of dried sample was added with 100 mg of potassium bromide in sample preparation. The mid FTIR region between 600 cm^{-1} to 4000 cm^{-1} was used in the analysis at 4 cm^{-1} resolution (Jamsai et al., 2019). The functional group were compared by identifying the spectrum peak of each sample.

3. RESULTS AND DISCUSSION

3.1 Production of Reducing Sugar from FPW

In this study, orange peel waste (OPW), banana peel waste (BPW), and pineapple peel waste (PPW) were tested for the production of reducing sugar through the application of microwave pretreatment, as shown by Figure 1. The results demonstrated that after enzymatic hydrolysis, pretreated PPW produced maximum reducing sugar which is 10.58 (g/L). Although BPW had higher cellulose content compared to PPW in lignocellulosic content analysis (Redondo-Gómez et al., 2020; Ali et al., 2020), lower lignin content in pineapple peel waste might be the main cause of higher reducing sugar production.

Microwave pretreatment caused the disruption on the surface area of the FPW through dielectric polarization, thus increase the accessibility surface area of cellulose to enzyme. In spite of the fact that microwave power has direct impact on fruit peel structural disruption, an increase in microwave power might degrade cellulose structure as well (Miller et al., 2021). From the results shown, microwave power of 450 Watt and pretreatment time for 5 min may have disrupted the lignin and hemicellulose in the PP. Thus, PPW was selected to undergo process optimization.

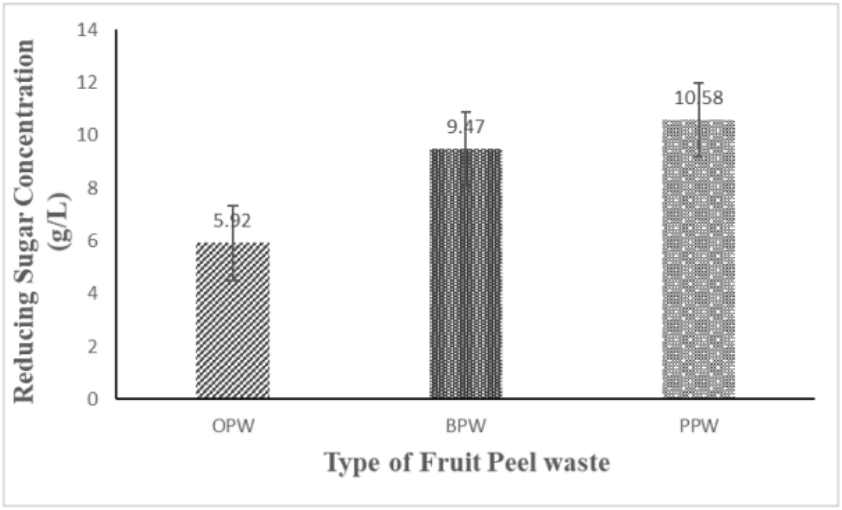


Figure 1: Reducing Sugar Production from Different Microwave Pretreated Fruit Peel Waste. OPW: Orange Peel Waste; BPW: Banana Peel Waste; PPW: Pineapple Peel Waste

3.2 Optimized Pretreatment Condition

In the present study, RSM was utilized to optimize key microwave pretreatment variables, including power, time, and biomass loading. Table 3 presents the Central Composite Design (CCD) matrix along with the corresponding experimental results. The highest reducing sugar concentration of 12.52 g/L was achieved at 450 W microwave power, 5 minutes exposure time, and 15% (w/v) biomass loading. In contrast, the lowest reducing sugar concentration of 9.48 g/L was observed at 180 W, 3 minutes, and 10% (w/v) biomass loading, suggesting that insufficient power and shorter irradiation time limited the hydrolysis efficiency of the biomass.

Table 3: Experimental Design for Microwave Pretreatment of PPW

	Parameters			Response
Run	**Power (watt)**	**Time (min)**	**Biomass Loading (% w/v)**	**Reducing Sugar Concentration (g/L)**
1	300	5	10	9.99
2	180	5	15	9.49
3	180	3	10	9.48
4	300	5	10	10.28
5	450	5	15	12.52
6	300	3	15	10.46
7	180	7	10	9.79
8	300	5	10	10.07
9	450	7	10	10.05
10	450	3	10	12.30
11	300	3	5	11.89
12	450	5	5	9.74
13	300	7	15	10.16
14	180	5	5	9.61
15	300	7	5	10.28

Analysis of Variance (ANOVA) is a statistical method used to test the difference between two or more means. This statistical method is used to measure the accuracy of the model that developed. In analyzing the model from Table 4, the p-value was less than 0.05 showing that the model developed was significant. In the model, the F-value of 97.69 indicated that the model was significant and there was only < 0.01% chance that the model F-value shown this large could occur due to noise. The ANOVA analysis also showed the lack of fit F-value of 5.54, implying the lack of fit is not significant relative to the pure error. There is a 16.08% chance that a lack of fit F-value this large could occur due to noise.

Table 4: ANOVA for Response Surface 2FI Model for Reducing Sugar Production

Source	Sum of Square	df	Mean square	F-value	p-value	
Model	13.74	6	2.29	97.69	<0.0001	significant
A-Power	2.73	1	2.73	116.41	<0.0001	
B-Time	2.57	1	2.57	109.78	<0.0001	
C- Biomass Loading	6.47	1	6.47	276.09	<0.0001	
AB	0.6155	1	0.6155	26.26	0.0009	
AC	1.37	1	1.37	58.29	<0.0001	
BC	9.5816	1	9.5816	24.81	0.0011	
Residual	0.1875	8	0.0234			
Lack of Fit	0.1769	6	0.0295	5.54	0.1608	Not significant
Pure Error	0.0106	2	0.0053			
Cor Total	13.93	14				

The 2FI model has been chosen to demonstrate the correlation for glucose production, as suggested by Design Expert Software version 11.1.2. Based on the experimental data, the final empirical model in terms of code factor for glucose production from PPW has been developed. The equation in terms of coded factors can be used to predict the response for given levels of each factor by comparing the factor coefficients. Using the regression coefficient values from the equation, a model was created as given by Equation (1);

$$Reducing\ sugar\ concentration = 10.44 + 0.5824A + 0.5689B + 0.9022C + 0.3911AB\ (4.1) + 0.5827AC + 0.3813BC \quad (1)$$

According to Table 5, the predicted R-squared value of 0.9472 (94.72 %) and the adjusted R-squared value of 0.9764 (97.64 %) are in reasonable agreement, indicating that the model is significant. In the model, adequate precision indicates the signal-to-noise ratio that is used to measure the range predicted response for the occurrence of possible errors. A ratio greater than 4.0000 is desirable based on the correlation of coefficient value. The adequate precision is 29.7684, indicating that the model is a sufficient signal that can be used to navigate the design space. In addition, low value of standard deviation (0.1531) was obtained showing a good precision and lower dispersion of the model. The coefficient of variation (CV) is a measure of spread that describe the amount of variability relative to the mean. In the developed model, CV obtained was 1.47 %. Overall, small values of the standard deviation and coefficient of variance, reflect the reproducibility of the model developed.

Table 5: R-squared Value for Response Parameter

Parameters	Value
Standard deviation	0.1531
Mean	10.41
CV%	1.47
R^2	0.9865
Adjusted R^2	0.9764
Predicted R^2	0.9472
Adequate precision	29.7684

Model analysis in RSM was utilized to visualize the relationship between the response and the variables. Each contour curve represents an infinite number of variable test combination permutations. The red region of the contour diagram represents the maximum predicted value.

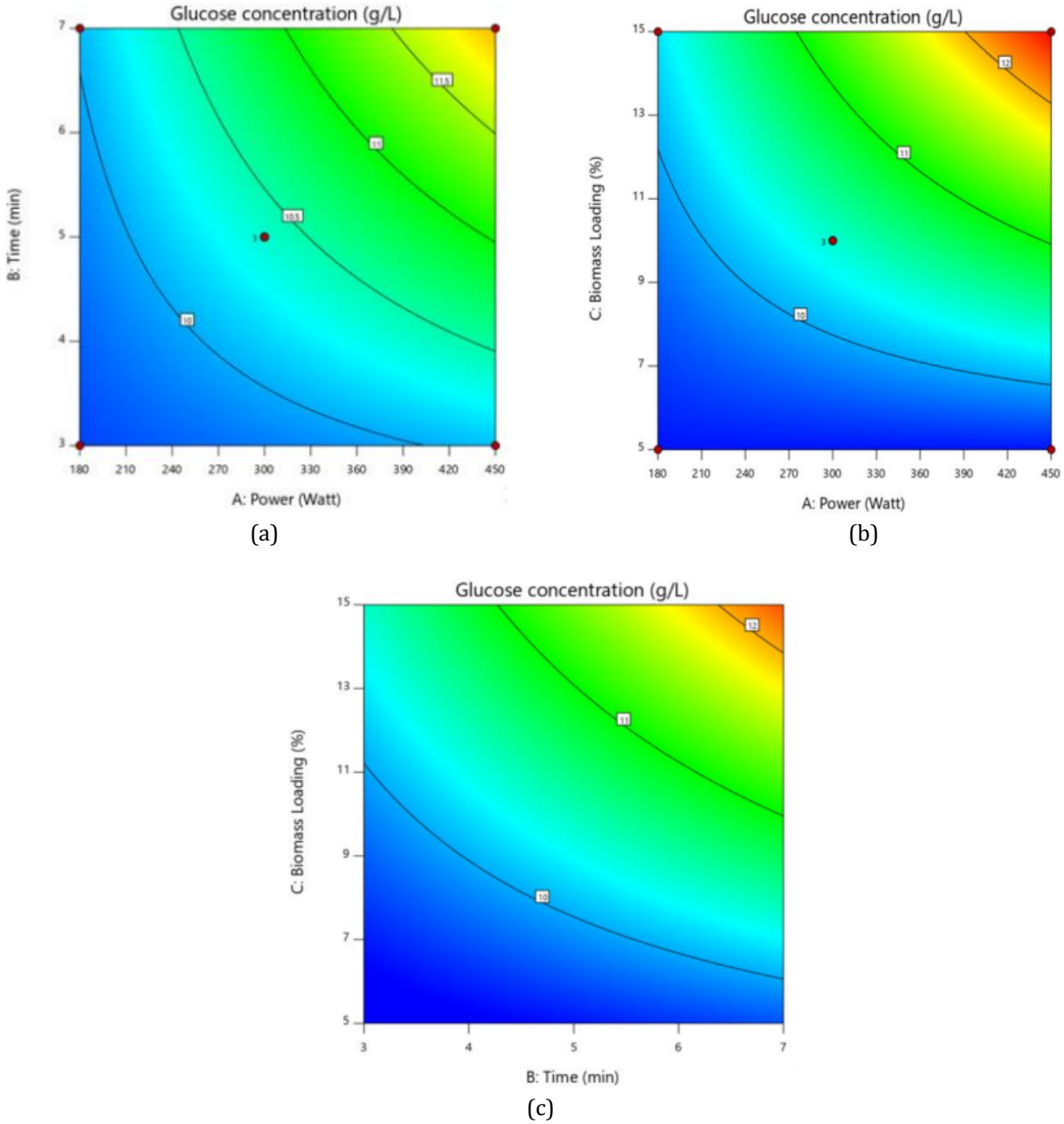


Figure 2: 2D Contour Plots Towards Reducing Sugar Production: (a) Microwave Power Versus Irradiation Time (b) Microwave Power Versus Biomass Loading, (c) Irradiation Time versus Biomass Loading

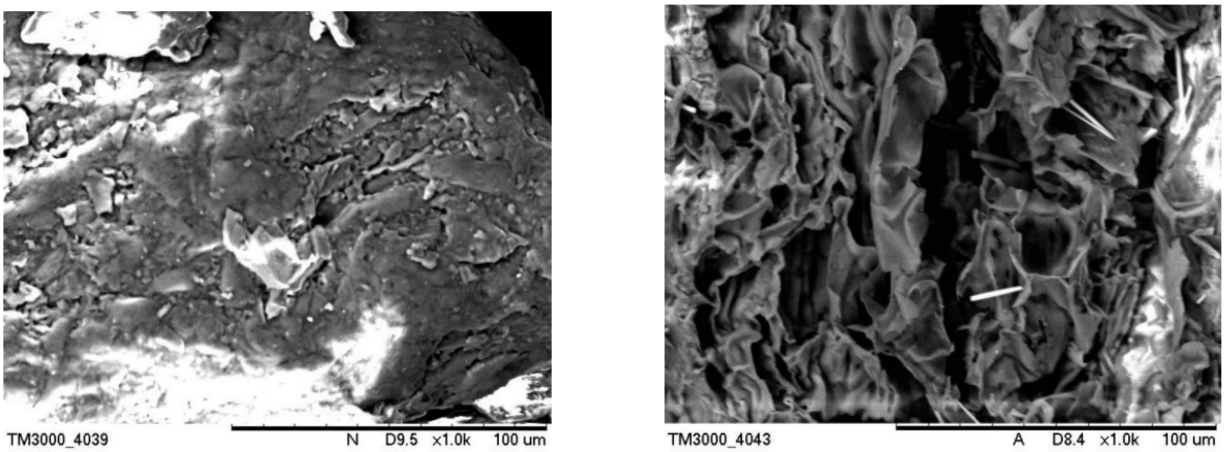
According to Figure 2 (a), the contour plot illustrating the interaction between microwave power and reaction time on glucose concentration exhibited an elliptical shape, indicating a significant reciprocal interaction between the two parameters. An increase in both power and time at a constant biomass loading of 10% (w/v) enhanced the reducing sugar yield. The optimum power was observed around 450 W, suggesting that lower power levels may limit the extent of cellulose disruption and consequently reduce the availability of hydrolysable cellulose, leading to a lower sugar yield [Indriani *et al*., 2021]. Conversely, higher power levels increased the surface area exposed to microwave radiation, enhancing cellulose degradation and sugar release (Hoang *et al.,* 2021). Furthermore, extending the reaction time from 3 to 7 min resulted in a corresponding increase in reducing sugar yield. Based on the observed trend, it can be inferred that increasing both reaction time and power beyond the studied ranges may further enhance reducing sugar production, although excessive conditions could risk sugar degradation [Ethaib *et al*., 2020].

Figure 2 (b) is the 2D contour plot for the combined effect of reaction power and biomass loading when the time was maintained at 5 min. From the model analysis, it indicated the mutual interaction between the power and biomass loading were in elliptical form. It can be observed that reducing sugar production increased with increasing power and biomass loading whereby the optimum biomass loading was 15 % (w/v). This is because high biomass loading would generate high cellulose availability for enzymatic hydrolysis. Lastly, Figure 2(c) illustrates the combined effect of reaction time and biomass loading on glucose production under a constant microwave power of 300 watts. The results demonstrated that increasing both time and biomass loading led to a higher yield of reducing sugars. As reported by Alexander *et al.,* (2020), extended microwave irradiation enhances delignification due to rapid molecular collisions and disruption of the lignocellulosic matrix, thereby facilitating greater sugar release.

3.3 Alteration of Surface Morphology and Functional Group

3.3.1 Surface Morphology

Surface morphology study on untreated and pretreated PPW was carried out by using Scanning Electron Microscopy (SEM) to analyze the microstructural changes of the PPW after microwave pretreatment. The PPW was observed under 100 times magnification to get the clear image of pores (Ali et al., 2020). Figure 3(a) shows that the surface of untreated PPW exhibiting minimal porosity, whereas Figure 3(b) reveals the surface of microwave-treated PPW containing significantly larger and more abundant pores. This indicated that microwave pretreatment effectively disrupted the PPW structure, resulting in increased porosity and surface area, thereby enhancing enzyme accessibility (Jamsai *et al*., 2019).

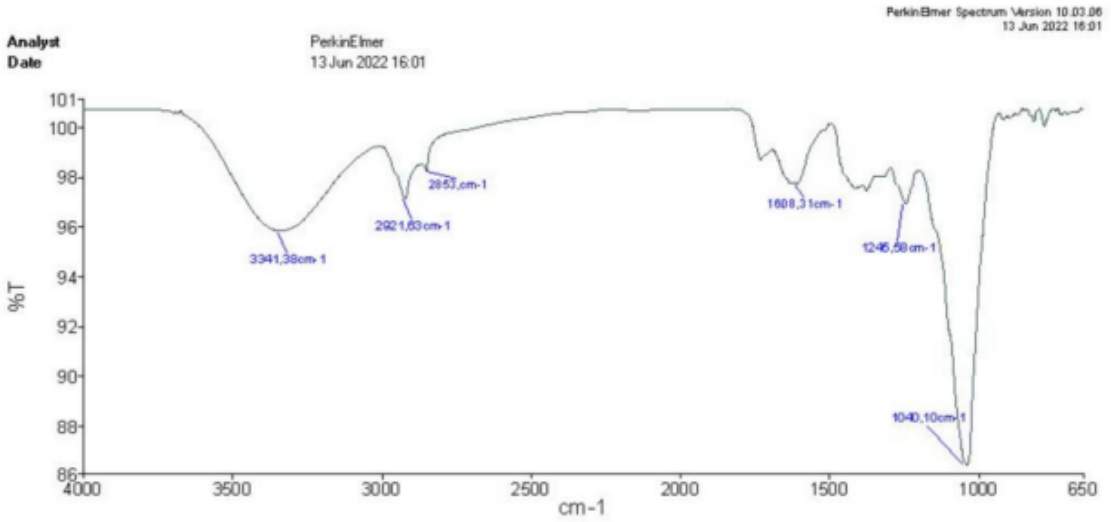


(a) (b)

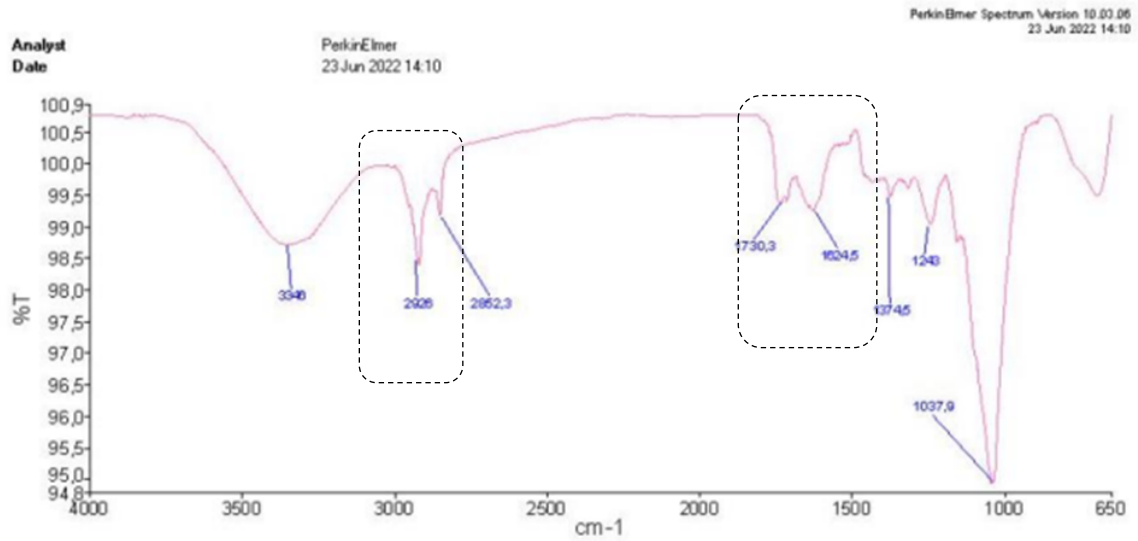
Figure 3: SEM Micrograph of (a) Untreated and (b) Pretreated PPW

3.3.2 Functional Group

Fourier-transform infrared spectroscopy (FTIR) was used to study the functional group of PPW and the change caused by the treatment. The spectra also provide the information suggesting the presence and the absence of compounds, also the intensity of an absorption band changes following treatment. Figure 4 (a) and (b) shows the FTIR spectra of untreated and pretreated PPW in the fingerprint region between 4000 – 650 cm^{-1}. Clear difference can be detected in the infrared spectra, both in different absorbance and shape of the bands and in their location.



(a)



(b)

Figure 4: FTIR Spectra of (a) Untreated PP and (b) Pretreated PPW

From the previous study of the PP, it was mainly composed of 83% cellulose, 10% lignin, 1% pectin, and 19% hemicellulose. Thus, the major assigned group of the PPW that took part in the microwave pretreatment process such as hydroxyl, polysaccharide, carbonyl and ester corresponded to the major composition of the PPW (Ali et al., 2020). The 3550-3200 cm^{-1} and 3000-2500 cm^{-1} bands in Figure 4 (a) and (b) corresponded to the hydroxyl groups (inter, intra, and free OH) and the stretching vibration of alkyne. C=C stretching in a conjugated alkene is responsible for the band at

1680-1600 cm^{-1}. The peak is the aromatic C=C of the aromatic rings of lignin. The band between 1275 and 1200 cm^{-1} is due to C–O–C stretching in aryl–alkyl ether (Casabar et al., 2019). This peak is typically observed when the lignin macromolecule subunit guayacil ring is present. The band between 1070 and 1030 cm^{-1} is due to S=O stretching in sulfoxide. Comparison of Figure 4 (a) and (b) indicated that the C-H stretching of methyl and methylene group of cellulose and lignin slightly increased at peak 2926 cm^{-1} and 2852.3 cm^{-1} after microwave pretreatment. In addition, the pretreated PPW spectrum (Figure 4 (b)) has two bands at 1730 and 1374 cm^{-1}. Peaks at 1730 cm^{-1} is attributed to carbonyl stretching in conjugated anhydride and O-H bending in phenol (Casabar et al., 2019). It is due to either the acetyl and uronic ester groups of hemicellulose or the ester linkage of the carboxylic groups of ferulic and p-coumeric acids of lignin and hemicelluloses. Besides, the peak at 1374 cm^{-1} represented the CH_3CH bending of cellulose in the FTIR spectra of pretreated PPW, implying that microwave pretreatment enhanced the cellulose content (Md Salim *et al*., 2021). The bands at 700-900 cm^{-1} for all samples represent C-H bending in -glycosidic sugar linkages.

4. CONCLUSION

This study demonstrated that microwave pretreatment is an effective strategy to enhance reducing sugar production from FPW, with PP showing the greatest potential. Microwave power, exposure time, and biomass loading were found to strongly influence sugar yield by governing the disruption of cellulose, hemicellulose, and lignin. Structural analyses via FTIR and SEM confirmed that controlling these parameters directly affects polymer breakdown, highlighting the importance of process optimization. Careful adjustment of these factors is therefore essential to maximize sugar release from agricultural residues. Overall, microwave pretreatment represents a promising approach to valorize fruit peel waste into fermentable sugars for biofuel production.

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