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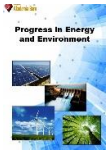

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Original Article

Investigation on the potential of bioethanol synthesis from honeydew melon rind

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Abstract

Honeydew melon rind is an abundant and low-cost fruit waste, which holds potential for glucose and eventual bioethanol synthesis. In this study, alkaline pre-treatment was introduced to break down the lignin structure before conducting acid hydrolysis to further breakdown the cellulosic components into glucose. The functional group of the pre-treated and raw samples were analysed using Fourier-Transform Infrared (FTIR) spectroscopy to evaluate the effectiveness of alkaline pre-treatment in lignin removal. Alkaline pre-treatment was found to effective in lignin removal from the rind, thus, improving the accessibility of cellulose and hemicellulose for acid hydrolysis. Using response surface methodology (RSM) based on the central composite design, a maximum glucose concentration of 9.847% w/v was obtained using an acid concentration of 6% w/v, a reaction temperature of 75°C for 60 minutes. The hydrolysate which was fermented using the Saccharomyces cerevisiae (baker's yeast) revealed the presence of ethanol as the major product with some traces of impurities. This shows that honeydew melon rind has the potential as lignocellulosic biomass source for bioethanol synthesis.

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1 Introduction

Over the last decades, human demand for mobility and transport has been significantly amplified as a result of urbanisation growth experienced by many countries in the world, which thus, leading to the increased dependence of automobile transportation [1-2]. Consequently, this phenomenon has directly placed pressure on energy demand, as the use of transportation is substantially energy-consuming [3]. At present, the vast majority of global transport's energy is still dominated by fossil fuels such as petroleum and diesel, which will continue to grow in the near future [4].

Meanwhile, there are some major issues with the continued use of fossil fuels as an energy supply for transportation [5]. As fossil fuels are inherently finite and will deplete with the passage of time, the assurance of a secure supply of fossil fuel is difficult to achieve as most countries still rely heavily on fossil fuel to feed their growing energy demand [6]. This will indeed accelerate the consumption of fossil fuel and poses a major threat to the world's natural energy resources in the future [7].

Moreover, the use of fossil resources as a transportation fuel is not regarded as sustainable and questionable from the ecology and environmental point of view due to its adverse effects of greenhouse gases (GHG) emissions on the environment, coupled with declining oil reserves [4]. Consequently, the interest has shift to the use of biofuels as an alternative to fossil fuel with the finite supply of fossil fuel and the environmental threats caused by the exploitation of non-renewable sources, particularly in terms of carbon emissions [8].

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In order to satisfy the global energy demand, bioethanol has been recognised as a viable alternative to fossil fuels which can offer a reduction in petroleum consumption, as it could be derived from any material containing starch and sugars [9]. However, the sustainability of starch - derived ethanol still remains as a primary concern due to their primary value as food supply [10]. Therefore, to secure long term sustainable energy supply while minimising the competition between fuels and food production, the change of focus to the use of lignocellulosic biomass and waste as bioethanol feedstock is gaining momentum [11].

The current research interest is often focusing on using low-cost and abundant availability of food waste as potential feedstock for bioethanol synthesis owing to its advantages in landfill waste diversion and GHG reduction [11]. The food waste selected for this study is honeydew melon rind, which is considered as a waste from one of the most abundant tropical fruits in the world due to its capacity to adapt to different kinds of soil and climates [12]. Moreover, several million tons of honeydew melon wastes are produced annually from factories [13]. After the consumption of honeydew, a large volume of honeydew melon rinds is generated as a by-product, which contributes about 15 - 20% of the whole fruit weight depending on the variety [14]. As the rind is currently not utilized for commercial purpose, it often ends up as a landfill waste and becomes a source of pollution [15].

Furthermore, waste generated from melon, which the scientific name is Cucumis melo, belongs to the family of Cucurbitaceae which includes cucumbers, watermelon, cantaloupe, squash, and pumpkins. This family of plant is a popular choice for bioethanol production due to its high percentage of sugar content that can be hydrolysed to glucose and subsequently fermented into ethanol [16]. A recent work by Salehi et al. [16] had successfully synthesized a maximum 49.5 g/L of bioethanol using 69 g/L of glucose obtained from 12 kg of cantaloupe rind. On the other hand, Bhandari et al. [17] also studied the amount of bioethanol obtained from different raw materials, which are pineapple, watermelon, jackfruit and muskmelon. They reported an ethanol yield of 4.64 g/L, 4.38 g/L, 3.08 g/L and 1.89 g/L from jackfruit, pineapple, watermelon, and muskmelon respectively. However, the present researches on bioethanol synthesis using honeydew melon rind as feedstock are very limited among most studies. Therefore, this research can be used to develop a further understanding on the bioethanol synthesis from lignocellulosic biomass by discovering the potential of the honeydew melon rind as a potential substrate for green energy production [18].

Other than that, the selection of an appropriate synthesis pathway is also another important aspect that would directly impact the conversion rate of the bioethanol and overall production cost [18]. There is a wide variety of emerging synthesis process to produce bioethanol, however, the practical bioethanol production process for scaling up should be able to offer advantages in terms of economical operation and technical simplicity such as low energy consumption, high production efficiency, and less environmental impact [19]. Since the synthesis process is closely linked to the nature of the feedstock and its recalcitrance, enormous research efforts have been done to identify the best option for economically effective bioethanol production [20].

Overall, the synthesis pathway of bioethanol can be divided into four main processes: pre-treatment, hydrolysis, fermentation and purification [21]. In this study, alkaline pre-treatment and dilute acid hydrolysis were selected owing to their technical simplicity and economical operation [20,22]. With that, our work aims to provide insight on the effectiveness of alkaline pre-treatment in the lignin removal, investigate the impact of variation of operating parameters on acid hydrolysis on glucose synthesis and hence evaluate the potential of honeydew melon rind in the bioethanol synthesis.

2 Methodology

2.1 Materials

Ultra-pure water with its particulates less than 0.22 μm (Milli-Q ®, Model Advantage A10) was used in performing sample preparation and chemical dilution. Sodium hydroxide pellets (R&M Chemicals) and sulphuric acid (Friendemann Schmidt Chemical) with a purity of 98.0% and 97.0% respectively were used in performing pre-treatment, hydrolysis, and pH adjustment. The construction of the calibration curve for glucose determination was done using 99.5% anhydrous D-glucose (R&M Chemicals). Ethanol with 99.9% purity (LiChrosolv) was used for Gas Chromatography Mass Spectrometer (GC-MS) injection as a standard peak to compare with the GC-MS result for potential bioethanol sample synthesized in this study.

2.2 Collection and Preparation of Sample

The honeydew melon rind was prepared from the fresh honeydew melon purchased Wangsa Walk Mall, Kuala Lumpur. The separated and cleaned honeydew melon rind was allowed to dry for 24 hours in an oven (Memmert, Model UFE 700). The drying temperature was maintained at 40°C to avoid denaturisation of the sample [23]. Before proceeding to the cutting process, the moisture measurement of the sample was performed using Moisture Analyser (Ohaus, Model MB 25) and summarized in Table 1 to ensure that the moisture content of the samples is within an acceptable range of 10% to 14% [24]. In this study, three batches of sample were being prepared, with each batch consisted of two honeydew melons rind, sufficient for 5 experimental runs. After that, the sample size was reduced into powder form using Universal Cutting Mill (Fritsch, Model Pulverisette 19) with a sieve cassette of 0.5 mm and stored in airtight glass sampling bottle before further usage for this work.

Table 1 Moisture content of the honeydew melon rind powder for each batch.

Batch number of sample	Moisture content of the sample	Average moisture content of the sample
First Batch	12.32	12.34
	12.42	
	12.29	
Second Batch	12.38	12.36
	12.27	
	12.43	
Third Batch	12.31	12.30
	12.25	
	12.34	

2.3 Experimental Set-Up

For alkaline pre-treatment, the honeydew melon rind powder was introduced into sodium hydroxide solution with a constant concentration of 3% w/v, which equivalent to 0.75 mol/L. The solid-to-liquid ratio was maintained at 1:10 g/mL under an agitation of 120 revolutions per minute (rpm) for 30 minutes using magnetic hotplate stirrer (V.go, Model 540C) [25-27]. The solution then underwent vacuum filtration to remove the hydrolysate. The washed solid sample was allowed to dry for another 24 hours in an oven at a drying temperature of 40 °C before proceeding to acid hydrolysis. The effectiveness of the lignin removal was verified using Fourier-Transform Infrared (FTIR) spectroscopy.

For acid hydrolysis, response surface methodology (RSM) based on the central composite design (CCD) was used for analysis of the parametric study. The RSM was performed using Design Expert ® Version 11 Software Trial (State-Ease, Inc., Minneapolis, USA). The pre-treated sample with a fixed solid-to-liquid ration of 1:10 g/mL was introduced with different concentration of dilute sulphuric acid (2, 4, and 6% w/v) and reaction duration (30, 45, and 60 minutes) at a reaction temperature of 25°C to 75°C under a constant stirring speed of 120 rpm [28]. Table 2 summarises the independent variables with respect to their levels for CCD experimental design. A total of 20 experiment runs were carried out. The liquid fraction was collected and subjected to glucose content analysis using benchtop refractometer (Reichert, Model Abbe Mark III). The liquid fraction with the highest glucose concentration was subsequently selected to proceed for investigation of the potential of the honeydew melon rind in bioethanol synthesis.

For bioethanol synthesis, the fermentation process was conducted using Saccharomyces cerevisiae (Baker's Yeast). The incubation temperature was maintained at 25°C inside incubator shaker (Lab Companion, Model IST-3075R) under neutral condition of pH 7 [29] with an agitation rate of 150 rpm [30] for 96 hours. All the apparatus used in this process also underwent ultraviolet (UV) sterilisation using UV Sterilizer Cabinet (DP, Model LALCO) for 5 minutes to prevent the growth of other microbes and hence reduce the risk of contamination [31].

The resulting slurry was centrifuged at 10,000 rpm and 25°C for 15 minutes using refrigerated centrifuge (Eppendorf, Model 5804) to separate into supernatant and precipitated fractions. The

precipitated fraction was removed while the supernatant fraction was purified using a rotary evaporator (Hei-VAP, Model Core) to separate ethanol from the mixture. The final product underwent qualitative analysis of ethanol using Gas chromatography-mass spectrometry (GC-MS) method.

Table 2 Independent variables and their respective levels used for CCD experimental design.

Parameters	Level		
	-1	**0**	**+1**
Acid Concentration (% w/v)	2	4	6
Duration (Minutes)	30	45	60
Reaction Temperature (°C)	25	50	75

2.4 Chemical Analysis

2.4.1 Functional Group Study on the Alkaline Pre-Treated Sample

Data were analysed using Microsoft Excel Data Analysis's single factor ANOVA (Analysis of Variance) for significant difference by setting 95% confidence level ($p < 0.05$). Result is expressed as mean value ± standard deviation ($n = 8$).

2.4.2 Qualitative Analysis of Glucose

The determination of the glucose content was carried out using benchtop refractometer (Reichert, Model Abbe Mark III). A calibration curve was developed using 7 glucose standard solution with concentration ranging from 0 to 12% w/v as shown in Appendix. The result obtained was aligned with the theoretical value of the refractive index of glucose solution, ranging from 1.33 to 1.50 [32]. The result obtained from acid hydrolysis was compared to the plotted calibration curve of known glucose concentration to determine the actual glucose concentration for each sample.

2.4.3 Qualitative Analysis of Ethanol

The distillate obtained from the rotary evaporator was analysed using Gas Chromatograph (GC) (Agilent, Model 7890a) fitted to Inert Mass Selective Detector (Agilent, Model 5975C) with Triple-Axis Detector for qualitative confirmation of bioethanol. A low bleed GC-MS column (Agilent J and W, Model HP5-MS) with 30 m (length) × 250 μm (internal diameter) × 0.25 μm (film thickness) consisted of 5% phenyl - 95% dimethylsiloxane coating was used for this analysis. GC-MS grade Helium (99.999% purity) was used as the carrier gas and its flow rate was set at 1 mL per minute. 1 μL of the sample was injected into the GC under split mode at a 50:1 split ratio. The temperatures for the injector, interface, and ion source were all kept at 250°C. The column oven temperature was initially held at 37°C for 2 minutes, then raised to 40°C at the rate of 2 ℃/min and maintained for 3 minutes. The retention times of the sample was then compared with the standard ethanol solution (99.9% purity) to identify the ethanol present in the synthesized sample.

3 Results and Discussion

3.1 Efficacy of Alkaline Pre-treatment

The evaluation of efficacy of alkaline pre-treatment in lignin removal was evaluated through the formation and disappearance of certain functional groups before and after the pre-treatment. Fig. 1 depicts the FT-IR spectra for (a) pre-treated and (b) raw honeydew melon rind powder while the assignment of each peaks corresponding to the functional groups of lignocellulosic biomass components according to the literature were tabulated in Table 3.

Based on Fig. 1, the FT-IR spectra between the raw and pre-treated samples showed some differences in terms of intensity and shape. The broadband at 3297 cm^{-1} and 2923 - 2852 cm^{-1} were attributed to stretching vibration O-H of hydrogen bond and C-H, respectively, which reveals the typical structure of hemicellulose, cellulose, and lignin [33]. A significant reduction in the intensity of these peaks

implies that some alcohol and alkane were cleaved after alkaline pre-treatment, which result in the reduction of lignin composition in the sample along with some rupture of the structure from the hemicellulose and cellulose [29,33].

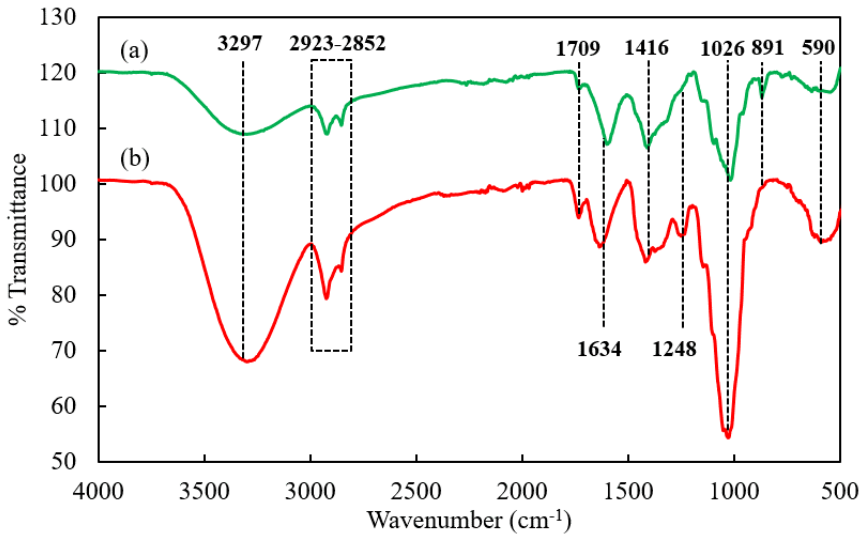

Fig. 1 FTIR spectra of (a) pre-treated, and (b) raw honeydew melon rind samples.

This is due to the fact that sodium hydroxide possessed the ability to cleave the aromatic linkage in lignin, creating more amount of free phenolic hydroxyl groups. As a result, the presence of these hydroxyl groups will increase the solubility of the lignin and hence reduce the crystallinity of the lignocellulosic structure, making them vulnerable for glucose synthesis [30].

In addition, the signal at 1416 cm^{-1} corresponding to the C-H bending of the methyl group also represents the characteristic of all three lignocellulosic components. However, no visible changes were noticed from the peak intensity, which indicates that the alkane groups remain present in the honeydew melon rind despite asymmetric deformation vibration [31]. Moreover, the broadband observed at 1026 cm^{-1} in both samples was assigned to C-O stretching vibration, which was exhibited as primary alcohol from lignin and cellulose. It is noticeable that the intensity of the peak becomes lower, indicating that the composition of lignin and cellulose decreased after the pre-treatment [34-35]. These findings are aligned with the existing findings in the literature describing the effectiveness of alkaline pre-treatment in lignin removal with minimal loss of hemicellulose and cellulose.

Other than that, the broadband presented at 1709 cm^{-1}, 1634 cm^{-1}, 1248 $cm^{-1,}$ and 590 cm^{-1} was an indication of the functional groups that were present in lignin [33]. More particularly, the band observed at 1709 cm^{-1} and 1634 cm^{-1} corresponded to the C=O and C-C stretching respectively, which belongs to the carbonyl and alkene group from lignin. The slight changes of these peaks implies that the functional groups were disrupted after the pre-treatment [29]. The sudden appearance of a peak at 1248 cm^{-1} from the spectrum (a) was attributed to the C-O stretching, which shows the characteristic of alkyl aryl ether in lignin. The absence of the signal in the spectrum (b) indicates that the lignin interlinkages were successfully broken under alkaline condition and hence increase the accessibility of hemicellulose and cellulose to the glucose synthesis [33].

Additionally, the peak of 891 cm^{-1} was noticed in the spectrum (b) after the pre-treatment, which attributed to the C-O-C stretching vibration [31,36]. This is mainly due to the presence of β-glycosidic linkage from hemicellulose and cellulose in the pre-treated sample which supported that fact that the breakdown of lignin structure can increase the content of hemicellulose and cellulose available for glucose synthesis [31,36]. Hence, the presence of this peak confirmed the effectiveness of sodium hydroxide in lignin removal of honeydew melon rind and promote the digestibility of hemicellulose and cellulose for glucose synthesis.

In short, the study on the changes in the chemical structure of the honeydew melon rind after alkaline pre-treatment demonstrated by FT-IR analysis confirmed that alkaline pre-treatment using sodium

hydroxide was effective in lignin removal of honeydew melon rind, which can subsequently increase the accessibility of hemicellulose and cellulose in glucose synthesis.

Table 3 Assignment and description of peaks corresponding to the functional group of lignocellulosic biomass components.

Wavenumber (cm^{-1})	Functional Group Assignment	Compound Class	Related Lignocellulosic Component
3297	O-H stretching	Alcohol	Hemicellulose, Cellulose, Lignin
2923 - 2852	C-H stretching	Alkane	Hemicellulose, Cellulose, Lignin
1709	C=O stretching	Carbonyl	Lignin
1634	C=C stretching	Conjugated Alkene	Lignin
1416	C-H bending	Alkane (Methyl Group)	Hemicellulose, Cellulose, Lignin
1248	C-O stretching	Alkyl Aryl Ether	Lignin
1026	C-O stretching	Primary Alcohol	Cellulose, Lignin
891	C-O-C stretching	Aliphatic Ether	Hemicellulose, Cellulose
590	C=C bending	Alkene	Lignin

3.2 Parametric Study on Acid Hydrolysis in Glucose Synthesis

3.2.1 Analysis of Variance (ANOVA) for the Response Surface Quadratic Model

The results of the glucose concentration (% w/v) obtained from the experimental run designed based on the Design Expert software is tabulated in Table 4. The significance of the parameters towards the response was analysed statistically using ANOVA.

The validity of the model fitted by Design Expert software was evaluated. The statistical significance was controlled by Fisher's statistical test (F-test) in order to avoid the generation of disingenuous results and hence identify the model that best fits the variables from which the data were sampled. Table 4 shows the ANOVA for the quadratic response surface model.

Based on the ANOVA results in Table 4, the model was observed to be significant, with a low probability value (P-value) of <0.0001 and Fisher value (F-value) of 57.04. The value of the predicted coefficient of determination, R^2 was 0.9809. It implies that approximately 98.09% of the variance is attributed to the variables, indicating the high significance of the model. The confirmation of the adequacy of the regression model can also be reflected by the good agreement between the experimental and the predicted values of the response variables, where the experimental glucose concentration ranged from 4.04 to 9.95% w/v and the corresponding predicted values were 4.19 to 9.55% w/v, respectively.

Meanwhile, the adequate precision is often used to measure the signal-to-noise ratio in which a ratio greater than 4 is desirable [37]. The value for this work is 32.4117, suggesting that the model is reliable and can be used to navigate the design space. Moreover, the accuracy and credibility of the model can be further evaluated using the coefficient of variation (C.V.) to investigate the dispersion of the experimental values in comparison with the predicted values. Practically, a C.V. value of less than 10 % would be favourable as it implies that the estimation is reasonable and precise [37]. With that, a C.V. value of 4.10 would ensure that all the experiments were conducted with a high degree of precision and reliability. Thus, it can be concluded that the second-order quadratic model equation provided a good estimation for the glucose production in the studied experimental range. The empirical interaction between the considered variables and experimental data obtained from CCD design model is as follows:

$$Y = 5.51 + 1.11A + 0.6495B + 0.8487C + 0.2037AB + 0.3090AC + 0.5170BC + 0.0127A^2 - 0.1473B^2 + 0.5337C^2 \quad (1)$$

where,

Y: Glucose Concentration (% w/v)
A: Acid Concentration (% w/v)
B: Reaction Duration (Minutes)
C: Reaction Temperature (°C)

Table 4 ANOVA of the fitted quadratic regression model equation.

Source	Sum of Square	Mean Square	F-Value	P-Value	Remarks
Model	28.08	3.12	57.04	< 0.0001	Significant
A - Acid Concentration	12.34	12.34	225.52	< 0.0001	Significant
B - Duration	4.22	4.22	77.12	< 0.0001	Significant
C – Reaction Temperature	7.20	7.20	131.68	< 0.0001	Significant
AB	0.3321	0.3321	6.07	0.0334	Significant
AC	0.7638	0.7638	13.96	0.0039	Significant
BC	2.14	2.14	39.09	< 0.0001	Significant
A^2	0.0004	0.0004	0.008	0.9301	Not Significant
B^2	0.0597	0.0597	1.09	0.3208	Not Significant
C^2	0.7832	0.7832	14.32	0.0036	Significant
Predicted R^2	0.9809	-	-	-	-
Adjusted R^2	0.9637	-	-	-	-
Adequate Precision	32.4117	-	-	-	-
C.V. (%)	4.10	-	-	-	-

3.3.2 Impact of Acid Concentration

The concentration of sulphuric acid played a significant role in determining the quantity of glucose during acid hydrolysis by breaking the linkage of hemicellulose and cellulose [38]. Fig. 2 depicts the impact of acid concentration on the glucose concentration under a reaction temperature of 75℃ for 60 minutes. A sharp increment was noticed on the glucose concentration the concentration of acid was increased from 2% w/v to 6% w/v. The highest glucose concentration obtained in this study condition was found to be 9.847% w/v. Similarly, Ramos-gonzalez et al. [35] also reported a maximum glucose concentration of 9.04% w/v from the acid hydrolysis of banana peel at 80℃ for 30 minutes when the higher acid concentration of 7.5% w/v was introduced. This trend can be explained by the fact that acid at higher concentration possessed greater strength in solubilising the structure of hemicellulose and cellulose, making them easier to be hydrolysed [39]. In other words, more H^+ ion can be supplied at a higher acid concentration to cleave the C-O-C bond and form glucose as a result [40]. Thus, an increase in acid concentration could provide a strong reaction for breaking down the chemical bonds inside honeydew melon rind and achieve higher glucose concentration in the hydrolysate.

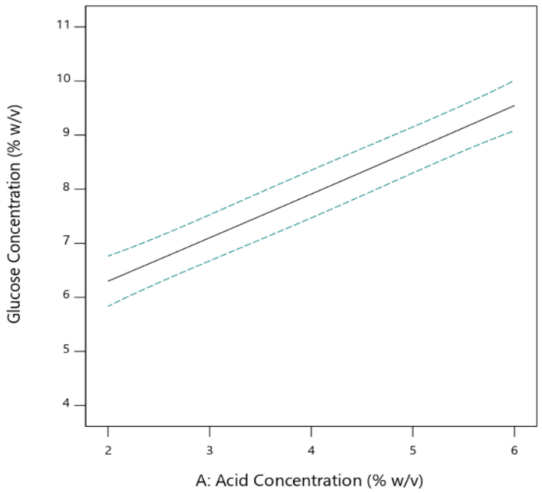

Fig. 2 The impact of acid concentration on the glucose concentration (Condition: temperature = 75°C; duration = 60 minutes).

3.2.3 Impact of Reaction Duration

Reaction duration is another important parameter affecting the glucose concentration in acid hydrolysis [38]. Fig. 3 depicts the impact of reaction duration on the glucose concentration using 6% w/v of sulphuric acid at 75°C. The glucose concentration was gradually increased from 6.03% w/v to 9.847% w/v

as the reaction duration increase from 30 minutes to 60 minutes. This trend was in agreement with the results obtained by Goud et al. [41] in the acid hydrolysis of sugarcane bagasse using 9%w/v of sulphuric acid at 80°C. The increasing reaction duration from 30 minutes to 60 minutes resulted in a significant increment in the glucose yield from 130 mg/g to 300 mg/g. This is because greater reaction duration can allow the hemicellulose and cellulose structure to contact with the more H^+ ion. As a result, more β-glycosidic linkage is allowed to be cleaved and hence more glucose can be produced [39].

Besides, the behavior of the result obtained also revealed that the acid hydrolysis reaction almost goes to completion, achieving equilibrium stage with the maximum glucose concentration. However, the extension of this operating parameter to more than 120 minutes might cause the glucose concentration to decrease. According to the study conducted by Aguilar and Ram [42] using sugarcane bagasse, the glucose concentration was found to be decreased when the acid hydrolysis occurred at a longer reaction duration of 180 minutes. This is because longer reaction duration will result in the continuous degradation of glucose into furan-related compounds such as furfural and 5-hydroxymethylfurfural (HMF) and significantly reduce the glucose produced [42]. Thus, it is suggested that the reaction duration must be precisely controlled to suppress the formation of furan-related compound.

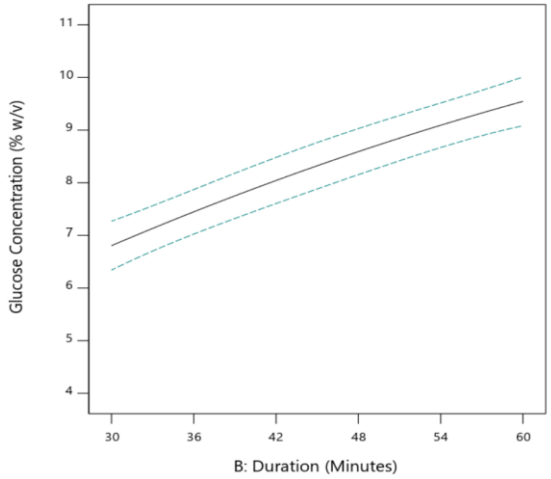

Fig. 3 The impact of reaction duration on the glucose concentration (Condition: acid concentration = 6% w/v; temperature = 75°C).

3.2.4 Impact of Reaction Temperature

Higher temperature can promote the rate of the hydrolysis reaction, but also might result in the thermal decomposition of cellulose and hemicellulose structure [43]. Thus, this operating parameter was taken into consideration to investigate how it affects the glucose concentration from honeydew melon rind. Fig. 4 depicts the impact of reaction temperature on the glucose concentration using 6% w/v of sulphuric acid for 60 minutes.

A higher glucose concentration of 9.847% w/v was obtained when higher reaction temperature was introduced. A similar trend was reported by Tian et al. [44], who performed acid hydrolysis of wheat straw using 4% w/v of acid concentration for 60 minutes. This is because the heat gained can promote the kinetic energy of the hydrogen molecule, causing them to accelerate at a higher speed. Consequently, the molecule collides more vigorously with the hemicellulose and cellulose structure, which subsequently increases the likelihood of bond breakage upon collision [45].

However, the study also revealed that a reverse trend on the glucose concentration was noticed after the reaction temperature exceed 120°C due to the thermal degradation of glucose into 5-HMF [44]. Thus, it can be concluded the reaction temperature of the acid hydrolysis must be controlled to prevent the decomposition reaction.

3.2.5 Optimisation for Glucose Concentration

The optimum condition for each of the parameters was identified using a desirability function from numerical optimisation. Based on the analysis from RSM, the maximum glucose concentration can be

obtained at acid concentration of 6% w/v reaction temperature of 75°C and duration of 60 minutes. Under this condition, the glucose concentration was predicted to be 9.545% w/v. A validation experiment was conducted to determine the percentage error of the actual values with the predicted values obtained from the model equation. The optimum conditions shown in Table 5 were cross validated experimentally to determine the glucose concentration.

The variation between the predicted and actual values was 3.07%. This indicates that the developed quadratic model is well-fitted, and the minimal percentage error indicates that the optimised operating parameters for maximum glucose concentration from acid hydrolysis process was reliable.

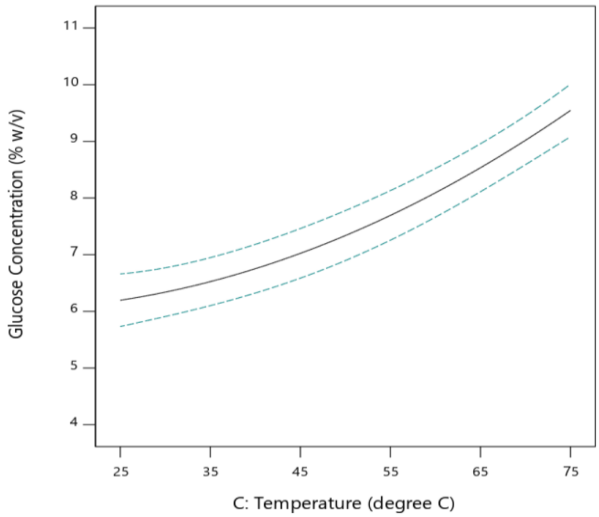

Fig. 4 The impact of reaction temperature on the glucose concentration (Condition: acid concentration = 6% w/v; duration = 60 minutes).

Table 5 Predicted and experimental values of the response at optimum conditions of acid hydrolysis.

Optimum Conditions	Glucose Concentration (% w/v)		Error (%)
	Experimental Value	Predicted Value	
Acid Concentration: 6% w/v Reaction Temperature: 75°C Reaction Duration: 60 minutes	9.847	9.545	3.07

3.3 Determination of Bioethanol Synthesis from Honeydew Melon Rind

Fig. 5 and Fig. 6 show the GC-MS chromatogram of pure ethanol and bioethanol samples from this study, respectively. Based on Fig. 5, it can be observed that the peak emerging at the retention time of 2.23 minutes shows the highest intensity, indicating that this compound is ethanol. On the other hand, a similar peak with the same retention time of 2.23 minutes was found in Fig. 6, which thus confirm the presence of ethanol in the sample. This finding is similar to the research done by Bakare et al. [46] that demonstrated bioethanol synthesis from rice bran, corn bran, and saw dust with the ethanol detected as the major product. Besides, Styarini et al. [47] also revealed that the fermentation of oil palm empty fruit bunch derived-glucose produced a significant amount of ethanol with some traces of organic impurities. Hence, these evidences confirmed the viability of honeydew melon rind for synthesis of bioethanol.

However, several unidentified peaks were present in Fig. 6, which implies the presence of organic impurities in the sample. According to Styarini et al. [47] , the common impurities that might exist in the bioethanol product would be acetaldehyde and pyruvic acid. These components normally appear as a by-product of yeast fermentation, which mainly caused by the incomplete hemicellulose and cellulose digestion. Besides, there could also be presence of furan-related compounds such as furfural, 2-acetylfuran, and 5-HMF in the bioethanol product due to the thermal degradation of cellulose from acid hydrolysis [48].

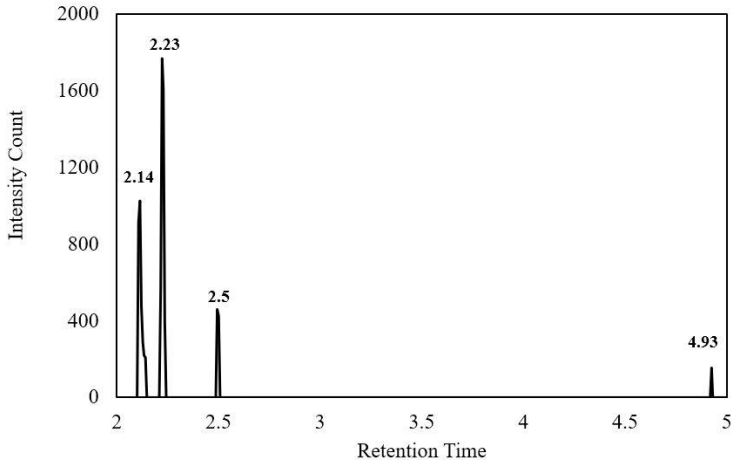

Fig. 5 GC-MS chromatogram of the pure ethanol sample.

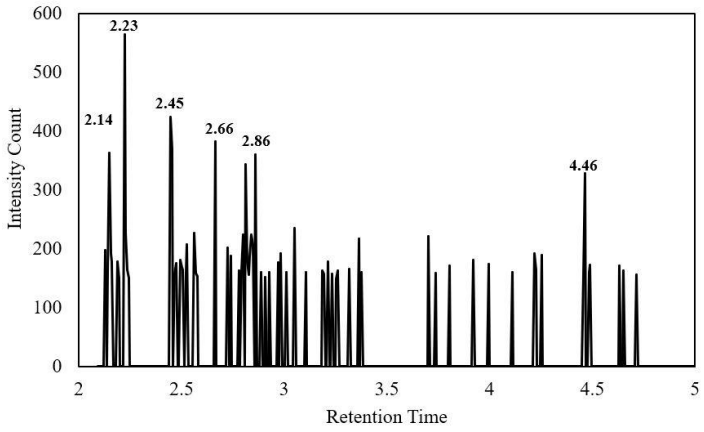

Fig. 6 GC-MS chromatogram of the bioethanol sample.

4 Conclusion

Based on FTIR analysis, the disappearance peak related to lignin confirmed a significant reduction in lignin content after the pre-treatment. This suggests that alkaline pre-treatment could be used in lignin removal for higher yield of glucose synthesis. From the parametric study on acid hydrolysis, it can be deduced that the glucose concentration increased with the increase of acid concentration, reaction duration, and temperature. The acid hydrolysis resulted in maximum glucose concentration of 9.847% w/v obtained under the identified optimal condition. Analysis via GC-MS confirmed the presence of ethanol as the major product from this study, with some traces of impurities. This indicates the feasibility of honeydew melon rind as a potential biomass source for bioethanol synthesis.

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Declaration of Conflict of Interest

The authors declared that there is no conflict of interest with any other party on the publication of the current work.

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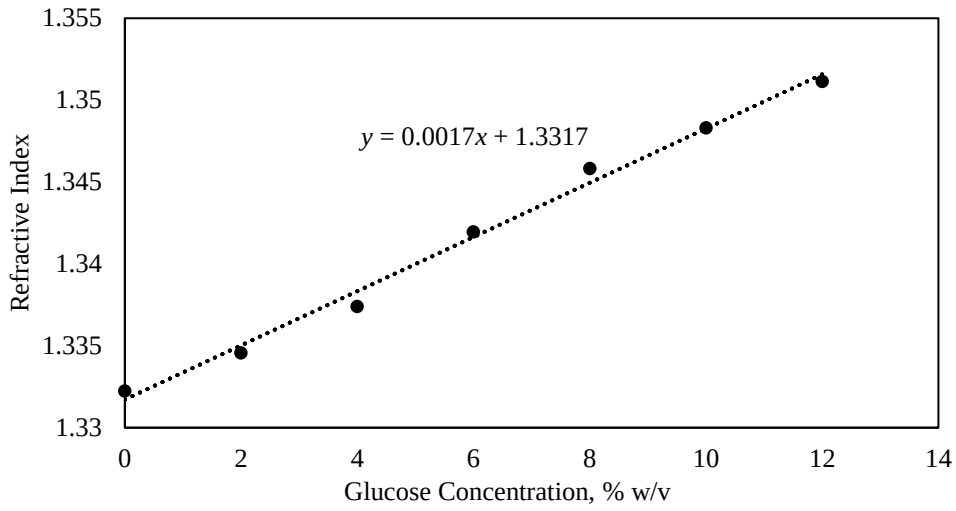
Appendix



Calibration curve of standard glucose solution developed in this study.