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Original research article

Effects of Aqueous Ethanol Concentration and Solid-to-Liquid Ratio in the Extraction of Organosolv Lignin from Mango (*Mangifera indica* L.) Seed Husk

Francis Dave C. Siacor*, Ian Dominic F. Tabañag, Camila Flor Y. Lobarbio, Evelyn B. Taboada

Department of Chemical Engineering, School of Engineering,
University of San Carlos, Cebu City 6000, Philippines

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ABSTRACT

The Philippines is abundant with agricultural residues that are seemingly underutilized and undervalued. One of these residues is mango seed husks (MSH) generated from the mango processing industry. The current practice of discarding MSH to open dumpsites poses health and environmental concerns. This ushered in the need to address this issue. As lignocellulosic biomass, the major components of MSH are cellulose (~56%), hemicellulose (~18%), and lignin (~12%), which exhibit potential in several applications. Lignin, a natural biopolymer, has become an important compound in biorefineries. The exploitation of MSH to recover lignin necessitates several methods, such as the organosolv process using aqueous ethanol. In this study, lignin from MSH was recovered via the ethanol organosolv process. Specifically, the effects of ethanol concentration (50%, 65%, 80%) and solid-to-liquid ratio (SLR; 1:5, 1:7, 1:10) under fixed reaction time (4 hours) and temperature (95°C) on the recovery of lignin, in terms of %acid-soluble lignin (ASL), was investigated. The results show that higher lignin recovery is obtained at lower SLR and ethanol concentration. Response surface modeling revealed the combined effects of %ethanol and SLR on %ASL, in which linear effects imparted negative influence on %ASL, while quadratic and two-way interaction effects have a positive impact on the response variable. Furthermore, elucidation of lignin constituents in the extract, as well as its properties and biological activities, is seen to be an important undertaking to assess the appropriate application of the recovered lignin.

Keywords: Acid-soluble lignin; Ethanol; Lignin; Mango seed husk; Organosolv process

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***Corresponding author:** 192805@usc.edu.ph

1. Introduction

The United Nations, through its Sustainable Development Goals, has emphasized the significance of shifting to a sustainable bio-economy that includes bio-refineries [1-2]. In the Philippines, bio-refineries have been focused on the production of biofuels due to insufficient capacities for fuel blending mandated by the Biofuels Act of 2007 [3]. Sugarcane has been the most exploited feedstock for biofuel production; however, there is a current shift towards exploring agricultural wastes [4]. As an agricultural country, the Philippines generates huge amounts of agricultural residues [5], such as mango processing wastes. These waste materials are composed of seeds (kernels and husks) and peels that are estimated to be 50% of the fruit mass combined [6]. About 30,000 MT of these wastes were generated during the third quarter of 2020 in the Philippines [7], and these are merely disposed of in dumpsites and landfills. This practice has become a serious issue because it has been shown to have detrimental environmental effects and health hazards. As these wastes undergo decomposition, they release a repugnant smell and secrete leachates that affect air and soil quality, respectively. The degraded matter also provides an avenue for the growth of pathogenic microorganisms that potentially harm residents living nearby. This calls then for urgent action to alleviate the problems of mango waste disposal through waste management and valorization.

Mango wastes are largely composed of the seed coating, otherwise known as mango seed husk (MSH), which is lignocellulosic. The cellulose (52-58%), hemicellulose (23-29%), and lignin (16-24%) content in MSH suggest that it is a potentially suitable raw material in bio-refineries [8]. The glucose and pentose molecules found in cellulose and hemicellulose can be utilized in the production of fermentation products and biomaterials. Lignin, on the other hand, remains an underutilized natural biopolymer despite its commercial value [9]. It is a complex biomacromolecule that consists of cross-linked phenylpropane monomers of *p*-coumaryl, coniferyl, and sinapyl alcohols [10-11]. Because of its structure, lignin has been considered in several applications, such as in the development of bioplastics, composite materials, adsorbents, and dispersants, as well as in electrochemical and biofuel applications [12]. The global lignin market size was about 954.50 million in 2019 and this is expected to grow at a 2% rate up to 2027. The increase in demand for lignin is due to its utilization not only in bio-refineries but also in the construction sector [13].

The manipulation of MSH for lignin extraction poses challenges because of the recalcitrant nature of lignocelluloses [14]. There is a need to deconstruct its matrix by cleaving ether linkages, which are resisted by carbon-carbon linkages in the lignin structure, to release lignin fragments [15]. At present, chemical-based processes that are employed in the paper and pulp industry are used to facilitate the isolation of lignin [16]. However, these processes have several disadvantages such as emission of rancid odor, production of strong bleaching effluents, and hazardous waste treatment processes [17]. To circumvent these issues, the use of organic solvents such as alcohols and ketones have been explored as cooking liquors. This paved the way for an organic solvent-based (organosolv) approach in lignin extraction [18]. In this process, disruption and solubilization of lignin are promoted, resulting in the removal of lignin while making celluloses easily digestible. Aside from a lignin-rich liquor (organosolv lignin), a hemicellulose-rich stream is also generated as a co-product in this process [19-20]. The use of organic solvents in lignin extraction has several advantages. It can extract lignin of high purity with a narrow molecular weight range and sulfurfree structure compared to chemical-based processes. The process also has a lower environmental impact since organic solvents are consumed instead of sulfur-containing compounds [18, 21]. However, there are drawbacks to this process such as the occurrence of side reactions (formation of inhibitors) and high material and energy cost due to solvent recovery [21].

Several solvents have been investigated for organosolv lignin extraction [18,22,23]. Among these solvents, ethanol is preferable because it is the least toxic with lower process complexity but higher reaction stability. However, its cost can be a potential economic drawback [24]. The use of aqueous ethanol for lignin extraction from biomass has been extensively investigated in several works [25-28]. Most of these studies are catalyzed by either sulfuric or hydrochloric acids at a ≤1% concentration. For example, the work of Mesa et al. [25] obtained 42.25% lignin yield from sugarcane bagasse using 50% ethanol at 175°C for 1.50 hours with minute levels of H_2SO_4. On a similar note, rice straw gave about 40% lignin yield using 75% ethanol at 180°C for 1 hour with lesser amounts of H_2SO_4.

Although there has been extensive literature citing the use of lignocelluloses in lignin extraction using ethanol, there is no reported use of MSH elsewhere. Moreover, studies elaborating the linear and combined effects of process conditions, particularly ethanol concentration and solid-to-liquid ratio (SLR), on the extent of lignin removal during its extraction from MSH are deficient. These parameters have been found to greatly affect lignin fragmentation and dissolution. The aim of this work then was to investigate lignin extraction from MSH via ethanol organosolv process, specifically the effects of %ethanol and SLR on the %acid-soluble lignin of the liquor, as well as the %sugar yield of the process. These would address the research gaps mentioned above and lead towards the value-addition of MSH.

2. Materials and Methods

2.1 Materials

Mango seeds were provided by Green Enviro Management Systems (GEMS) Inc. in Lapu-lapu City, Cebu, Philippines. Ethanol and sulfuric acid were purchased from Sigma-Aldrich Pte. Ltd. (Singapore). All chemicals were reagent grade and used without further purification.

2.2 Preparation and subsequent characterization of mango seed husk

Mango seeds were meticulously washed with water and dried at 65°C ± 10°C until they were brittle. Afterward, the seeds were cracked open to separate the husks (MSH) from the kernels inside. MSH was then cut into strips and further dried at 60°C for over 6 hours to remove residual moisture. Dried MSH was size-reduced to ≤ 1 mm using a Wiley mill (Thomas Scientific, USA) and then stored in air-tight containers at room temperature until use. Fig. 1 shows the prepared MSH powder before extraction. MSH was then characterized for its proximate composition, as well as its lignocellulosic components.

Fig. 1. Untreated mango seed husk powder before extraction.

The determination of proximate composition, namely moisture, ash, volatile matter, and fixed carbon, of MSH, was based on ASTM D1762-84 [29]. About 2 g of MSH was constantly dried in a convection oven at 105°C to obtain its moisture content. Then, the dried matter was incinerated in a furnace at 950°C for about 11 minutes to measure its volatile matter content. Subsequently, the residue after incineration was further heated at 575°C for 4 hours to calculate its ash content. Finally, the fixed carbon content was computed by difference.

Moreover, the lignocellulose components of MSH which are hemicellulose and α-cellulose were measured based on TAPPI T19m-54 [30] with modifications, while lignin was computed by difference. In obtaining its holocellulose content, MSH was first moistened with water at a 1:40 (w:v) ratio. About 0.25 mL of glacial acetic acid and 5.0 g of sodium hypochlorite were then added and the solution was heated at 70°C for 1 hour. The addition of the aforementioned compounds and the subsequent heating was repeated twice. The final solution was then chilled, and the solid was filtered and washed with water and methanol to neutral pH. The washed residue was dried to constant weight at 40°C, representing the holocellulose. Furthermore, the dried residue was mashed with 7 mL of 17% NaOH for 8 minutes. After mashing, the same amount of NaOH was added. The mashed residue was added with water and then mixed. Afterward, the solid was filtered and washed with water and 10% acetic acid until its color becomes off-white. The washed residue was then dried to constant weight at 40°C. The dried residue is the α-cellulose, and the hemicellulose is computed based on the difference between holocellulose and α-cellulose.

2.3 Experimental design

A one-factor-at-a-time approach was applied in investigating the effects of independent variables, namely solvent concentration, and SLR, under fixed reaction temperature and time to the response variables during the extraction of acid-soluble lignin from MSH. The response variables are the % acid-soluble lignin (%ASL) content of the liquor and the %sugar yield (%Y_S) of the process. Three levels were considered for each variable: 50%, 65% and 80% for aqueous ethanol concentration and 1:5, 1:7 and 1:10 for SLR. There were three replicates for each level and combinations are repeated three times for statistical validation of the process.

2.4 Organosolv lignin extraction

Lignin extraction via organosolv method proceeded by contacting MSH with aqueous ethanol of specific concentration at a determined SLR at 95°C for four hours. The temperature was based on the ethanol boiling point diagram so that solvent volatilization was minimized during the process. Moreover, the extraction time was based on Borand & Karaosmanoğlu [19] which suggested that pulping times can be performed at a maximum of 5 hours. The extraction experiments were carried out batchwise in reaction tubes with heating provided by a controlled water bath (WNB 7, Memmert GmbH Co.). After extraction, the tubes were cooled to room temperature. The pulps were filtered and then washed with the solvent, while the filtrate (liquor) was set aside. The washings were collected and were pooled together with the liquor. The pooled liquor was analyzed for its acid-soluble lignin (ASL) content.

2.5 Determination of acid-soluble lignin content of liquor and sugar yield

ASL content, expressed in percentage (%), of pooled liquor, was determined based on the method by Sluiter et al. [31]. The liquor was diluted with 4% H_2SO_4 until an absorbance reading of 0.7-1.0 at 240 nm wavelength was obtained. The first dilution of the liquor samples serves as the positive control of the analysis, while a blank sample made up of only water was considered as the negative control. ASL was then calculated using Eq. (1),

$$\%\mathrm{ASL} = \frac{\triangle \mathrm{AL}_{240\mathrm{nm}} \cdot \mathrm{V} \cdot \mathrm{D}}{\boldsymbol{\varepsilon} \cdot \mathrm{W} \cdot \mathrm{L}} \times 100, \qquad (1.1)$$

where $\Delta A_{240\ nm}$ is the absorbance reading, V is the volume of liquor (mL), D is dilution factor, ε represents absorptivity at 240 nm (25 L/g·cm), W is the mass of liquor (g) and L is the cell pathlength (cm).

The total carbohydrate content (C_L) of the pooled liquor was also measured to compute for $\%Y_S$ during lignin extraction. This was done through the phenol-sulfuric colorimetric method based on the work of Nielsen [32]. Approximately 2 mL of diluted liquor was added with 2 mL of phenol solution (5% w/w). Afterward, concentrated H_2SO_4 (5 mL) was quickly added. The solution was made to sit for 10 minutes and then mixed. The next step was the incubation of the solution at 30°C for 2 minutes. Finally, the absorbance at 490 nm was measured using a spectrophotometer (Spectroquant Pharo 100, Merck, USA). Glucose was considered as a standard to compute C_L (g/L) of the liquor. The $\%Y_S$ is then calculated using Eq. (1.2),

$$\%Y_s = \frac{C_L \cdot V_L}{m_s} \times 100, \tag{1.2}$$

where V_L is the liquor volume and m_s is the mass of MSH used. The glucose standard solutions were considered the positive control of the analysis, while a blank sample consisting of only water was the negative control.

2.6 Statistical analysis of data

The effects of ethanol concentration and SLR on the response variables %ASL and $\%Y_S$ were assessed by performing the statistical F-test via Microsoft Excel at a significance level of 0.05. The results of the statistical analysis are indicated as $F_{test} > F_{crit}$ (significant) or $F_{test} < F_{crit}$ (not significant), in which $F_{test} = 5.143$ for an experimental design with degrees of freedom of 2 (between variation) and 6 (within variation).

3. Results and Discussion

3.1 Chemical composition of mango seed husk

The chemical composition of MSH is presented in Table 1. In terms of its lignocellulosic components, MSH is principally composed of α-cellulose which is an indication that it can be a suitable raw material for bioethanol production. The repeating unit of α-cellulose is cellobiose, a dimer of glucose molecule [33]. Another important component is hemicellulose, which is utilized in xylitol production due to pentose units. The hemicellulose content of MSH is comparable to that of rice and wheat straw [34]. On the other hand, the proximate composition of MSH, namely: moisture, volatile matter (VM), ash, and fixed carbon are also shown in Table 1. Proximate analysis is done to determine the potential of MSH for biomass energy use, and it is shown that VM is the highest while ash is the lowest. Agricultural residues are anticipated to have high VM content and low ash levels because of their inherent fuel characteristics [35]. Furthermore, total lignin is a combination of acid-insoluble and acid-soluble lignin. Acid-insoluble lignin, otherwise known as Klason lignin, is the insoluble residue after ash, while acid-soluble lignin refers to the lignin degradation products, such as syringyl lignin, and lignin-carbohydrate compounds [36] that are dissolved in solvents. It can be noted from Table 1 that the total lignin content of MSH is almost 12%, and is nearly comparable with the lignin content of most agricultural biomass such as Napier grass and other forages [37].

Table 1. Chemical characteristics of mango seed husks.

Components	% (w/w, dry-basis)
Holocellulose	74.08 ± 5.55
α-cellulose	55.93 ± 4.58
Hemicellulose	18.14 ± 5.07
Total lignin	11.91 ± 2.34
Moisture	5.57 ± 0.17
Ash	1.04 ± 0.06
Volatile matter	75.14 ± 0.11
Fixed carbon	18.24 ± 0.13

3.2 Effect of ethanol concentration on the removal of lignin from MSH

The effect of increasing aqueous ethanol concentration at various levels of SLR on the removal of lignin from MSH is presented in Table 2 and Figs. 2a-2c. In this study, removal of lignin is measured by the concentration of ASL in the liquor. It can be noted from Fig. 2a that %ASL of the liquor at 1:5 SLR significantly increased (F_{test} > F_{crit}) as %ethanol is increased from 50% to 65%; however, it significantly decreased (F_{test} > F_{crit}) as %ethanol is increased up to 80%. However, the %ASL of the liquor was not significantly affected (F_{test} < F_{crit}) by the increase in %ethanol at 1:7 SLR as shown in Fig. 2b. Moreover, it can be observed from Fig. 2c that %ASL of the liquor at 1:10 SLR significantly decreased (F_{test} > F_{crit}) as %ethanol is increased from 50% to 65%; however, it significantly increased (F_{test} > F_{crit}) as %ethanol is increased up to 80%.

Table 2. %Acid-soluble lignin and %sugar yield during lignin extraction from mango seed husk at different ethanol concentration and solid-to-liquid ratio.

%Ethanol	%Acid-soluble lignin	%Sugar yield
Solid-to-liquid ratio = 1:5		
50%	0.161 ± 4.88x10^{-3}	9.802 ± 0.027
65%	0.167 ± 5.53x10^{-3}	11.231 ± 0.041
80%	0.153 ± 4.77x10^{-3}	12.121 ± 0.032
Solid-to-liquid ratio = 1:7		
50%	0.169 ± 5.09x10^{-3}	9.575 ± 0.021
65%	0.167 ± 5.35x10^{-3}	7.615 ± 0.085
80%	0.170 ± 5.41x10^{-3}	12.495 ± 0.170
Solid-to-liquid ratio = 1:10		
50%	0.173 ± 5.51x10^{-3}	12.630 ± 0.073
65%	0.152 ± 4.64x10^{-3}	9.174 ± 0.005
80%	0.159 ± 4.91x10^{-3}	10.808 ± 0.091

It can be inferred from the results that there is an improvement in the extraction of lignin when aqueous ethanol concentration is increased while increasing SLR at the same time. However, there exists a limitation in increasing the amount of ethanol, as well as SLR, and this is manifested by the decrease in %ASL. The combination of ethanol and water as solvent mixture stimulates the removal of lignin by disrupting first the lignocellulosic matrix so that low molar mass lignin fragments are formed. These fragments are then dissolved

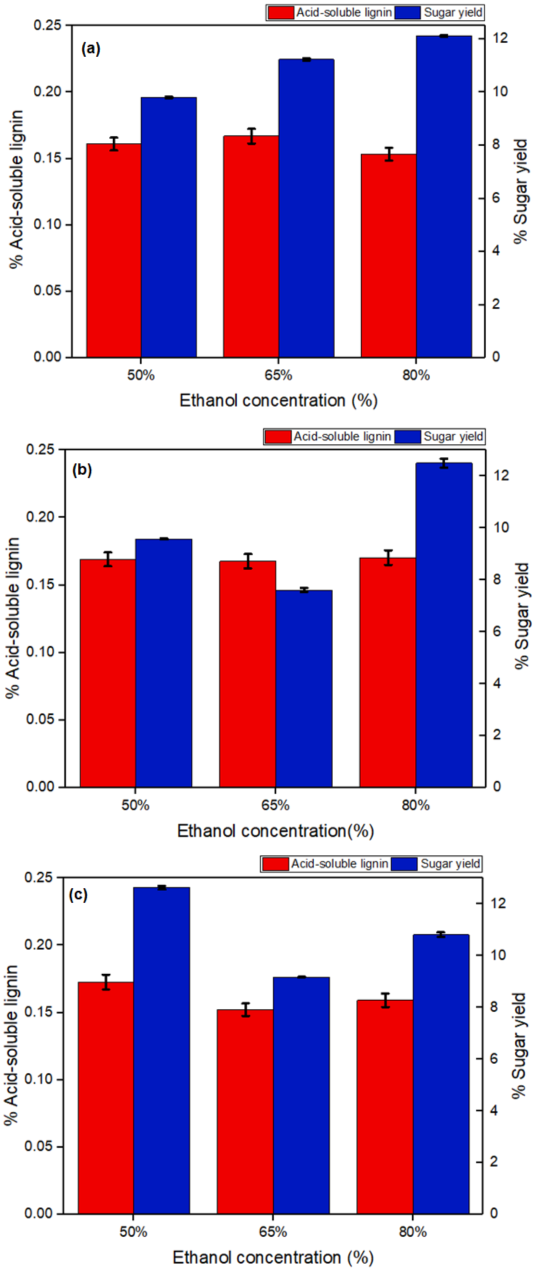

Fig. 2. %Acid-soluble lignin of liquor and %sugar yield during lignin extraction from mango seed husk at varying %ethanol and at (a) 1:5; (b) 1:7; (c) 1:10 solid-to-liquid ratio.

into the solvent system [38]. The effect of solvent composition can be elaborated in the dissolution stage because of the maximum solubility of lignin fragments in the ethanol-water system; hence, mass transfer is restricted. In the work of Ye et al. [39], %delignification is maximum at around 70%-80% ethanol because it is in this solvent concentration that the values of the solubility parameter of both extracted lignin and mixed solvent are nearly equal. At this point, poor dissolution of lignin into the solvent system happens.

The effect of solvent composition on sugar yield is also presented in Fig. 2. This is to validate the co-existence of hemicellulose solubilization and the possibility of cellulose hydrolysis [19] during organosolv lignin extraction from MSH. It can be noted that sugar yield significantly increased ($F_{test} > F_{crit}$) as %ethanol is increased at 1:5 and 1:7 SLR. On the other hand, increasing %ethanol led to a significant decrease ($F_{test} > F_{crit}$) in sugar yield when SLR is at 1:10. This would then imply that the organosolv process can also be used as a pretreatment strategy in recovering sugar monomers from MSH; however, restrictions to solvent composition are still present. These sugar monomers can be further used in the production of fermentation products, such as ethanol, butanol, and xylitol [40].

Table 3. %Acid-soluble lignin and %sugar yield during lignin extraction from mango seed husk at different solid-to-liquid ratio and ethanol concentration.

Solid-to-liquid ratio	%Acid-soluble lignin	%Sugar yield
%Ethanol = 50%		
1:5	0.161 ± 4.88x10^{-3}	9.802 ± 0.027
1:7	0.169 ± 5.09x10^{-3}	9.575 ± 0.021
1:10	0.173 ± 5.51x10^{-3}	12.630 ± 0.073
%Ethanol = 65%		
50%	0.167 ± 5.53x10^{-3}	11.231 ± 0.041
65%	0.167 ± 5.35x10^{-3}	7.615 ± 0.085
80%	0.152 ± 4.64x10^{-3}	9.174 ± 0.005
%Ethanol = 80%		
50%	0.153 ± 4.77x10^{-3}	12.121 ± 0.032
65%	0.170 ± 5.41x10^{-3}	12.495 ± 0.170
80%	0.159 ± 4.91x10^{-3}	10.808 ± 0.091

3.3 Effect of solid-to-liquid ratio on the removal of lignin from MSH

The effect of increasing SLR from 1:5 to 1:10 at different aqueous ethanol concentrations on the removal of lignin from MSH is presented in Figs. 3a-3c. The %ASL of the liquor is not significantly affected ($F_{test} < F_{crit}$) by the increase in SLR at 50% and 65% aqueous ethanol as presented in Figs. 3a and 3b, respectively. However, %ASL of the liquor significantly increased ($F_{test} > F_{crit}$) as SLR was increased from 1:5 to 1:7 at 80% aqueous ethanol as shown in Fig. 3c. As SLR is further increased to 1:10 at 80% ethanol, %ASL significantly decreased ($F_{test} > F_{crit}$).

The implication of these results is similar to the effect of solvent composition during lignin extraction. Although it is favorable to increase SLR and at the same time ethanol concentration to recover more lignin from MSH, limitations to these process conditions are expected. This is due to mass transfer limitations attributed to the solubility of lignin in an ethanol-water solvent system [39, 41]. In the work of Evangelina et al. [41], it was found that delignification yield is maximum at lower SLR. During lignin extraction, α-aryl and β-aryl ether bonds are cleaved by hydronium ions first. The fragments that are released are then dissolved in the solvent system; hence, the volume of solvent, as well as its concentration, should be given consideration [38, 41].

The effect of SLR on sugar yield is also presented in Fig. 3. It can be noted that the sugar yield significantly increased ($F_{test} > F_{crit}$) as SLR is increased at 50% aqueous ethanol. At 65% and 80% ethanol concentration, sugar yield significantly decreased ($F_{test} > F_{crit}$) as SLR is increased. This supports the validation in the previous section that dissolution of celluloses simultaneously occurred during the extraction process. This asserts that organosolv lignin extraction can also be an appropriate technique in recovering sugars in MSH for various applications; however, there exists a limitation in terms of SLR.

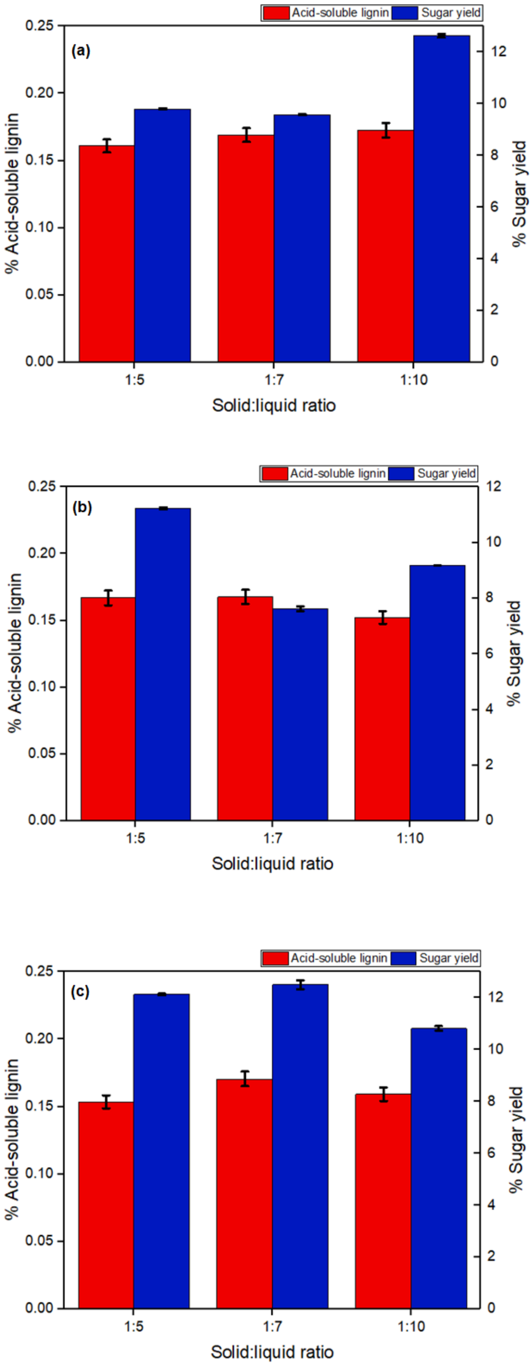

Fig. 3. %Acid-soluble lignin of liquor and %sugar yield during lignin extraction from mango seed husk at varying solid-to-liquid ratio and at (a) 50%; (b) 65%; (c) 80% ethanol.

3.4 Combined effect of ethanol concentration and solid-to-liquid ratio on the removal of lignin from MSH

The %ASL, as a response variable, of liquor obtained after each extraction runs were correlated with the process parameters through the second-order quadratic polynomial equation presented in Eq. (1.3)

$$y = ax_1 + bx_2 + cx_1^2 + dx_2^2 + ex_1x_2 + f. \quad (1.3)$$

In Eq. (1.3), y refers to the response variable, while x_1 and x_2 denote %ethanol and SLR, respectively. The coefficients a-b, c-d, and e represent linear, quadratic, and two-way interaction regression coefficients, respectively. The constant f denotes a residual error. Eq. (1.3) was fitted to the experimental data via Levenberg Marquadt or damped least-squares method in OriginPro 2017 software to determine values of the regression coefficient and the linear contribution of independent variables to the response variable. Moreover, fitting also verifies the contribution of quadratic and interaction effects on the response variable. The fitting results generated by the software are presented in Table 4. Based on the standard error, Eq. (1.3) was able to approximate the linear and quadratic effects of %ethanol with greater precision than the linear and quadratic effects of SLR. In addition, the two-way interaction effect was estimated with better precision. The values of regression coefficients and t-values revealed that both linear effects imparted negative contribution to the response, while quadratic and two-way interaction effects exhibited positive contribution. On the other hand, the coefficient of determination (R^2) revealed that the strength of the relationship (size effect) between the independent and response variables is moderate to almost strong. Furthermore, a closer examination of the adjusted R^2 showed that additional input variables or predictors improve the model by less than expected by chance. These variables do not possess a contributing effect to the model since they have an extremely small value [42].

Table 4. Regression coefficients of the second-order polynomial equation for % acid-soluble lignin with statistical parameters.

Coefficient (Term)		**Value**	**Standard Error**	**T**
a	(x_1)	-1.249	0.682	-1.831
b	(x_2)	-210.828	158.744	-1.328
c	(x_1^2)	0.008	0.005	1.657
d	(x_2^2)	429.996	469.326	0.916
e	($x_1 \cdot x_2$)	1.287	1.076	1.195
f	(constant)	63.552	25.455	2.497
Coefficient of determination (R^2)				0.659
Coefficient of determination, adjusted (R^2)				0.091
Reduced χ^2				2.624

Incorporating the coefficients from Table 4 in Eq. (1.3), organosolv lignin extraction from MSH can then be represented by Eq. (1.4). This statistical model provided a good fit to the experimental data, as shown by the coefficient of determination (R^2) and the reduced χ^2 values in Table 4. Moreover, this model would commence approximation and subsequent optimization of the response variables. Through this equation, the response can be predicted at any given process conditions; hence, this has importance in the design and development of organosolv-based lignin extraction [43]

$$y = -1.249x_1 - 210x_2 + 0.008x_1^2 + 429x_2^2 + 1.287x_1x_2 + 63.552. \quad (1.4)$$

A contour plot of the experimental data was generated and is presented in Fig. 4, where the response (%ASL) is plotted as a function of %ethanol (x_1) and SLR (x_2). The regions in the contour plot with different color intensities represent the concentration of acid-soluble lignin at a specific %ethanol and SLR. It can be seen from Fig. 4 that the regions within the range of %ethanol and SLR considered in this study did not display a maximum %ASL. The response only increases along and beyond the limits of both ranges. This would imply that the curvature has not yet peaked. This could then be attributed to the incomplete fragmentation and dissolution of lignin from MSH within 50% - 80% ethanol and 1:5-1:10 SLR. Moreover, a red to yellow region indicating higher %ASL concentrations can be noted at these ranges: <55% ethanol, <1:8 SLR, and >77% ethanol and >1:7 SLR. This implies then that optimum values for %ethanol and SLR can be observed outside the ranges deliberated in this study.

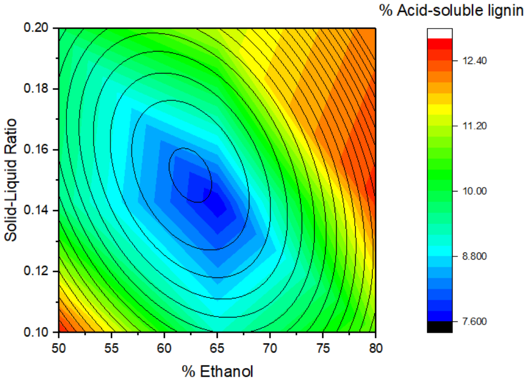

Fig. 4. Response contour plots showing the response behavior of %ethanol and solid-to-liquid ratio on %acid-soluble lignin of liquor.

4. Conclusion

The results of this study indicate that organosolv process, particularly using aqueous ethanol as solvent, can be an effective method in extracting lignin from MSH. The results also show that to obtain higher recoveries of lignin, which is measured by the acid-soluble lignin concentration in liquor, a lower SLR and lower ethanol concentration is favorable. The application of response surface modeling led to the evaluation of the combined effects of %ethanol and SLR in terms of %ASL. The linear effects influence the response variable negatively, while the contribution of quadratic and two-way interaction showed otherwise. Furthermore, an investigation on the optimum conditions of %ethanol and SLR would entail extending further the %ethanol and SLR range tested in this study.

This study also shows that mango seed husk has potential in the production of several high-value products, particularly lignin. Aside from its usual application, lignin has been utilized in the development of biomaterials and biofuels. However, the acid-soluble lignin obtained in this study needs to undergo characterization to elucidate the lignin constituents present in the extract. This would also determine its properties and biological activities to further assess its appropriate application. Furthermore, lignin extraction from MSH demonstrates utilization and value-addition of a seemingly useless waste material that has been deemed to have negative environmental and health impacts.

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References

[1] United Nations. A bio-based, reuse economy can feed the world and save the planet - UN agency [Internet]. [cited 2020 March 18]. Available from: https://www.un.org/sustainabledevelopment/blog/2018/04/a-bio-based-reuse-economy-can-feed-the-world-and-save-the-planet-un-agency/

[2] IEA Bioenergy. Bio-based chemicals: value added products from biorefineries [Internet]. [cited 2020 March 18]. Available from: https://www.ieabioenergy.com/publications/bio-based-chemicals-value-added-products-from-biorefineries/

[3] USDA Foreign Agricultural Service Global Agricultural Information Network. Philippines' biofuels situation and outlook [Internet]. [cited 2020 March 23]. Available from: https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=Biofuels%20Annual_Manila_Philippines_11-28-2018.pdf

[4] Aditiya HB, Mahlia TMI, Chong WT et al. Second generation bioethanol production: a critical review. Renew Sust Energ 2016;66:631-53.

[5] Bioenergy Consult. Agricultural wastes in the Philippines [Internet]. [cited 2020 March 19]. Available from: https://www.bioenergyconsult.com/agricultural-resources-in-philippines/

[6] Philippine Statistics Authority. Performance of Philippine agriculture April-June 2017 [Internet]. [cited 2020 March 23]. Available from: https://psa.gov.ph/content/performance-philippine-agriculture-april-june-2017

[7] Philippine Statistics Authority. Major fruit crops quarterly bulletin, July-September 2020 [Internet]. [cited 2020 December 9]. Available from: https://psa.gov.ph/content/major-fruit-crops-quarterly-bulletin

[8] Torres-Leon C, Rojas R, Contreras-Esquivel JC et al. Mango seed: functional and nutritional properties. Trends Food Sci Technol 2016;55:109-17.

[9] Lora JH, Glasser WG. Recent industrial applications of lignin: a sustainable alternative to nonrenewable materials. J Polym Environ 2002;10:39-48.

[10] Lee HV, Hamid SBA, Zain SK. Conversion of lignocellulosic biomass to nanocellulose: structure and chemical process. Sci World J 2014;631013.

[11] Dessbesell L, Paleologou M, Leitch M et al. Global lignin supply overview and kraft lignin potential as an alternative for petroleum-based polymers. Renew Sust Energ Rev 2020;123:109768.

[12] Norgren M, Edlund H. Lignin: recent advances and emerging applications. Curr Opin Colloid Interface Sci 2014;19:409-16.

[13] Grand View Research. Lignin market size, share and trends analysis report by product, by application, by region, and segment forecasts, 2020-2027 [Internet]. [cited 2020 March 23]. Available from: https://www.grandviewresearch.com/industry-analysis/lignin-market

[14] Ge X, Chang C, Zhang L. Conversion of lignocellulosic biomass into platform chemicals for biobased polyurethane application. In: Li Y, Ge X, editors. Advances in Bioenergy. Volume 3. Amsterdam, Netherlands:Elsevier; 2018. p. 161-213.

[15] Hon DNS, Shiraishi N. Wood and cellulosic chemistry. New York, NY: Marcel Dekker; 2001.

[16] Shafiei M, Kumar R, Karimi K. Pretreatment of lignocellulosic biomass. In: Karimi K, editor. Lignocellulose-based bioproducts. Switzerland: Springer International Publishing; 2015. p. 85-154.

[17] Nimz HH, Casten R. Chemical processing of lignocellulosics. Holz als Roh-und Werkstoff 1986; 44:207-12.

[18] Johansson A, Aaltonen O, Ylinen P. Organosolv pulping - methods and pulp properties. Biomass 1987;13(1):45-65.

[19] Borand MN, Karaosmanoğlu F. Effects of organosolv pretreatment conditions for lignocellulosic biomass in biorefinery applications: a review. J Renew Sustain Energy 2018;10(3).

[20] Bajpai P. Pretreatment of lignocellusic biomass for biofuel production. Singapore:Springer; 2016.

[21] Zhao X, Cheng K, Liu D. Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Appl Microbiol Biotechnol 2009;82(5):815.

[22] Lindner A, Wegener G. Characterization of lignins from organosolv pulping according to the organocell process part 1. elemental analysis, nonlignin portions and functional groups. J Wood Chem Technol 1988;8(3):323-40.

[23] Parajó, JC, Alonso JL, Vázquez D, Santos V. Optimization of catalyzed acetosolv fractionation of pine wood. Holzforschung 1993;47(3):188-96.

[24] Pye E, Lora J. The Alcell process: a proven alternative to kraft pulping. Tappi J 1991;74(3):113-8.

[25] Mesa L, Gonzáles E, Ruiz E et al. Preliminary evaluation of organosolv pre-treatment of sugarcane bagasse for glucose production: application of 23 experimental design. Appl Energy 2010;87(1):109-14.

[26] Amiri H, Karimi K, Zilouei H. Organosolv pretreatment of rice straw for efficient acetone, butanol and ethanol production. Bioresour Technol 2014;152:450-6.

[27] Salapa I, Katsimpouras C, Topakas E, Sidiras D. Organosolv pretreatment of wheat straw for efficient ethanol production using various solvents. Biomass Bioenergy 2017;100:10-6.

[28] Gong D, Holtman KM, Franqui-Espiet DO et al. Development of an integrated pretreatment fractionation process for fermentable sugars and lignin: application to almond (*Prunus dulcis*) shell. Biomass Bioenergy 2011;35(10):4435-41.

[29] ASTM D1762-84. Standard test method for chemical analysis of wood charcoal. West Conshohocken, PA:ASTM International; 2013.

[30] Trindade WG, Hoareau W, Megiatto JD et al. Thermoset phenolic matrices reinforced with unmodified and surface-grafted furfuryl alcohol sugar cane bagasse and curaua fibers: properties of fibers and composites. Biomacromolecules 2005;6:2485-96.

[31] Sluiter A, Hames B, Ruiz R et al. Determination of structural carbohydrates and lignin in biomass [Internet]. [cited 2020 March 19]. Available from: https://www.nrel.gov./docs/gen/fy11/42618.pdf

[32] Nielsen SS. Phenol-sulfuric acid method for total carbohydrates. In: Nielsen SS, editor. Food Analysis Laboratory Manual Food Science Texts Series. Boston: Springer; 2009. p. 47-53.

[33] Bañuelos JA, Velazquez-Hernandez I, Guerra-Balcazar M, Arjona N. Production, characterization and evaluation of the energetic capability of bioethanol from *Salicornia bigelovii* as a renewable energy source. Renew Energy 2018;123:125-34.

[34] Prasad S, Singh A, Joshi HC. Ethanol as an alternative fuel from agricultural, industrial and urban residues. Resour Conserv Recy 2007;50:1-39.

[35] Singh YD, Mahanta P, Bora U. Comprehensive characterization of lignocellulosic biomass through proximate, ultimate, and compositional analysis for bioenergy production. Renew Energy 2017;103:490-500.

[36] Yasuda S, Fukushima K, Kakehi A. Formation and chemical structures of acid-soluble lignin I: sulfuric acid treatment time and acid-soluble lignin content of hardwood. J Wood Sci 2001;47:69-72.

[37] Kou L, Song Y, Zhang X, Tan T. Comparison of four types of energy grasses as lignocellulosic feedstock for the production of bioethanol. Bioresource Technol 2017;241:424-9.

[38] Pasquini D, Pimenta MTB, Ferreira LH, Curvelo AAS. Extraction of lignin from sugarcane bagasse and *Pinus taeda* wood chips using ethanol-water mixtures and carbon dioxide at high pressures. J Supercrit Fluids 2005;36:31-9.

[39] Ye Y, Liu Y, Chang J. Application of solubility parameter theory to organosolv extraction of lignin from enzymatically hydrolyzed cornstalks. Bioresources 2014;9(2):3417-27.

[40] Mussatto SI, Teixeira JA. Lignocellulose as raw material in fermentation process. In: Mendez-Vilas A, editor. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. United Kingdom:Formatex Research Center, The Book Depository Ltd.; 2010. p. 897-907.

[41] Evangelina M, Dib M, Cristina M, Aprigio A. Low liquid-solid ratio fractionation of sugarcane bagasse by hot water autohydrolysis and organosovl delignification. Ind Crop Prod 2014;65:349-53.

[42] Zikmund WG. Business research methods 6th ed. San Diego, CA:Harcourt College Publishers; 2000. p. 513.

[43] Senaras AE. Parameter optimization using the surface response technique in automated guided vehicles. In: Kumar K, Zindani D, Davim P, editors. Sustainable Engineering Products and Manufacturing Technologies, Cambridge, MA:Elsevier; 2019, pp. 187-97.