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Optimizing the Extraction of Phenolic Compounds with High Antioxidant Activity from Mango Seed Kernel Wastes Using Response Surface Methodology

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Abstract

Mango seed kernels (MSK), which are waste streams in the mango processing industry, are good sources of phenolic compounds with high antioxidant, anti-bacterial, and anti-viral properties. These phenolic compounds are reported to have an increasing demand over the years in the quest for healthy ready-to-eat food and nutraceuticals. To recover these compounds from MSK, solid-liquid extraction (SLE) can simply be applied, although there is a knowledge gap in the systematic exploration of this process for mango-based phenolic compounds. In this work, phenolic compounds were extracted from MSK through SLE using ethanol-water solvent system. A statistical-based approach was used to evaluate and optimize the extraction conditions in relation to the yield of phenolic compounds from MSK. The central composite design together with response surface methodology was adopted to assess the effect of extraction temperature (30ºC, 45ºC, and 60ºC) and ethanol concentration (25%, 50%, and 75%) under fixed extraction time (105 min) and solid-to-solvent ratio (1:10) on the extraction yield. Both temperature and ethanol concentration provided positive effects on the yield and the optimum conditions of temperature and ethanol concentration were obtained at 63.21ºC and 53.21% ethanol, respectively. A second-order polynomial equation was obtained describing the extraction process, and a validation test of this response model showed that it sufficiently described the process. Furthermore, the extracts obtained at optimal conditions possess a potential antioxidant activity at IC_{50} = 45 ± 0.002 µg GAE mL^{-1} extract. The results indicated that phenolic compounds in MSK can be recovered through extraction using aqueous ethanol. This study also promotes value-addition of a seemingly useless waste material while reducing its health and environmental impact.

Keywords: Phenolic compound; Mango seed kernel; Solid-liquid extraction; Response surface methodology; Antioxidant activity

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Introduction

Mango (*Mangifera indica* L. Anacardiaceae) is among the major fruit crops of the Philippines in terms of volume and value. As of 2019, mango production for the entire country is over 700,000 MT. The domestic market captures more than 90% of annual mango production while the rest are for export [1–2]. In the production of commercial goods from mangoes such as dried mangoes, juice, purees, and candies, only the pulp is consumed and utilized. The peels, seed husks and kernels, estimated to be 50% of the fruit mass combined, are left as waste materials and are simply disposed to landfills. Based on 2019 production, more than 350,000 MT of these waste materials are generated in the country [2]. The mere dumping of these wastes in landfills has turned into a rising issue due to its increasing production and its adverse health and environmental hazards since it emits offensive smell, offers a platform for the cultivation of unwanted and pathogenic microorganisms and affects soil quality during degradation of the waste components [3].

Among the waste materials, mango seed kernel (MSK) shows great potential in food and pharmaceutical applications. Based on its proximate composition reported in various literature [4–5], the carbohydrate and protein levels in MSK indicate that it can be a suitable feedstock for flour and amino acids production, respectively. Furthermore, MSK is also a good source of other commercially viable products, particularly phenolic compounds [4–6]. Currently, phenolic compounds have gained significant attention and popularity due to their health benefits in the prevention of cardiovascular and neurodegenerative diseases, as well as cancer [7]. Most importantly, the economic potential of phenolic compounds is promising. The worldwide market of phenolic compounds was equal to US $1.28 million in 2018 and is growing at a compounded rate of 7.2% in 2019 up to 2025 according to Grand View Research [8].

Phenolic compounds are found in the cell vacuoles of the seed coat of MSK, as evident by the presence of significant amounts of tannin-containing cells [6]. The constituents of phenolic compounds present in MSK are largely comprised of tannin and vanillin, with considerable levels of coumarin, cinnamic acid and ferulic acid. In addition, caffeic acid, gallic and mangiferin are significantly present [4–9]. To expedite the discharge of these phenolic compounds, an array of preliminary processes is initially done to MSK, such as homogenization, size reduction, defatting, drying and combinations thereof. Afterwards, a preferable extraction technique is involved [9–10]. The most typical method exploited in the extraction of phenolic compounds from plant-based materials is solid-liquid extraction (SLE) using organic or inorganic solvents [11]. This technique is expansively used in obtaining various food products in different processes since it permits the efficient release of the target components from the raw material [12].

SLE is a phenomenon involving mass transfer in which the compound of interest (regarded as solute) confined in the feedstock is transported into the surrounding solvent. Specifically, the mechanism of solute (phenolic compounds) transport from the inner portion of MSK to the bulk solvent begins with the rapid washing stage. In this stage, the solid surface is being swiftly washed by the solvent from the bulk solution. The next stage is the slow diffusion stage, wherein the solvent penetrates into the solid structure, permitting the solute within the solid structure to be dissolved in the solvent. The solute-solvent interaction is reflected in this stage, and this is also considered as the rate-limiting step of the entire SLE operation. After a sufficient amount of time, an equilibrium condition exists between solid and solvent phases [13–14].

The transport phenomenon described above is improved, in terms of extraction yield, through adjustments of the SLE process factors, leading to an optimized value of the yield. These factors

are comprised of the following: extraction time, temperature, solvent-to-feedstock ratio and type of solvent. Furthermore, the optimum conditions for the recovery of phenolic compounds are dependent on the type and variety of plant feedstock with varying bioactive compounds [15–16]. Among these factors, the choice of solvent, as well as temperature, heavily influences extraction yield. Several studies employing SLE in the extraction of phenolic compounds from various sources using different solvents are summarized in Table 1. It can be noted that a wide array of organic solvents can be used, but the most common is aqueous ethanol. Molecular polarities of phenolic compounds have a profound effect on their solubility in a particular solvent since it determines the strength and types of inter-molecular forces of attraction at work in a system. With the exemption of tannins, phenolic compounds are more soluble in ethanol, methanol and acetone than in water [18–19]. The preference of phenolic compounds in aforementioned solvents is possibly attributed to the non-polar portion of the compound and the aliphatic fragment of the alcohols and ketone; while their preference to water is due to the aromatic ring of the compound that is well surrounded with three hydroxyls and one carboxyl group that may display dipole-dipole interactions with the solvent [20]. This means then that in order to extract simultaneously most of the phenolic compounds present in a feedstock, a mixture of water and polar organic solvents is recommended. Among these solvents, ethanol and its aqueous form is still preferred over other organic solvents for food and pharmaceutical applications because of lesser restrictions than organic solvents. In terms of human consumption, ethanol is acceptable in specific residual percentages compared to other organic solvents because it is less toxic [21].

Table 1 Solid-liquid extraction of phenolic compounds from various plant sources

Plant Source	Extraction Conditions					References
	Temp. (°C)	Time (min)	Solvent (Conc.)	Solid to Solvent Ratio (w:v)	Phenolics[a] Yield (%)	
Tiger uts	35	180	Ethanol (50%)	1:15	0.22	[22]
Peach	25	180	Acetone (60%)	1:33	0.36	[23]
Pink guava	30	180	Methanol (60%)	1:17	0.02	[24]
Choke berries	30	60	Ethanol (50%)	1:20	2.8	[25]
Spent coffee grounds	47	150	Ethanol (57.7%)	1:48	97.8	[26]
Bitter melon	80	5	Water	1:40	1.10	[27]
Barley	45	120	Ethanol (30%)	1:30	4.8	[28]
Mango kernels	30	60	Ethanol (50%)	1:10	10	[9]
	28	180	Hexane	1:15	0.6[b]	[29]

[a] based on gallic acid equivalence

[b] based on tannic acid equivalence

The extraction of phenolic compounds from MSK through solid-liquid extraction in a systematic approach is still inadequate at present. As presented in Table 1, only the works of Lim et al. [9] and Yoswathana & Eshiaghi [29] explored MSK as a raw material. However, the former focused on characterization of phenolic constituents in the extracts while the latter employed hexane as solvent, which has health and safety implications as far as food and pharmaceutical applications are concerned. Hence, there is still an existing knowledge gap on the optimum conditions for the extraction of phenolic compounds from MSK in relation to the extraction yield. The extraction conditions of the various feedstock shown in Table 1 can then be considered as baseline data during optimization since these studies were focused on phenolic compounds identification and anti-microbial activity screening.

The main objective then of this study was to optimize the yield of phenolic compounds from MSK in relation to SLE process conditions employing response surface methodology as an optimization tool. In this study, the solvent system used was ethanol-water mixture. Specifically, the effects of extraction temperature (30ºC – 60ºC) and binary solvent composition (25% – 75% v/v ethanol) to the phenolic compounds yield of the SLE process were investigated at fixed extraction time and feedstock-to-solvent ratio (1:10 w:v). Furthermore, optimal values for extraction temperature and binary solvent composition were obtained.

Experimental

1) Materials

The following reagent-grade chemicals were procured from Sigma-Aldrich Pte. Ltd. (Singapore): Folin-Ciocalteau reagent, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium carbonate and ethanol. These chemicals were readily used during the conduct of the experiment. Mango seeds were kindly given by Green Enviro Management Systems (GEMS) Inc., Lapu-lapu, City, Cebu, Philippines.

2) Preparation of mango seed kernels

Mango seeds were washed with tap water prior to solar-drying at 60ºC – 70ºC until the seed husks became breakable. Subsequently, the seeds were split open to obtain the wet mango seed kernels (WMSK) within the husks. Then, WMSK was subjected to grinding to obtain ground mango seed kernels (GMSK). GMSK was stored in dark containers at cold temperature (~4ºC) until use. In addition, GMSK was characterized for their particle size, moisture content, water- and ethanol-extractives content, as well as lipid content.

3) Determination of equilibrium extraction time

Extraction was initially carried out with pure water and ethanol as solvents at the following extraction temperatures: 30ºC, 45ºC and 60ºC over a period of 300 min to determine the equilibrium extraction time. To 100 mL of the solvent, 10 g of wet GMSK was added and the resulting mixture was shaken at 150 rpm and at the specified temperature. Then, approximately 0.1 mL of the liquid extract was pipetted at time intervals for 4–5 h. The total phenolic content (TPC), expressed as mg of gallic acid equivalence (GAE) per g of extract of these samples were analyzed via the Folin-Ciocalteau colorimetric method. The extraction yield (Y), expressed as the amount of extracted phenolic compounds (mg GAE) to the amount of GMSK in dry-basis (g dry matter or dm), per time was then computed using Eq. 1. The extraction yields were plotted versus time, and the empirical Peleg model was fitted to the data using the solver function of the Microsoft Excel software to obtain a regressed curve. Peleg model was used since it can sufficiently describe SLE phenomenon and is extensively employed in various studies. Finally, the equilibrium extraction time was determined from the regressed curve. The longest extraction

time among the experimental runs was taken as the equilibrium extraction time in proceeding with the extraction experiments.

4) Design of experiment: central composite design

A central composite design (CCD) was used to investigate the effects of temperature (x_1) and % of ethanol (x_2) at fixed SLE time and solid: solvent ratio (1:10) on the recovery of phenolic compounds from MSK. The CCD consisted of a full 2^2 full factorial design with two blocks, while the corner points were replicated twice. The design also included a center point for every block. A block was included in the design to account for deviations in the response variable caused by other uncontrollable factors, such as variety and characteristics of feedstock. These design parameters resulted to 6 center points, in which 3 points are located within the cube and 3 points are located in axial positions. The α was set at 1.414, which denotes the distance of the axial points from the central point. The experimental design layout and the observed response values are shown in Table 2 while the coded factor levels are presented in Supplementary Material (SM) 1. Experiments were carried out based on the run order in Table 2.

$$\text{Yield, Y}\left(\frac{\text{mg GAE}}{\text{g dm}}\right) = \frac{\text{TPC}_{\text{extract}} \times \text{mass of extract}}{\text{mass of GMSK used}_{\text{dry-basis}}} \quad \text{(Eq. 1)}$$

Table 2 Central composite design layout and computed responses (yield)

Standard order	Run order	Factor levels		Yield (%)
		Temperature, x_1 (°C)	%Ethanol, x_2 (%)	
24	1	45	15	9.80 ± 0.51
12	2	45	50	12.66 ± 0.80
14	3	45	50	13.32 ± 1.61
22	4	25	50	10.57 ± 0.81
10	5	45	15	9.28 ± 0.61
8	6	25	50	10.60 ± 0.72
26	7	45	50	12.14 ± 0.65
28	8	45	50	12.83 ± 0.56
13	9	45	50	12.85 ± 0.61
27	10	45	50	13.25 ± 0.43
11	11	45	85	10.63 ± 0.74
9	12	66	50	13.13 ± 0.32
23	13	66	50	13.39 ± 0.39
25	14	45	85	10.91 ± 0.42
18	15	60	75	13.72 ± 1.99
6	16	45	50	14.23 ± 1.89
7	17	45	50	13.59 ± 0.68
20	18	45	50	14.67 ± 2.46
19	19	45	50	13.85 ± 0.53
1	20	30	25	11.21 ± 0.90
17	21	30	75	10.85 ± 1.53
2	22	60	25	12.98 ± 1.51
3	23	30	75	11.24 ± 1.30
15	24	30	25	11.14 ± 1.60
4	25	60	75	14.42 ± 2.35

The statistical software Minitab 17 (Minitab Inc., USA) was used in the design and analysis of experiments. A second-order polynomial equation (model) was then fitted to the responses to generate the model equation and the optimum values for each factors.

5) Solid-liquid extraction of phenolic compounds

Phenolic compounds from GMSK were extracted using aqueous ethanol under batch mode in shake-flasks. Similar with Section 3, about 10 g of wet GMSK were added with 100 mL of aqueous ethanol of known composition. The resulting mixture was shaken at 150 rpm and at a specified temperature for a time period equal to the extraction time obtained in Section 3. Immediately after the extraction process, the extract was collected by filtration and then its mass was measured. A portion of the extract was then obtained and analyzed for TPC using Folin-Ciocalteau assay. From the phenolic compounds content, the %extraction yield (Y) for every run based on Table 3 was computed using Eq. 2. The yield was the response variable of the extraction process in the response surface regression analysis.

6) Response model validation

To evaluate the validity of the model, the SLE process was duplicated using the optimum values obtained for each factors. Besides the extraction yield, the potential antioxidant activity of the extract in terms of its scavenging activity on DPPH free radical was measured. The response (Y) for this validation experiment was compared to the predicted response from the model equation. A 5% difference between the values was preferably set as acceptable.

7) Analytical methods

TPC of extract samples were determined using the Folin-Ciocalteau method [30] with slight modifications. A 1.0 mL portion of the liquid extract was added with water until 7 mL diluted solution was obtained. The solution was then added with 0.5 mL Folin-Ciocalteau phenol reagent. After 5 min, 1.0 mL saturated sodium carbonate solution was added. The mixture was then diluted to 10 mL with water. The sample was incubated for 2 h at room temperature and the absorbance was determined at 765 nm using a spectrophotometer (UV-1700, Shimadzu Corp.). A standard calibration curve using gallic acid was also prepared to obtain TPC of the samples, expressed as mg of gallic acid equivalence (GAE) per g of extract. On the other hand, the anti-oxidant activity assay in terms of scavenging activity of DPPH free radical of extracts were determined based on colorimetric method by Libran et al. [31] with slight modifications. Approximately 2.9 mL of DPPH-ethanol solution (0.1 mM) and 0.1 mL of liquid extract were mixed vigorously and the resulting mixture was kept in the dark for 30 min at 30°C. The absorbance was measured at 515 nm using a spectrophotometer (UV-1700, Shimadzu Corp.). The %inhibition, which is based on the change of absorbance, and the half maximal inhibitory concentration (IC_{50}) was then computed. On the other hand, the moisture content and particle size distribution were measured according to AOAC [32] while the extractives and lipid content were quantified according to Sluiter et al. [33].

$$\text{Yield, Y (\%)} = \frac{\text{TPC}_{\text{extract}} \times \text{mass of extract}}{\text{mass of GMSK used}_{\text{dry-basis}}} \times 100 \qquad \text{(Eq. 2)}$$

Table 3 Physicochemical properties of mango seed kernels

Parameter	Value
[a]Moisture content (%)	68.17 ± 0.50
[b]Total extractives (%)	46.45 ± 0.54
[b]Water-soluble extractives (%)	26.33 ± 1.26
Phenolic content in water-soluble extractives (%)	20.78 ± 0.80
[b]Ethanol-soluble extractives (%)	20.12 ± 0.72
Phenolic content in ethanol-soluble extractives (%)	11.71 ± 0.35
[b]Total phenolic content (mg/g)	78.24 ± 0.64
[b]Lipid content (%)	7.98 ± 0.12
Average particle size (mm)	4.45 ± 0.35

[a] wet basis; [b] dry basis

Results and discussion

1) Physicochemical properties of mango seed kernel

The physicochemical properties of mango seed kernel are shown in Table 3. The moisture content of MSK is high since it was utilized as fresh and did not undergo further pretreatment processes like drying. Although it is known to improve storage and handling, several studies have demonstrated that application of drying can possibly degrade and reduce the phenolic compound content of a specific feedstock [34]. The lipid content of MSK is generally lower, which is preferable since high amounts of lipids can potentially obstruct complete extraction of phenolic compounds [12]. The total extractives represent the non-structural components in the sample, for which water-soluble extractives is usually higher than ethanol-soluble extractives. The determination of total extractives provides an initial assessment of the maximum phenolic compounds that can be extracted from MSK. From Table 3, the maximum concentration of extractable phenolic compounds expected in MSK (dry-basis) is 78.24 ± 0.64 mg g^{-1}. Furthermore, the average particle diameter of MSK is found to be 4.45 ± 0.35 mm, which is relatively large than other feedstock due to limitations with size reduction processes. The particle size distribution presented in SM 2 reveals that over 53% and 34% of the particles retained on an average mesh size of larger than 6.30 mm and 4.73 mm, respectively. About 12% of the particles were retained on smaller mesh sizes.

2) Equilibrium extraction time in SLE of phenolic compounds from MSK

The change in extraction yield (mg GAE g^{-1} dm^{-1}) over time during the extraction of phenolic compounds from MSK at three extraction temperatures (30ºC, 45ºC and 60ºC) and at constant solid-to-solvent ratio (1:10) using water and ethanol are shown in Figures 1 to 3. These profiles serve as basis in fixing the extraction time in the ensuing optimization experiments. To obtain a smooth (regressed) curve for each settings, the two-parameter, empirical model developed by Peleg (1988) was fitted to the experimental data. The SLE phenomena have been usually described empirically due to the complex nature of solute compounds in the feedstock. Among the empirical methods developed, Peleg model is widely considered in several studies in describing SLE kinetics of organic compounds from plant sources [15, 35]. Although it has limitations in providing insight on fundamental principles, Peleg model is found to adequately represent the relationship between extraction solvent and feedstock [15]. This model is shown in Eq. 3, where Y(t) refers to the extraction yield (mg GAE g^{-1} dm^{-1}) at a certain time, while K_1 and K_2 are Peleg rate constant (min g mg^{-1} GAE^{-1}) and Peleg capacity

constant (g mg^{-1} GAE^{-1}), respectively [36]. As noted in Eq. 1, the experimental and empirical (Peleg) yield at this point was expressed as mass ratio of total phenolic compounds to dry MSK (mg GAE g^{-1} dm^{-1}).

$$Y(t) = \frac{t}{K_1 + (K_2 \cdot t)} \quad \text{(Eq. 3)}$$

The extraction time for each profile was determined from the approximated curves, which is the point when the difference in yield between time interval is 1% or less. Then, the extraction times among the settings are compared and the highest value is the fixed extraction time for the SLE process.

The Peleg model parameters obtained after fitting to the experimental data are shown in Table 4, including the statistical parameters residual sum of squares (RSS), coefficient of determination (R^2) and the root mean squared error (RMSE). These statistical parameters specify the goodness-of-fit of the regression model to the experimental data. It can be noted from Table 4 that the Peleg model fits better the experimental data at an extraction temperature of 60°C using the pure ethanol solvent (R^2 = 0.6884; RSS = 7.50 x 10^3; RMSE = 13.51). Generally, the Peleg model is able to describe 43% to 68% of the data variation that is due to the increase in temperature and changes in solvent.

On a different note, it is shown in Table 4 that K_1 generally decreased to approximately 50% as temperature is increased from 30°C to 60°C when using water only. Moreover, K_1 decreased to about 34% as temperature is increased from 30°C to 60°C when using ethanol only as solvent. These results indicate that the initial mass transfer rate of phenolic compounds to the solvents increases as temperature increases [36–37]. In contrast, K_2 decreased to approximately 17% as temperature is increased from 30°C to 60°C when using water. However, K_2 increased to 26% as temperature is increased from 30°C to 60°C when using ethanol as solvent. These results indicate that the capacity of water in extracting phenolic compounds from MSK increases as temperature is increased, whereas the capacity of ethanol decreases as temperature is increased. There is an expected increase in yield as temperature is increased since the contact between phenolic compounds and solvent is enhanced. However, the decrease in capacity of ethanol can be attributed to the degradation of phenolic compounds, particularly flavanol dimers, at elevated temperatures [22]. This is in agreement with various studies presented in Table 1, where the optimum temperature is within 25°C – 35°C during extraction of phenolic compounds.

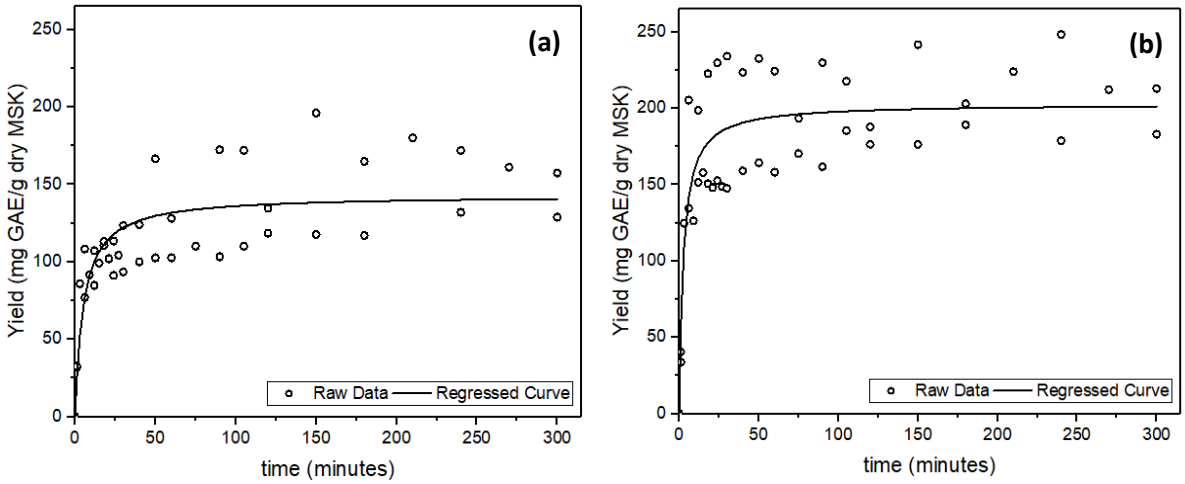

Figure 1 Phenolic compounds yield vs. time profile in the extraction of phenolic compounds from MSK using water (a) and ethanol (b) at 30°C (symbols: experimental data; lines: approximation via Peleg model).

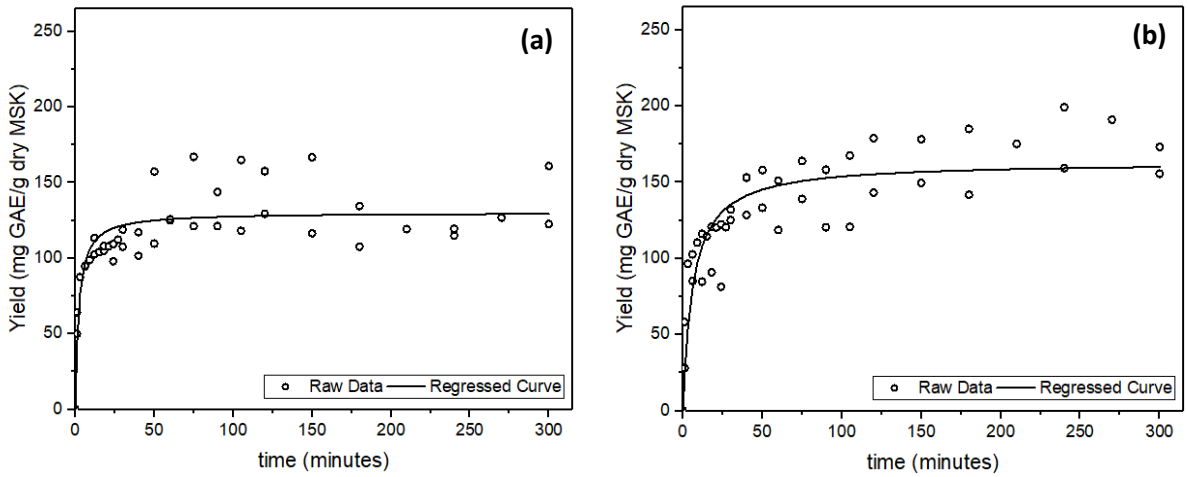

Figure 2 Phenolic compounds yield vs. time profile in the extraction of phenolic compounds from MSK using water (a) and ethanol (b) at 45°C (symbols: experimental data; lines: approximation via Peleg model).

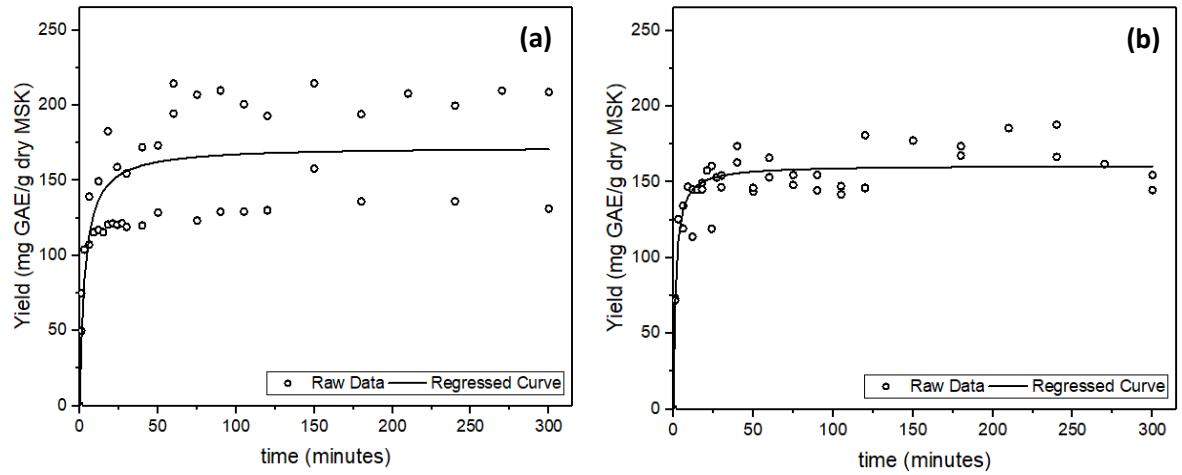

Figure 3 Phenolic compounds yield vs. time profile in the extraction of phenolic compounds from MSK using water (a) and ethanol (b) at 60°C (symbols: experimental data; lines: approximation via Peleg model).

Table 4 Extraction times for SLE of phenolic compounds from MSK at three extraction temperatures using water and ethanol as solvents (solid-to-solvent ratio = 1:10)

Solvent	Temp. (°C)	K_1 (min g mg^{-1} GAE^{-1})	K_2 (g mg^{-1} GAE^{-1})	Extraction time (min)	R^2	RSS (mg GAE g^{-1} $dm^{-1})^2$	RMSE (mg GAE g^{-1} dm^{-1})
Water	30	3.59×10^{-2}	7.00×10^{-3}	105	0.56	2.20×10^{4}	23.45
	45	1.59×10^{-2}	7.70×10^{-3}	50	0.53	1.18×10^{4}	16.94
	60	1.81×10^{-2}	5.80×10^{-3}	60	0.43	3.99×10^{4}	31.19
Ethanol	30	1.27×10^{-2}	4.90×10^{-3}	60	0.58	3.67×10^{4}	29.94
	45	3.79×10^{-2}	6.10×10^{-3}	105	0.68	1.75×10^{4}	20.68
	60	8.40×10^{-3}	6.20×10^{-3}	50	0.69	7.50×10^{3}	13.51

It is also presented in Table 4 the extraction times obtained from regressed curves of Figures 1 to 3. These extraction times are found at the equilibrium state of the extraction process. Therefore, the extraction yield referred to in the subsequent optimization results is the equilibrium yield. Furthermore, the highest extraction time noted from Table 4 is 105 min at an extraction temperature of 30ºC for water and 45ºC for ethanol, and a solid-to-solvent ratio of 1:10. This is in agreement with the range of extraction times observed in several studies [25–26, 38] and in Table 1, which is from 30 min to 24 h. The fixed extraction time is also expected for a conventional SLE of compounds from a complex feedstock matrix. Normally, the extraction time for the said process is extended (>60 min) to most, if not all, phenolic compounds in plant-based feedstock.

3) Model fitting and response surface analysis

The extraction yields for each run in CCD is presented in Table 3 and is expressed as mass percentage (%) of phenolic compounds and MSK in accordance to Eq. 2. These were correlated in terms of the process factors (temperature and solvent ratio) by the second-order polynomial equation (referred as the full model) shown in Eq. 4.

Eq. 4 was fitted to the yield (responses) for each run presented in Table 2 through regression analysis, specifically the method of least-squares, to allow approximation of model coefficients. The analysis of variance (ANOVA) showed that the two-way interaction effect ($p = 0.409$) was insignificant at both 90% confidence level ($p > 0.10$) and 95% ($p > 0.05$), while the rest of the effects are significant at both confidence levels. Hence, the two-way interaction effect was eliminated to construct a simplified model with an enhanced prediction capability. The estimated regression coefficients of the reduced model, together with other statistical parameters (standard error and t-statistics aside from p-value), are then summarized in Table 5. The ANOVA results are also shown in SM 3. Based on p-values of linear and interaction factors in SM 3, the statistical model, in general, was able to explain variations in the responses at 95% confidence level ($p > 0.05$) even at relatively high p-values of x_2. Moreover, the p-value for lack-of-fit revealed that the latter is statistically significant. This could be attributed to the exclusion of two-way interaction effects. Nevertheless, the reduced model was able to estimate the linear effects with greater precision than the quadratic effects based on the standard deviation or error value. On the other hand, t-statistics (t-stat) shows that the linear and quadratic effects have significant difference.

The results presented in Table 5 revealed that the main (linear) factors temperature (x_1) and solvent ratio (x_2) exhibited a positive effect on the equilibrium yield of phenolic compounds of MSK in this order: $x_1 > x_2$. Moreover, the quadratic dependencies of both factors influenced the response variable in a negative manner. Furthermore, each factor exerted its effect independently of the others since the two-way interaction effect was not significant.

$$y = \beta_o + \sum_{i=1}^{2} \beta_i x_i + \sum_{i=1}^{2} \sum_{j=1, i<j}^{2} \beta_{ij} x_i x_j \sum_{i=1}^{2} \beta_{ii} x_i^2 + \epsilon \qquad \text{(Eq. 4)}$$

In the equation above, y represents the equilibrium yield (process response variable), x_i and x_j are the coded independent variables (i = temperature; j = aqueous ethanol concentration), β_o is the intercept, β_i, β_{ii} and β_{ij} are the linear, pure quadratic and interaction regression coefficients, respectively, and ϵ is the experimental (residual) error.

Table 5 Coefficients of the simplified polynomial model for phenolic compounds extraction yield with relevant statistical parameters

Coefficient	Term	Value	Standard error	t-stat	p
β_o	Intercept	0.12	0.239	56.52	0.000
β_1	x_1	0.2554	0.293	5.33	0.000
β_2	x_2	0.2265	0.293	1.50	0.147
β_{11}	$x_1 \cdot x_1$	-0.002021	0.431	-2.11	0.046
β_{12}	$x_2 \cdot x_2$	-0.002141	0.431	-6.22	0.000
			Coefficient of determination (R^2)		0.7579
			Coefficient of determination (R^2), adjusted		0.7158
			Coefficient of determination (R^2), predicted		0.6298
			S-value		0.8274

In terms of decoded factors, the simplified model assumes the form presented in Eq. 5. The model provided a tolerable fit to the data, as manifested by the difference (5.71%) between its coefficient of determination (R^2) and the adjusted-R^2. The R^2 and the adjusted-R^2 values are 75.79% and 71.58%, respectively, while the predicted-R^2 is 62.98%. This means that the factors temperature and solvent ratio explain 75.79% of the variability in yield. Furthermore, the reduced model can predict the phenolic compounds yield within ±1.65 with a 95% confidence level based from the obtained value of S. Although there has not been an established standard for the acceptability of R^2, the values reported in this study are comparably lower than those reported in previous literature dealing with optimization [25–26]. This might have a negative implication to the reliability of model fitting. However, R^2 can be affected by several factors, such as effect of sample size, degree of randomness and predictors considered in the analysis [39]. In order to assess further the sufficiency of fitting, the normal probability plot of residuals shown in SM 4 is evaluated. It can be seen that residuals closely form a linear pattern with minimum dispersion. This indicates normal distribution of residuals, which justifies the adequacy of R^2 values in describing variability of responses.

The graphs of the resulting response surface are presented in Figures 4 to 5, where the model response (yield) is plotted as a function of temperature and aqueous solvent concentration. The surface plot is shown in Figure 4, while the contour plot is presented in Figure 5.

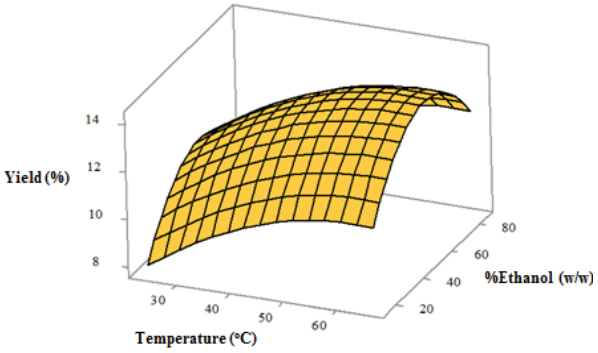

Figure 4 Response surface plot showing the response behavior of temperature (x_1) and %ethanol (x_2) on the phenolic compounds yield.

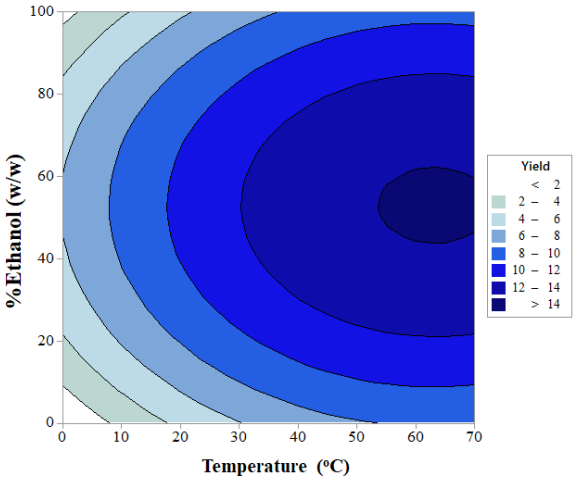

Figure 5 Contour plot showing the response behavior of temperature (x_1) and %ethanol (x_2) on the phenolic compounds yield.

$$y = 0.12 + 0.2554x_1 + 0.2265x_2 - 0.002021x_1^2 - 0.002141x_2^2 \quad \text{(Eq. 5)}$$

The positive effect of temperature is more defined in Figure 4 based on the surface curvature, while the range of optimum values for the extraction yield can be easily obtained from Figure 5. The equilibrium yield reached optimum values at temperatures approximately between 53ºC – 68ºC, above which the trend could not be directly determined. However, a decline in yield can be expected as temperature is further increased based on the curvature of the surface.

The encouraging effect of temperature can be attributed to its positive influence on the solubility of phenolic compounds to the binary solvent system. An increase in extraction temperature favors extraction by enhancing the solubility of the solute being extracted to the solvent. However, there is a limitation in terms of increasing further the extraction temperature. Phenolic compounds may experience thermal degradation under elevated temperatures, generally above 65ºC – 70ºC [12, 40–41]. This means that there is a certain temperature range at which optimum values for extraction yield exist. Beyond that range, a significant decrease in yield is most likely to be expected.

The positive effect of aqueous ethanol concentration is also more defined in Figure 4 based on the curvature of the surface plot, while the range of optimum values for the extraction yield can also be easily determined from Figure 5. An optimal aqueous ethanol concentration exists at about 55 – 60% ethanol. This range is in agreement with the results from several studies [25–26]. This result can be explained by the chemical characteristics (i.e. solubility), polarities and affinities (in terms of activity coefficients) of the various phenolic compounds present in MSK with a specific ethanol-water mixture. It is reported in various works that MSK specifically contains tannin, vanillin, coumarin, gallic acid and some other phenolic compounds of significant amounts [4, 9]. The solubility and activity coefficient values of these compounds in organic solvents, such as ethanol and water, suggest that phenolic compounds possess a solubility preference to solvents with intermediate polarity rather than solvents that are relatively polar. Hence, a mixture of ethanol and water would provide such requirement. In addition, the use of an ethanol-water system would allow simultaneous extraction of most phenolic compounds present in MSK. Another factor that would explain the results is related to the type and nature of ethanol and water as solvent system [42]. A comparison of polarity indices show that water tends to have a higher degree of interaction with phenolic compounds than ethanol. However, an enhanced extraction yield of phenolic compounds is obtained when a mixture of ethanol-water is used as the solvent system instead of water alone. This is demonstrated by the studies of Radojkovic et al. [43] and Gironi & Piemonte [44].

4) Optimization of process factors

The characteristics of the plots shown in Figures 4 and 5 propose that the recovery of phenolic compounds from MSK can be optimized by suitable selection of extraction conditions. Maximization of yield was performed numerically using the gradient descent method, giving the following results: 63.21ºC and 53.21% for extraction temperature and aqueous ethanol concentration, respectively. At these optimum conditions, the maximum yield is equal to 14.18%. Statistically, the factor settings optimized the response variable well based on the composite desirability (D) value that is 0.91. In ideal cases, D should be close or equal to 1. The standard error of fit, which assesses the discrepancies in the estimated mean response for the factor settings, is 0.37. Finally, the maximum response variable (yield) range for a 95% confidence interval and 95% predictor interval are 13.42% – 14.95% and 12.31% – 16.06% respectively. The confidence interval presents a range of probable values for the mean yield given the specified settings of factors, while predictor

interval is a range that is likely to contain a single future response (yield) for a selected combination of factor settings. Both intervals are helpful when assessing the practical significance of the results.

The maximum yield obtained in this study is relatively higher than most of the extraction yields of several types of feedstock shown in Table 1. This solidifies the potential of MSK as an interesting raw material in the production of phenolic compounds. In terms of process conditions, the optimum aqueous ethanol concentration is similar to those studies employing ethanol-water solvent. Moreover, the optimum extraction temperature obtained in this study is much higher than most of the studies presented in Table 1. This suggests that degradation of phenolic compounds did not transpire at the aforementioned process conditions.

5) Validation of response model

The solid-liquid extraction of phenolic compounds from MSK was repeated under the optimized conditions (temperature = 64°C; %ethanol = 53.21%) and the fixed conditions (time = 105 min; solid-to-solvent ratio = 1:10). The phenolic compound concentration of the extract produced is 5.77 ± 0.05 mg GAE g^{-1} MSK^{-1}. This value corresponds to an extraction yield of 14.88 ± 0.29%, which differs by about 4.98% from the value (14.18%) predicted by the model shown in Eq. 5 using the optimum conditions.

Moreover, the antioxidant potential of the extract is assessed in order to evaluate its possible activity against oxidative stress via DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. This assay is based on the measurement of the scavenging capacity of antioxidants towards the stable free radical DPPH [45]. DPPH assay is usually expressed as IC_{50}, which denotes the concentration of sample extract, required to scavenge 50% of the DPPH free radical. The IC_{50} value after assay is inversely proportional to the activity, which means that the antioxidant activity is higher at lower IC_{50} values. In this study, the antioxidant activity of the extract prepared under optimum extraction conditions and fixed extraction conditions has an IC_{50} value of 45 ± 0.002 μg GAE mL^{-1}. This value is relatively lower compared to the IC_{50} values of mango kernel extracts in methanol obtained from the study of Arogba & Omede [46], in which the IC_{50} values of the extracts from two mango varieties Mangifera indica and Irvingia gabonensis are 143.36 μg GAE mL^{-1} and 177.22 μg GAE mL^{-1}, respectively. On the other hand, the IC_{50} value of the extract in this work is higher than the IC_{50} value of the mango kernel (Mahachanok cultivar) extract in hexane, which is 8.05 μg GAE mL^{-1} [47]. However, there are no established standards yet for antioxidant activity based on DPPH assay for product quality and acceptability.

6) Utilization of spent MSK

An impending waste material in the form of spent MSK is generated as phenolic compounds are extracted. From an environmental and economic perspective, there is an urgent call to reuse these wastes through the zero-waste model in waste management. In this model, waste materials are given value by identifying their desired application [48]. Aside from its physicochemical properties presented in Table 4, MSK contains about 7% protein and >30% of carbohydrates [49]. During extraction, only the lignin-containing compounds are degraded to release phenolic compounds; hence, the aforementioned components are still present in the matrix of spent MSK. This would imply then that spent MSK has a potential application in the production of food products. The carbohydrate content of spent MSK indicates that it can be an acceptable source of energy when consumed; hence, it can be a raw material in flour and feed mix production [50]. Moreover, most of the essential amino acids comprise the amino acid profile of MSK even at low protein concentration [49].

Conclusions

The results of this study indicate that the phenolic compounds present in mango seed kernel can be recovered through solid-liquid extraction with a mixture of ethanol-water solvent. Application of central composite design and response surface modeling allowed evaluation of the contribution of the two process variables temperature (x_1) and %ethanol (x_2) and optimization of the extraction process to the recovery of phenolic compounds, in terms of yield. The linear (x_1 and x_2) and quadratic (x_1^2 and x_2^2) effects of the process variables had a significant contribution to the response, while the two-way interaction was insignificant. Both linear effects imparted a positive influence to the yield, whereas the quadratic effects had a negative impact. The optimum process conditions are as follows: temperature = 63.21ºC and %ethanol = 53.21% at 105 min and 1:10 solid-to-solvent ratio. Furthermore, response model validation showed that the model sufficiently describes the extraction process.

This study also presents the utilization of a waste material in the mango processing industry that has human health benefits and an economic potential. Hence, this study follows the framework of a zero-waste environment in which wastes are used as raw material for new products and application. This framework is also considered in the case of spent MSK, in which it has potential in several applications. In addition, this study supports the principle of 5R's in waste management – reduce, reuse, recycle, recover and residuals (rot) as a means to mitigate the problems on waste disposal, which is always an environmental concern.

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