Optimisation of Extraction Parameters for Phenolic Content in Banana (*Musa* sp.) Peels

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Abstract: Banana peels are unused by-product and form about 18-40% of the whole fruit. With a great advantage of exploiting banana peels as a source of valuable compound, this study was carried out to optimise the extraction condition for phenolic content from two varieties of local banana (*Musa* sp.) peels using response surface methodology (RSM). The varieties are including *Pisang Nangka*, and *Pisang Tanduk*. A single factor of central composite rotatable design (CCRD) was applied to determine the effect of extraction temperature (°C) and extraction time (min) on total phenolic content (TPC) from the unripe banana peels. Based on the results, the optimum condition of extraction for *Pisang Nangka* is 65.80 °C for 74.36 minutes, and *Pisang Tanduk* is 58.37 °C for 79.27 minutes and the total phenolic content (TPC) obtained were 1305.02 mg GAE/100 g DW, and 3573.13 mg GAE/100 g DW respectively. The results also showed that TPC was significantly influenced by both factor studied.

Keywords: Banana Peels, Total Phenolic Content (TPC), Extraction Parameters, Response Surface Methodology (RSM)

Introduction

Ample attention has been engrossed on the activity of natural antioxidant in fruits and fruit's by-product because their high potential in reducing the level of oxidative stress (Tan *et al.*, 2012). Fruit wastes are highly perishable and producing problem to processing industries. These problem can be reduced by utilizing its value compounds that has great potential as natural food preservative. This would be the safer and healthier alternative to replace the artificial preservative.

Banana is a tree-like plant of genus *Musa* in the family Musaceae. Banana (*Musa* sp.) is a delightful nutritious fruit which can provide a good source of nutrients and energy. Banana plants are considered as one of the world's biggest herbs which grown abundantly worldwide. After citrus, banana is found to be the second largest produced fruit which contributing to 16% of world's total fruit production (Annu *et al.*, 2015). In Malaysia, banana is considered as one of the largest fruit crop cultivation after durian for both domestic and exported market which covering about 29 000 hectares of plantation area with a total production of 289 000 metric ton (Malaysia Department of Agriculture, 2012). Kanazawa and Sakakibara (2000) has classified banana as one of strong oxidative food. Many of previous study have mentioned that peel fraction of fruits may potentially contain more antioxidant qualitatively or quantitatively than pulp fraction (Marina & Noriham, 2014). According to Someya *et al.* (2002), banana peel contained stronger antioxidant activity, pooled more quantity of phenolic compounds, and higher mineral content compared to banana pulp.

Peel is the main by-product in banana which represent up to 40% of total weight of fresh banana and in average, it is estimated that about 220 tons of banana waste can be produced per hectare every

year as one banana plant contributed up to 80% of total plant mass (Malaysia Department of Agriculture, 2012). Previous study by Hernandez-Carranza *et al.* (2015) shows that banana peels is rich in phytochemical compound mainly in antioxidant capacity such as phenolic compound like catechin, epicatechin and galocatechin, anthocyanin (delphinidin and cyaniding), carotenoids (β -carotenoids, α -carotenoid, and xanthophylls), catecholsmine, sterols, and triterpines. In the past decade, Kanazawa and Sakakibara (2000) reported that banana pulp and peels contains catecholamines, dopamine, and flavonoids. In 2011, study Nagarajaiah and Prakash stated the components of banana peels consist of 0.9% protein, 1.7% lipid, 8.9-12.96% ash, 59% carbohydrates, and 31.7% fibre. High percentage of ash content indicated the high concentration of mineral content such as potassium, calcium, sodium, iron, manganese, and bromine.

Based on the data collection by Malaysia Agricultural Research and Development Institute (MARDI), there are more than 50 edible banana cultivars in Malaysia (Sulaiman *et al.*, 2011). Among the numerous cultivars, two plaintain including *PisangNangka* and *PisangTanduk* will be selected for this study. This selection of cultivars is based on the most common cultivars that used in food production (producing chips) in Malaysia and contributed to more unutilised peels.

The aim of extraction process should be to provide maximum yield of phenolic content at the optimum extraction condition (temperature and time of extraction. The condition of extraction may be differ in every different plant sources as the yield of phenolic can be influenced by several factors including chemical nature of sample, solvent used, agitation, solvent ratio, time and temperature of extraction (Khoddami *et al.*, 2013, Alberti *et al.* 2014). Khoddami *et al.* (2013) also stated that among all those factors, the time and temperature of extraction are found to be the most crucial parameters as the increasing of time and temperature during extraction endorse analyte solubility.

To date, no comparative study has been done to measure the optimum temperature and time of extraction on selected species of bananas. Exploring the ideal condition of each species can ensure the efficiency of extraction. Therefore, this study is aimed to determine the optimum condition (temperature and time) of extraction for total phenolic content of banana peels using Response Surface Methodology (RSM) approach.

Materials and Methods

Sample Preparation

The peels from two cultivars of plantain (*Nangka*, and *Tanduk*) were used in this research. Fresh banana were taken freshly from farm located in Pontian, Johor. The fruits chosen were in maturity index III and uniform in size. The blemished peel were eliminated. The method of sample preparation was obtained from Hernandez-Carranza *et al.* (2015) with some modification. The banana were washed with tap water, and rinsed with distilled water. The peels were separated from the pulp and cut into cube. Then, peels were dry in cabinet dryer (Vision Scientific Enterprise) at 60°C for 36 hours. The dried peels was ground using grinder to make it into powder and passed through the sieve shaker (Retsch Model AS2000) at 50 mesh. Powdered peels will be stored in hermetic bottle in chiller prior to analysis (Gonzalez *et al.*, 2010a, 2010b).

Solvent Extraction

Extraction of phenolic content from dried unripe banana peels were conducted based on Sulaiman *et al.* (2011) with slight modifications. Exactly 10 g of powdered sample was extracted with 100 ml of 80% ethanol in conical flask. The conical flask was fully covered with aluminium foil to prevent light exposure. The extraction was done in incubator shaker (New Brunswick Scientific, Model 5420) under constant agitation at 80 rpm with required temperature and time of extraction. After that, the extract was filtered through a muslin cloth to remove residue. Then, filtered extract was centrifuged (Kutoba Corporation, Model 5420) at 3000 rpm for 15 minutes for separation in order to discard the precipitate. The supernatant was concentrated under reduce pressure at

50°C using rotary evaporator (Chemophram) until about 95% of the filtrate had been evaporated. The concentrated extract were kept in amber bottle and store at -20°C prior to analysis.

Determination of Total Phenolic Content

The total phenolic content (TPC) was determined using Folin-Ciocalteu reagent according to the method described by Fatemeh *et al.* (14) with slight modification. Approximately, 10 mg of crude extraction was dissolved in 2 ml solvent. Then, exactly 100 µl of the mixture was mixed with 0.5 ml Folin-Ciocalteu reagent and 7.9 ml distilled water. The mixture was kept in room temperature for five minutes. After that, 1.5 ml of 20% (w/v) sodium carbonate (Na₂CO₃) was added to mixture. Later, the mixture was left to stand for two hours in dark place at room temperature. The absorbance was measured against blank sample at 765 nm using UV-VIS light spectrophotometer (Genesys 20, Model 4001/4, Thermo Fisher Scientific). Each extract was analysed triplicate. The TPC was expressed as Gallic Acid Equivalent (mg GAE/ 100 g of dried weight) and was determined on the basis of standard curve using Gallic acid as standard (0 – 1000 ppm).

Optimization Condition of Phenolic Compounds Extraction

The experimental design was generated using software Design Expert (Stat-Ease Version 6.0.4). Optimisation of extraction parameters of banana peels was carried out by employing the response surface methodology (RSM) using central composite rotatable design (CCRD). CCRD with two-factors (X_1 and X_2) and five level ($-\alpha$, -1, 0, 1, + α) was applied. The complete CCRD comprised of thirteen experiment with eight factorial point, and five centre point (Table 2). The temperature of extraction (°C), and time of extraction (minutes) were recorded as independent variables while total phenolic content (TPC) was set as the dependant variable. Each experiment was performed triplicate and the average was account as response. Experimental data was fitted with statistical models to produce response surface. Models were deemed suitable when it is significant based on ANOVA, insignificant lack of fit and r^2 of more than 0.75. The chosen models were subsequently optimised based on optimisation criteria of optimum temperature and time while TPC were set for maximum (Chan *et al.*, 2009, Deniz *et al.*, 2007a, 2007b). Table 1 shows the symbol and coded factor levels for the independent variables and table 2 displays the thirteen experimental combination that generated by the Design Expert Software. All experiments were done according to the condition described in table 2.

Table 1: Coded and uncoded levels for independent variable for experimental design

Symbols	Independent variable factor	Coded and uncoded levels				
		$-\alpha$	-1	0	1	$+\alpha$
		-1.414				+1.414
X_1	Temperature (°C)	25.68	35.00	57.50	80.00	89.32
X_2	Time (minute)	0.50	21.00	70.50	120.00	140.50

Table 2: Actual and Coded levels of Temperature (X_1) and time (X_2) constructed using software.

Run	X_1 A:Temperature (°C)	X_2 B:Time (min)
1	35.00 (-1.000)	21.00 (-1.000)
2	80.00 (1.000)	21.00 (-1.000)
3	35.00 (-1.000)	120.00 (1.000)
4	80.00 (1.000)	120.00 (1.000)
5	25.68 (-1.414)	70.50 (0.000)

6	89.32 (1.414)	70.50 (0.000)
7	57.50 (0.000)	0.50 (-1.414)
8	57.50 (0.000)	140.50 (1.414)
9*	57.50 (0.000)	70.50 (0.000)
10*	57.50 (0.000)	70.50 (0.000)
11*	57.50 (0.000)	70.50 (0.000)
12*	57.50 (0.000)	70.50 (0.000)
13*	57.50 (0.000)	70.50 (0.000)

*replication of centre point

Result and Discussion

Fitting the model

The result of Total Phenolic Content (TPC) for banana peels from *Pisang Nangka* and *Pisang Tanduk* using central composite rotatable design (CCRD) are shown in Table 3. The statistical results suggested that quadratic model was the most suitable model for TPC for both samples. The response surface equation for the fitting of TPC data based on quadratic models are shown in Table 5. Referring to variance analysis, the models for both sample were significant due to low probability value where ($p < 0.05$). The independent variables of X_1 , X_{11} and X_{22} were found to be the significant model terms at the probability level of 95%. The fitness and adequacy of the models were judged by the coefficient of determination r^2 and the significance of lack-of-fit. The value of r^2 explained the difference to the total variation and the closer the r^2 value to unity, the better the empirical model fits the actual data (Fatemeh *et al.*, 2009). For this study, the r^2 value for models were higher than 0.75, which indicating a good fit. The r^2 value for *Pisang Nangka* peel was 0.8604 and 0.9803 for *Pisang Tanduk* peel. The lack-of-fit test for both samples were not significant ($p > 0.05$) representing a good fit between experimental data and strengthen the reliability the model.

Table 3: Actual levels of independent variables along with observed values for TPC

Run	X_1 Temperature (°C)	X_2 Time (min)	TPC mg GAE/ 100 g DW	
			<i>PisangNangka</i>	<i>PisangTanduk</i>
1	35.00	21.00	980.01	2050.28
2	80.00	21.00	1117.06	2297.21
3	35.00	120.00	890.72	2606.60
4	80.00	120.00	1230.61	2498.70
5	25.68	70.50	1010.03	2128.04
6	89.32	70.50	1150.32	2301.73
7	57.50	0.50	1076.10	2117.45
8	57.50	140.50	1138.48	2659.38
9*	57.50	70.50	1339.00	3423.44
10*	57.50	70.50	1210.56	3599.10
11*	57.50	70.50	1349.01	3694.74
12*	57.50	70.50	1332.34	3417.59
13*	57.50	70.50	1378.01	3544.45

*replication of centre point

Table 4: Analysis of coefficient models used to fit TPC experimental data

Independent variable	PisangNangka peel			PisangTanduk peel		
	TPC Coefficient	F	Prob<F	TPC Coefficient	F value	Prob<F
Intercept	1321.95	12.32	0.0017*	3535.86	99.46	<0.0001*
Temperature, X ₁	84.42	11.32	0.0099*	48.08	1.53	0.2505
Time, X ₂	14.06	0.31	0.5905	190.53	24.09	0.0012*
X ₁₁	-130.67	23.59	0.0013*	-645.10	240.19	<0.0001*
X ₂₂	-117.11	18.95	0.0024*	-558.34	179.92	<0.0001*

*significant p<0.05

Table 5: Model equation fitted for TPC experimental data

Samples	Model equation	Model significance	Lack-of-fit	R ²
PisangNangka peel	Actual equation: -4.75382 + 33.43487X ₁ + 7.02327X ₂ - 0.25811X ₁₁ - 0.047796X ₂₂	0.0017 (Significant)	0.3740 (not significant)	0.8604
	Coded equation: +1321.95 + 84.42X ₁ + 14.06X ₂ - 130.67X ₁₁ - 117.11X ₂₂			
PisangTanduk peel	Actual equation: -2204.01823 + 148.67886X ₁ + 35.97865X ₂ - 1.27428X ₁₁ - 0.22787X ₂₂	<0.0001 (Significant)	0.6192 (not significant)	0.9803
	Coded equation: 3535.86 + 48.08X ₁ + 190.53X ₂ - 645.10X ₁₁ - 558.34X ₂₂			

Analysis of response surface plot

The response surface three dimensional diagram were employed to describe an interactive effect of temperature and time of extraction towards total phenolic content (figure 1). By referring to figure 1 (a), the TPC for *Pisang Nangka* peel extract was readily increased with the increasing of temperature up to 68.75 °C and followed by slight decrease afterwards. The similar trend also showed in figure 1 (b) where the TPC of *Pisang Tanduk* peel increased with the increasing of temperature up to 60 °C and start to decrease subsequently. This suggested that incubation in slightly higher temperature did improve phenolic extraction, and gentle enough to avoid heat degradation of the target phenolic antioxidant. According to literature, mild heating might soften the plant tissues, deteriorate the cell wall integrity, hydrolyse the bond of phenolic compounds including phenol-protein and phenol-polysaccharide, and boost the phenolic solubility, therefore more phenolic would distribute to the solvent (Li *et al.*, 2006, Spigno *et al.*, 2007, Fatemeh *et al.*, 2009).

With regard to time of extraction, the trend showed that TPC of both samples increased with the increasing time up to 70 to 80 minutes of extraction and gradually degraded afterwards. From the results, it shows that the longer extraction time may compensate the beneficial effects of moderate temperature by inducing oxidation as well as degraded the yield of TPC.

Figure 1: 3D contour plot of response surface for the interaction between temperature and time of extraction that affect total phenolic content for Pisang Nangka (a) and Pisang Tanduk (b).

Verification of predicted model

The suitability of the model equation for predicting the response value was verified by conducting the extractions under recommended optimal condition. In this study, a numerical optimisation method was adopted to find a point that maximised the responses. The verification was selected based on desirability and suitability. At the end, the experimental and predicted results of TPC were compared to support the optimised data as given by the statistical modelling according to the solutions determined by the reduced model with higher desirability. Referring to the results in table 6, the experimental results for both peel samples were very close to the each predicted values. This indicated that there was a high fit degree between the experimental values and the values which predicted by regression model. Thus, the response surface modelling could be applied effectively to predict extraction phenolic compounds from peels of *Pisang Nangka* and *Pisang Tanduk*.

Table 6: Optimum condition, predicted and experimental value of response under those condition

Sample	Optimum condition		TPC (mg GAE/ 100 g DW)	
	Temperature (°C)	Time (min)	Experimental ^a	Predicted
<i>Pisang Nangka</i> peel	65.80	74.36	1395.54	1321.97
<i>Pisang Tanduk</i> peel	58.34	78.98	3470.29	3573.13

Conclusions

The data of this study showed that the temperature and time of extraction had significant effect ($p < 0.05$) on the total phenolic content of banana peels. The use of RSM has effectively optimised the extraction condition. Optimised condition of extraction for *Pisang Nangka* is 65.80 °C for 74.36 minutes, and *Pisang Tanduk* is 58.37 °C for 79.27 minutes and the total phenolic content (TPC) obtained were 1305.02 mg GAE/100 g DW, and 3573.13 mg GAE/100 g DW respectively. The results revealed that moderate temperature (50 – 70 °C) and shorter time (70 – 80 min) consist of high concentration of phenolic compounds. The obtained results concluded that banana peels has a wide range of phenolic compounds and will affected by the extraction condition.

Conflict of interest

Authors state that they have no conflict of interest regarding this submission.

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References

- Alberti A., Zielinski A.A.F., Zardo D.N., Demiate I.M., Nogueira A., Mafra L.I., (2014). Optimisation of the Extraction of Phenolic Compounds from Apples using Response Surface Methodology, *Journal of Food Chemistry*, 149, 151-158
- Annu A., Baskar R., &Sivasankari C. (2015). Optimization of Flavonoid Extraction from the peel of Poovan variety banana (*Musa acuminata*) using L16 Orthogonal Design, *Journal of Chemical and Pharmaceutical Science*, volume 8, Issue 4
- Babbar, N., Oberoi, H.S., Uppal, D.S., &Patil, R.T. (2011). Total phenolic content and antioxidant capacity of extracts obtained from six important fruit residues. *Food Research International*, 44, 391–396.
- Baskar R., Shrisakthi S., Sathyapriya, Shyampriya R., Nithya R. &Poongodi O. (2011), Antioxidant potential of peel extracts of banana varieties (*Musa sapientum*)
- Chun O.K., Dae-Ok K., Smith N., Schroeder D., Jae T.H., & Chang Y.L., (2005). Daily consumption of phenolics and antioxidant capacity from fruit and vegetables in American diet. *Journal of the Science of Food and Agriculture* 85:1715-1724.
- Chan S.W., Lee C.Y., Yap C.F., Wan Aida, W.M. &Ho C.W., (2009), Optimization of extraction conditions for phenolic compounds from Limau Purut (*Citrus hystrix*) peels, *International Food Research Journal* 16: 203-213.
- Deniz B., &Ismail H.B. (2007a), Modeling and optimization II: Comparison of estimation capabilities of response surface methodology with artificial neural networks in a biochemical reaction, *Journal of Food Engineering* 78 (2007) 846–854
- Deniz B., &Ismail H.B., (2007b), Modeling and optimization I: Usability of response surface methodology, *Journal of Food Engineering* 78 (2007) 836–845
- Fatemeh S.R., Saifullah R., Abbas F.M.A. &Azhar M.E., (2012). Total phenolics, flavonoids, and antioxidant activity of banana pulp and peel flours: influence of variety and stages of ripeness, *International Food Research Journal* 19 (3): 1041-1046.
- González-Montelongo, R., Lobo, M. G., & González, M. (2010a). The effect of extraction temperature, time, and number of steps on the antioxidant capacity of methanolic banana peel extract, *Separation and Purification Technology* 71, 347-355
- González-Montelongo, R., Lobo, M. G., & González, M. (2010b). Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds. *Food Chemistry*, 119, 1030–1039
- Hernandez-Carranza P., Avila-Sosa R., Guerrero-Beltran J.A., Navarro-Cruz A.R., Corona-Jimenez E. & Ochoa-Velasco C.E. (2015). Optimization of antioxidant compounds extraction from fruit by-products: apple pomace, orange, and banana peel, *Journal of Food Processing and Preservation* ISSN 1745-4549.
- Kanazawa K. &Sakakibara H., (2000) High content of dopamine, a strong antioxidant, in Cavendish Banana. *Journal of Agriculture and Food Chemistry* 48:844-848.
- Khoddami A., Wilkers M.A., Roberts T.H., (2013). Techniques for analysis of plant phenolic compounds. *Journal Molecules*, 18, 2328-2375.
- Li L., Li X., Ban Z., & Jiang Y. (2014) Variation in antioxidant metabolites and enzyme of Red Fuji apple pulp and peel during cold storage. *International Journal of Food Properties* 17(5): 1067-1080.
- Malaysia Department of Agriculture (2012), Fruit Crops Statistic, pg248.

- Marina Z. and Noriham A., (2014), Quantification of total phenolic compound and in *vitro* antioxidant potential of fruit peel extract, *International Food Research Journal* 21(5): 1925-1929.
- Nagarajaiah, S. B., Prakash, J. (2011). Chemical composition and antioxidant potential of peels from three varieties of banana. *As. J. Food Ag-Ind.*, 4(1), 31–46.
- Someya, S., Yoshiki, Y., & Okubo, K. (2002). Antioxidant compounds from bananas (*Musa cavendish*). *Food Chemistry*, 79, 351–354.
- Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effect of extraction time, temperature, and solvent concentration and antioxidant activity of grape marc phenolics. *Journal of Food engineering*, 81(1), 200-208.
- Sulaiman S.F., MdYusoff N.A., Eldeen I.M., Seow E.M., Sajak A.A.B., Supriatno&Ooi K.L., (2011) Correlaion between total phenolic and mineral content with antioxidant activity of eight Malaysian banana (*Musa sp.*) *Journal of Food Composition and Analysis* 24:1-10.
- Tan S.E., Aminah A., Khalid H.M., Mohamad Y.M., & Maaruf A.G., (2012) Antioxidant properties of three banana cultivars (*Musa acuminata* Berangan, Mas, and Raja) extracts. *Sains Malaysiana* 41(3):319-324.