Response Surface Methodology Optimization for Extraction of Phenolics and Antioxidant Capacity in Defatted *Dabai* Parts
(Pengoptimuman Kaedah Sambutan Permukaan Bagi Pengekstrakan Fenolik dan Kapasiti Antioksidan dalam Bahagian Dabai Nyahlemak)

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ABSTRACT

This study aimed to determine the total phenolics and antioxidant capacity of defatted dabai *parts based on liquid extraction and optimized using response surface methodology (RSM). A two-level factorial design was applied to determine the effect of two independent variables (extraction time: X_1 and % methanol: X_2) on three response variables (total phenolic content: Y_1, total flavonoid/anthocyanin content: Y_2 and Trolox equivalent antioxidant capacity: Y_3). The optimum conditions for extraction time and percent methanol were 36 min or 1 min and 62.25% or 53% for the defatted* dabai *pulp or peel, respectively. The RSM optimized extraction was compared with sonication-assisted extraction. Optimization results showed that defatted* dabai *parts had high total phenolic content and antioxidant capacity. Sonication-assisted extraction utilized the optimized extraction conditions had further increased the total phenolic content and antioxidant capacity of defatted* dabai *peel, but not in the pulp. Therefore, optimization of different extraction methods for the defatted fruit parts is recommended for future studies.*

Keywords: Antioxidant capacity; Canarium odontophyllum*; response surface methodology; sonication; total phenolics*

ABSTRAK

Kajian ini bertujuan menentukan jumlah fenolik dan kapasiti antioksidan buah dabai nyahlemak berdasarkan prosedur pengekstrakan cecair yang dioptimumkan menggunakan kaedah sambutan permukaan (RSM). Satu reka bentuk dua-peringkat faktorial telah digunakan untuk menentukan kesan daripada dua pembolehubah bebas (masa pengeskstrakan X_1 dan % metanol: X_2) ke atas tiga pembolehubah respon (jumlah kandungan fenolik: Y_1, jumlah kandungan flavonoid/antosianin: Y_2 dan kapasiti antioksidan setara Trolox: Y_3). Keadaan optimum untuk tempoh pengekstrakan dan peratusan metanol adalah 36 min atau 1 min dan 62.25% atau 53%, masing-masing untuk isi atau kulit dabai nyahlemak. Pengekstrakan yang dioptimumkan oleh RSM telah dibandingkan dengan pengekstrakan bantuan-sonikasi. Hasil kajian menggunakan keadaan pengekstrakan optimum menunjukkan bahawa bahagian dabai nyahlemak mengandungi jumlah kandungan fenolik dan kapasiti antioksidan yang tinggi. Pengekstrakan bantuan-sonikasi menggunakan keadaan pengekstrakan optimum didapati meningkatkan jumlah kandungan fenolik dan kapasiti antioksidan kulit dabai nyahlemak tetapi tidak bagi bahagian isinya. Oleh itu, pengoptimuman kaedah pengekstrakan yang berlainan untuk bahagian buah-buahan nyahlemak adalah dicadangkan untuk kajian masa hadapan.

Kata kunci: Canarium odontophyllum*; jumlah fenolik; kaedah sambutan permukaan; kapasiti antioksidan; sonikasi*

INTRODUCTION

Dabai is a type of olive-like fruit. In Sarawak, *dabai* (*Canarium odontophyllum* Miq.) is known as 'Sibu olive'. Similar to olive, *dabai* peel exhibited the highest antioxidant capacity compared with other *dabai* parts, which was highly correlated with total phenolic content (Shakirin et al. 2010). Defatted *dabai* parts are considered as by-products of *dabai* oil extraction, where these by-products are potential source of antioxidants. Besides, previous study found that defatted *dabai* pulp and peel contained flavonoids and anthocyanins as major phenolic compounds, respectively (Khoo et al. 2012a).

Optimization of extraction method to obtain higher total phenolics and antioxidant capacity from defatted *dabai* is essential for future nutraceutical product development. Until now, there is no optimized extraction procedure for the defatted *dabai* parts. Various factors are needed to be considered for preservation of antioxidant properties in the defatted *dabai* parts. De Rensis and Scaramuzzi (2003) and Lim and Murtijaya (2007) revealed that increase in temperature is the potential cause of antioxidants loss. On the other hand, heat generated from sonication-assisted extraction may contribute to decrease in total phenolics (Xu et al. 2010). Conversely, sonication-assisted extraction has been shown to increase extraction yield and offered a higher recovery (Vilkhu et al. 2008).

Based on previous literature, the response surface method has been successfully optimized the phenolic

content and extract yield in fruit samples (Prasad et al. 2012). Central composite design was applied in determination of optimum conditions for extraction. Extraction time and water-methanol ratio are two important factors controlled for obtaining an extract with optimal total phenolics and antioxidant capacity. These two independent variables (time and percent methanol) has also been studied by Mané et al. (2007) for extraction of phenolics from grapes using RSM, which showed a significant interaction for these two factors.

Our initial study showed that methanol and water extracts of defatted *dabai* parts demonstrated high phenolic contents and antioxidant capacity as compared to other extraction solvents (Khoo et al. 2012b). In this study, the extraction time and percentage of methanol were considered for optimization of total phenolics and antioxidant capacity using RSM. Other parameters such as pH, temperature and solid to liquid ratio were not taken into consideration. Although these parameters seem important in food analysis especially food product development and yield harvesting; however, for antioxidant analysis, temperature and pH could affect the reproducibility of antioxidant properties obtained from plant sources. Besides, phenolic compounds are stable at pH7 and decomposition occurs during pH changes (Xia et al. 2010).

MATERIALS AND METHODS

CHEMICALS

Ethanol, methanol and n-hexane were purchased from Fisher Scientific (Leicestershine, UK). Acetic acid and $FeCl_3.6H_2O$ were obtained from Merck (Darmstadt, Germany). Hydrochloric acid was purchased from Scharlau (Barcelona, Spain) and other chemicals were obtained from Sigma Chemical Co. (Missouri, USA). All solvents are analytical grades.

SAMPLE PREPARATION

Fresh *dabai* fruits were collected from few different locations in Sarawak, Malaysia. The pulp and peel of *dabai* fruits were freeze dried using a bench top freeze dryer (Virtis, New York, USA). The lyophilized sample powders were defatted using hexane before subjected to different extraction methods. The defatting procedure was adapted from Khetarpaul et al. (2004).

EXPERIMENTAL DESIGN

A two-level factorial central composite design (CCD) was developed to obtain an optimize extraction method for determination of total phenolics and antioxidant capacity in defatted *dabai* parts. Two selected independent variables (uncoded) were determined, namely extraction time (X_1: 1–30 min), percentage of methanol (X_2: 50–100%) on total phenolic content (Y_1) and total flavonoid content/ total anthocyanin content (Y_2) and Trolox equivalent

antioxidant capacity (Y_3). The response optimizer was applied for both graphical and numerical optimizations to obtain optimum conditions and predicted values for the response variables.

SAMPLE EXTRACTION BASED ON RSM

The extraction procedure was adapted from a method described by Earp et al. (1981). Defatted *dabai* part powders (1.0 g each) were added with 10.0 mL of different percentages of aqueous methanol which based on RSM design (50–100%). A solvent to solid ratio of 10:1 is more than enough to obtain an optimum yield (Pinelo et al. 2005). The sample extraction was carried out by shaking on a vortex for 1–30 min (RSM design) at room temperature (~25°C) and filtered using a funnel. The funnel was rinsed with 1 mL aliquot of extraction solvent before the filtration. All samples were extracted once for each RSM experiment.

SONICATION-ASSISTED EXTRACTION

The liquid-solvent extraction was determined based on a method described by Le Floch et al. (1998) with some modifications. Defatted *dabai* powder (1.0 g each) was added with 10 mL of the optimized methanol concentration and sonicated based on the optimized extraction time using a Power Sonic 405 Ultra-sonicator (Hwashin Technology Co., Seoul, Korea) at 40 kHz to facilitate the extraction. After each step, the mixture was filtered using a funnel and the extract was separated.

TOTAL PHENOLIC CONTENT

Analysis of total phenolic content (TPC) was performed using Folin-Ciocalteu reagent assay (Hajimahmoodi et al. 2009) which were modified from the method of Singleton and Rossi (1965). Defatted *dabai* pulp or peel extracts (200 μL) were mixed with 1.5 mL of Folin-Ciocalteau reagent (10-fold dilution) and allowed to stand at room temperature for 5 min. Then 1.5 mL of sodium bicarbonate (Na_2CO_3) solution (60 g/L) was added to the mixture. The absorbance was measured at 750 nm using a UV–Vis spectrophotometer (RS232, Cedex, France) after 90 min incubation at room temperature. All samples determined based on triplicate analysis except RSM experiments.

TOTAL FLAVONOID CONTENT

Total flavonoid content (TFC) was determined using aluminum chloride colorimetric method (Chang et al. 2002). Quercetin was used for standard calibration curve at concentration of 5–200 μg/mL. The diluted defatted *dabai* pulp extract or standard (0.5 mL) was added with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. After 30 min incubation at room temperature, absorbance of the reaction mixture was read at 415 nm using a UV–Vis spectrophotometer (RS232, Cedex, France). For blank,

10% aluminum chloride was used. All tests were performed in triplicate except the RSM experiments.

TOTAL ANTHOCYANIN CONTENT

Total anthocyanin content (TAC) of defatted *dabai* peel extract was determined based on a method described by Xu et al. (2007). Briefly, the extract was diluted with 60% ethanol (pH3.0). Absorption value was measured at 535 nm. All tests were performed in triplicate except RSM experiments. TAC was calculated as shown in:

$$TAC = \frac{A_{535\ nm} \times V \times N}{98.2 \times m}, \tag{1}$$

where TAC is total anthocyanin content in extract (mg/g), $A_{535\ nm}$ is absorption value measured at 535 nm, V is total volume (mL), N is dilution multiple, 98.2 is extinction coefficient at 535 nm and m is mass of the sample.

ANTIOXIDANT CAPACITY

Antioxidant capacity was performed based on ferric-reducing antioxidant power (FRAP) assay (Benzie & Strain 1996). FRAP reagent was prepared by mixing 0.3 M acetate buffer (pH3.6), 0.01 M TPTZ [2,4.6-tris(2-pyridyl)-s-triazine] and 0.02 M $FeCl_3.6H_2O$ at a ratio of 10:1:1 at 37°C. Defatted *dabai* pulp or peel extract (0.2 mL) was mixed with 3 mL of FRAP reagent and incubated at 37°C. Change in absorbance was measured at 593 nm after initial mixing and at 30 min. Trolox solution (0.2–1.0 mM) were used for calibration. All tests were performed in triplicate except RSM experiments.

STATISTICAL ANALYSIS

RSM design was created and analyzed using a statistical package, Minitab 15 (Minitab Inc., PA, USA). Fitness to RSM model between theoretical and practical values of the predicted dependent variable was determined by 2-sample *t*-test at significant value of $p<0.05$. The important criteria for choosing an adequacy model for this design were regression analysis (r^2) and ANOVA analysis ($p<0.05$). Total phenolics and antioxidant capacity of the defatted *dabai* pulp and peel were expressed as means ± standard deviations.

RESULTS AND DISCUSSION

RSM MODELS VALIDATION

Fourteen randomized analyses were determined based on a second order CCD with six centre points. The experimental design obtained from the CCD is presented in Table 1. Determinations of TPC (Y_1), TFC/TAC (Y_2) and FRAP (Y_3) were carried out using the optimized extraction conditions. Verification of the response surface model was performed by comparing experimental values to predicted values obtained from the optimized model. The analysis using 2-sample t-test showed that there were

no significant differences ($p=1.00$) between theoretical values and predicted values for all dependent variables (Y). Therefore, theoretical validations of all reduced models were confirmed. The data fit the second-order of polynomial models, which was confirmed based on coefficients of determination (r^2) (>0.9) (Prasad et al. 2011). We concluded that the models explain more than 90% of the response variation. The results also showed that the reduced model was fit ($p\geq0.05$). The data obtained from the optimal extraction conditions were applied for experimental validation. Analyses using 1-sample t-test showed that the experimental means were not significantly different.

OPTIMIZATION FOR DEFATTED *DABAI* PULP EXTRACT

Applying the optimized extraction time (36 min) and percentage of methanol (62.25%), TPC (1382.95 mg/100 g), TFC (30.99 mg/100 g) and FRAP (13.55 mM/100 g) should be obtained. However, the optimized conditions obtained from combined parameters of dependent variable have yielded lower TPC, TFC and FRAP values compared to individual parameter optimization. The regression (2) – (4) for defatted *dabai* pulp are as follows:

$$TPC:\quad Y = 65.44X_1 + 162.41X_2 - 0.89X_1^2 - 1.16X_2^2 \\ - 0.29X_1X_2 - 4797.53 \tag{2}$$

$$TFC:\quad Y = 0.716X_1 - 0.151X_2 + 0.011X_1^2 \\ - 0.012X_1X_2 + 25.992 \tag{3}$$

$$FRAP:\quad Y = -0.006X_1 + 1.976X_2 + 0.004X_1^2 \\ - 0.014X_2^2 - 60.331 \tag{4}$$

Interaction effects of extraction time and methanol (%) for defatted *dabai* pulp are demonstrated in Figure 1(a). TPC and TFC for the defatted *dabai* pulp showed significant interaction between extraction time and percentage of methanol, while FRAP value showed no significant interaction effect ($p\geq0.05$) between the extraction time and percentage of methanol (4). Respond surface plot showed an increase in extraction time has a gradual increase in TPC value. A high TPC can be obtained from moderate points of extraction time and percentage of methanol. Minimum and maximum points for the independent variables (extraction time and percent methanol) are shown in Table 1. For TFC, increasing in the extraction time and percentage of methanol have resulted in decreasing and an increasing TFC, respectively. Nevertheless, a combination of moderately high extraction time and percentage of methanol could yield a maximum TFC value (Figure 1(a)).

The optimized extraction procedure has shown to yield maximal TPC, TFC and FRAP values in defatted *dabai* pulp. An optimized condition of 36 min extraction has yielded an optimal TFC where flavonoids are rich in the defatted *dabai* pulp, but not in the defatted *dabai* peel. For the percentage of methanol, 62.25% methanol is best to be used in extraction of defatted *dabai* pulp. Higher percentage of

TABLE 1. Experimental design and the results for defatted *dabai* parts

| Run	Block	Extraction Time (min) (X₁)	Methanol % (X₂)	Defatted *Dabai* Pulp			Defatted *Dabai* Peel		
				TPC (Y₁)	TFC (Y₂)	FRAP (Y₃)	TPC (Y₁)	TAC (Y₂)	FRAP (Y₃)
1	1	1	50	443.98	17.70	0.48	4673.60	95.01	12.34
2	1	30	95	476.63	9.90	2.14	2731.02	46.44	7.10
3	1	1	95	166.95	10.65	0.04	1739.18	25.36	3.91
4*	1	15.5	72.5	1350.40	15.85	8.34	3294.29	71.18	9.44
5	1	30	50	1130.40	31.90	4.21	2253.47	40.33	4.81
6*	1	15.5	72.5	1342.40	15.00	7.99	3318.78	64.77	9.41
7*	1	15.5	72.5	1322.40	16.05	7.77	3310.61	65.07	9.64
8	2	<1	72.5	927.40	15.90	12.53	4353.60	76.68	13.28
9	2	36	72.5	1374.80	25.85	17.85	3713.60	68.13	11.32
10*	2	15.5	72.5	1375.40	16.35	14.28	3686.12	73.93	10.56
11	2	15.5	100	255.20	7.51	1.90	1865.71	28.72	3.11
12*	2	15.5	72.5	1378.40	16.30	13.69	3641.22	70.57	10.66
13	2	15.5	40.7	539.90	27.40	1.05	3208.57	66.60	5.78
14*	2	15.5	72.5	1400.40	15.30	13.07	3714.69	70.88	10.61

* Center points
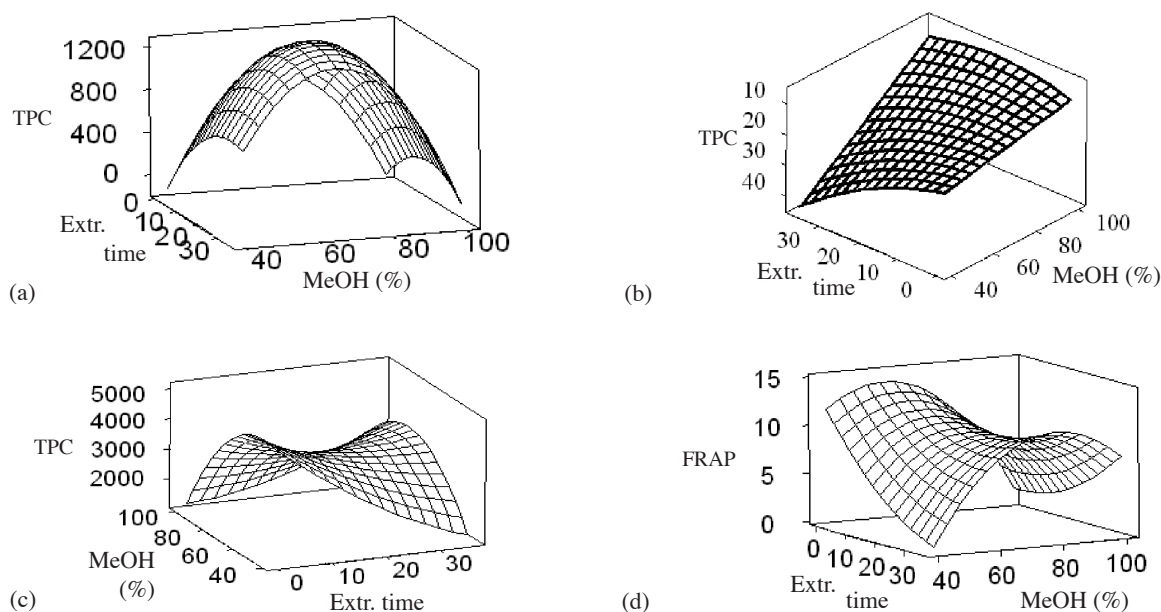
FIGURE 1. Response surface plot explaining the extraction parameters (i) extraction time (min); and (ii) methanol (%) on total phenolic content (TPC) and antioxidant capacity (FRAP) for defatted *dabai* (a,b) pulp and (c,d) peel. TPC: total phenolic content (mg/100 g); FRAP: ferric reducing antioxidant power (mM/100 g); Extr. time: extraction time (min)

methanol (>50%) is needed for flavonoids extraction in defatted *dabai* pulp due to some of the flavonoids have low solubility in water (Marfak et al. 2002). From these observations, extraction time and percentage of methanol have greatly influenced the proportions of phenolic and flavonoid compounds extracted.

OPTIMIZATION FOR DEFATTED *DABAI* PEEL EXTRACT

Applying the optimized extraction time (1 min) and percentage of methanol (53%), TPC (4847.63 mg/100 g),

TAC (95.17 mg/100 g) and FRAP (13.3 mM/100 g) should be obtained (Table 2). Similar as found for defatted *dabai* pulp, TPC and FRAP values obtained using the optimized conditions were lower in defatted *dabai* peel. Regression equations (5–7) for the defatted *dabai* peel are as follows:

$$\text{TPC:} \quad Y = -251.26X_1 + 131.05X_2 + 1.21X_1^2 \\ - 1.38X_2^2 + 2.61X_1X_2 + 1889.77 \quad (5)$$

$$\text{TAC:} \quad Y = -4.636X_1 + 2.679X_2 - 0.03X_1^2 \\ + 0.058\,X_2^2 + 38.4165 \quad (6)$$

TABLE 2. Total phenolics and antioxidant capacity of defatted *dabai* parts based on RSM optimized extraction parameters

Extraction	Defatted *Dabai* Pulp			Defatted *Dabai* Peel		
	TPC	TFC	FRAP	TPC	TAC	FRAP
Model	1382.95[A]	30.99[A]	13.55[A]	4774.7[A]	93.55[A]	15.31[A]
Experimental	1382.0[A,a]	31.84[A,a]	13.66[A,a]	4847.63[A,a]	95.17[A,a]	13.3[B,a]
Sonication-assisted liquid extraction	83.17[b]	34.02[a]	1.01[b]	7251.88[b]	95.86[a]	19.82[b]

Values are expressed as per 100 g basis. Different upper and lower case letters show a significant deviation between the two values, in which significant value set at $p<0.05$ and $p<0.01$, respectively

FRAP: $Y = -0.847X_1 + 0.837X_2 + 0.006X_1^2 - 0.007X_2^2 + 0.008X_1X_2 - 10.507$ (7)

Interaction effects between the extraction time and percentage of methanol for defatted *dabai* peel are demonstrated in Figure 1(b). The dependent variables of these models showed significant interaction between extraction time and percentage of methanol, except TAC in the defatted *dabai* peel which showed no significant interaction effect ($p \geq 0.05$) between extraction time and percentage of methanol (6). Based on the respond surface plot, highest extraction time and lowest percentage of methanol applied or vice versa would have yielded lowest TPC and FRAP values. A maximum TPC value can be obtained by applying lower extraction time and percentage of methanol. Similar as found for TPC, extraction time of ~1 min and ~50% methanol should have yielded a maximal FRAP value.

The optimized extraction procedure was also shown to yield maximal TPC, TAC and FRAP value for the defatted *dabai* peel. An optimized condition of 1 min extraction had also yielded optimal TAC, where anthocyanins are rich in defatted *dabai* peel. Based on the optimized method, about 1:1 ratio of methanol and water was needed for extraction of anthocyanins from defatted *dabai* peel. As reported by Mantell et al. (2002), variation in the yield of anthocyanin obtained was due to the degree of solubility of anthocyanins in different percentages of methanol. Thus, 53% methanol used is able to yield a maximum amount of anthocyanin. The result also showed no significant interaction between extraction time and percentage of methanol for TAC. Therefore, these optimized parameters may have no effect on TAC in defatted *dabai* peel.

SONICATION-ASSISTED EXTRACTION

Sonication-assisted extraction at 1 min had shown to significantly increase the TPC in defatted *dabai* peel, which also significantly enhanced the antioxidant capacity. The result also showed that TFC in the defatted *dabai* pulp has increased due to the increasing sonication time. However, no significant increased in TFC was found for 36 min extraction. A non-significant increased in TAC was observed for defatted *dabai* peel using sonication-assisted extraction as compared with non-sonication extraction. Sonication for 1 min may not help in increase the yield of anthocyanin, while longer sonication time should increase the extraction yield. However, prolonged sonication might cause a loss of phenolic compounds, where water temperature in water bath had been proven to increase to 40°C (Xu et al. 2010). As there are limitations for introducing ultra-sound in liquid extraction, further studies for optimization of sonication-assisted extraction are recommended. Therefore, the results obtained can be applied for extraction of many other types of food sample.

Sonication has helped to degas and remove oxygen that may potentially increase oxidation of phenolic compounds. By comparing the total phenolics and antioxidant capacity between solid-liquid extraction and sonication-existed extraction, an increase in sonication time had resulted in degradation of phenolic compounds, especially lost of TPC. TPC degradation in defatted *dabai* extract may lead to decrease in antioxidant capacity.

Vassilakis et al. (2004) supported our finding that longer duration of exposure to low-frequency sonic wave produced from ultra-sound was resulted in degradation of phenolic compounds, especially *p*-coumaric acid. The degradation is possibly linked to a series of aromatic and ring cleavage intermediates with OH radical-mediated reactions. Madhujith and Shahidi (2006) also revealed prolonged extraction time and increased in heat treatment (as observed in sonication-assisted extraction) enhanced the probability of oxidative degradation of phenolic compounds.

CONCLUSIONS

The use of RSM has effectively optimized the extraction conditions. Optimized extraction parameters of 36 min extraction time and 62.25% methanol had successfully yielded optimal TPC, TFC and FRAP value in defatted *dabai* pulp, while for defatted *dabai* peel, 1 min extraction time and 53% methanol had resulted in optimal TPC, TAC and FRAP value. Based on RSM optimized extraction conditions, additional use of sonication-assisted extraction has helped to increase the TPC and FRAP values obtained from defatted *dabai* peel by 50% and 0.5%, respectively. In term of antioxidant capacity of natural products, the optimization can be studied using other antioxidant methods.

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