Analysis of Antioxidant Property from Water Extraction of *Garcia Mangostana* Using Response Surface Methodology

Tsair-Wang Chung* and Irwan Saleh Kurniawan

***Abstract*—Mangosteen *(Garcia mangostana)* is an exotic fruit that can be found widely in Southeast Asia. Mangosteen pericarp contains bioactive compound that has pharmacological properties, including antioxidants, anticarcinogenic, and also suggested its applicability for skincare products. Water extraction is more applicable for industry due to simple process, low cost, and neutral reaction. In this study, water extraction on the pericarp of mangosteen was applied and the operating parameters were discussed by using Response Surface Methodology (RSM) for high recovery of antioxidant extract from the mangosteen pericarp. The experimental design used three factors, solid-to-liquid ratio (g/ml), temperature (ºC) and extraction time (hour), were analyzed to discuss two responses, DPPH radical scavenging effect (DPPH) and Ferric Reducing Antioxidant Power (FRAP). Under the operating conditions, the highest FRAP is 0.818 abs at the factors of 1:10 (g/ml), 65 ºC, and 3-hour. DPPH is significantly high for all RSM pattern. The optimum parameters determined by using RSM are at 1:10 (g/ml), 59.74 ºC, and 2.87 hours with DPPH 81.01% and FRAP 0.789 abs.**

***Index Terms*—Garcia Mangostana, water extraction, antioxidant, Response Surface Methodology (RSM)**

I. Introduction

The edible portion of mangosteen is quite small for about 40% as its flesh and the other part is pericarp for about 60% [1]. However, mangosteen pericarp is usually disposed as waste due to its unpleasant taste of bitterness [2]. Several studies found that mangosteen pericarp contains bioactive compound that has pharmacological properties, including antioxidants [3], antiproliferative [4], anti-inflammatory [5], anticarcinogenic [6], antimicrobial [7], antibacterial [8], and also suggested its applicability for cosmetics products [9, 10]. Numerous extraction techniques from mangosteen pericarp have been conducted to investigate [11, 12]. Compared to water extraction, those methods are complex and difficult to be applied at industrial scale. Therefore, water extraction is more applicable for industry due to its simple process. [13] The Response Surface Methodology (RSM) is applied for discussion of the extraction of antioxidant from mangosteen pericarp. In this study, solid-to-liquid ratio, temperature, and extraction time were analyzed to compare the antioxidant properties of DPPH radical scavenging effect (DPPH) and Ferric Reducing Antioxidant Power (FRAP).

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II. Materials and Methods

A. Materials

Mangosteen (Garcia mangostana) was purchased from the herbal powder raw materials (Indonesia). Mangosteen Pericarp Powder (MPP) was sieved in the size of 45 mesh and stored in 4 ºC for analyses.

B. Water Extraction of Mangosteen Pericarp Powder

Hot plate magnetic stirrer was employed for the extraction process, which has 5 × 7-inch Pyroceram top, digital temperature and stirring speed displays. The reaction process by placing Mangosteen Pericarp Powder (MPP) in beaker glass containing magnetic stirring bar and adding water solvent. One single factor extraction was conducted before deciding extraction variables. The range for extraction variables were determined by one-factor-at-a-time experimental and values of the range for each factor were shown in Table I. After water extraction, the crude extract was cooling in room temperature and centrifuged at 5000 rpm for 10 min [2]. Supernatant was kept and filter using 5B Advantec 90 mm filter paper, and stored at 4 ºC. Mangosteen Pericarp Extract (MPE) was analyzed for DPPH and FRAP.

C. Determination of DPPH

MPE was examined for its DPPH radical scavenging activity (DPPH) by following Shimada *et al.* method [14] and was done with some adjustment. Sample solution was obtained by mixing 0.1 mM DPPH reagent (3 ml) and MPE (6 ml, 20 times dilution). Deionized water (3 ml) and MPE (6 ml, 20 times dilution) was mixed to provide blank solution. Control solution was developed by adding deionized water (4 ml) to 0.1 mM DPPH. All solution were kept in dark condition for 30 minutes and the absorbance was analyzed using Chromtech® CT-2200 UV-Vis spectrophotometer at 517 nm. The DPPH radical scavenging effect of the MPE was measured as DPPH inhibition (%) as displayed in Eq. (1).

$$Scavenging\ effect\ (\%) = \left(1 - \left(\frac{sample - blank}{control}\right)\right) \times 100\% \quad (1)$$

D. Determination of FRAP

Ferric Reducing Antioxidant Power (FRAP) of MPE was determined according to FRAP assay established by Oiyaizu [15] method but with some alternation. MPE (1 ml, 20 times dilution) was mixed with phosphate buffer 2.5 M (2.5 ml), and potassium hexacyanoferrate 1% (2.5 ml) in test tube, then incubated for 20 minutes at 50oC to complete reaction. Tricholoro acetic acid 10% (2.5 ml) was added into the test tube, and centrifuged at 5000 rpm for 10 min. Supernatant (2.5 ml) was withdrawn from the mixture and

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mixed with deionized water (2.5 ml). Ferric chloride 0.1% was added to diluted reaction mixture, and incubated in dark condition for 10 minutes. Blank solution contained the same solution mixture without plant extract and it was incubated under the same conditions. The absorbance of total mixture and blank solution was analyzed using Chromtech® CT-2200 UV-Vis spectrophotometer at 700 nm. FRAP of MPE was reported as amount of absorbance (abs) and calculated using Eq. (2). High absorbance in the reaction mixture indicated high antioxidant power.

$$\text{Reducing power (abs)} = \text{absorbance value of sample} - \text{absorbance value of blank} \quad (2)$$

E. Experimental Design and Statistical Analysis

It is designed in 3 factors, 3 responses and 3 levels by Box-Behnken experimental design methodoilogy. The 3 factors are solid-to-liquid ratio (X1), temperature (X2), and extraction time (X3). The responses are DPPH radical scavenging effect (Y1) and ferric reducing antioxidant power (Y2). The 3 levels (−1, 0, +1) were listed in Table I and the effect of each variable and interaction between variables were analyzed.

TABLE I: THE SELECTED RANGE OF FACTORS FOR RSM

Level	X_1 (ml/g)	X_2 (ºC)	X_3(hr)
−1	10	25	2
0	15	45	3
+1	20	65	4

A quadratic (second-order) polynomial model was fitted to the factors and response with the equation expressed in Eq. (3). Three-factor and two-factor effect on the two responses of DPPH and FRAP can be discussed from the regression equation to observe the influence of the factors and their correlative relation on all three responses [16].

$$Y=a+bX_1+cX_2+dX_3+eX_1X_2+fX_1X_3+gX_2X_3+hX_1^2+1X_2^2+jX_3^2 \quad (3)$$

III. RESULTS AND DISCUSSION

A. Parameters Selection for RSM Optimization

This study attempted to find the most effective condition of water extraction for high antioxidant properties, DPPH and FRAP, by considering, solid-to-solvent ratio (X1), temperature (X2), and extraction time (X3). The higher solid-to-liquid ratio could enhance the extraction yield as the mass transfer of the immersed solutes into the solution allows a steeper concentration gradient [17]. Considering all these factors and results from previous studies, 1:10 to 1:20 solid-to-liquid ratio was selected and used in this study for RSM design. Moreover, in industry doing extraction at room temperature 25 ºC are more preferable due to cost and easy to handle. Considering these factors and result from previous experiment, the extraction temperature ranges from 25 ºC to 65 ºC were used. Considering all factors and previous experiment, extraction time from 2 h to 4 h is used for the RSM design.

TABLE II: THE EXPERIMENTAL DATA USING BOX-BEHNKEN DESIGN

Run	Pattern	X_1 (ml/g)	X_2 (ºC)	X_3 (hour)	DPPH (%)	FRAP (abs)
1	0−+	15	25	4	71.6699	0.4467
2	++0	20	65	3	75.6985	0.5647
3	0++	15	65	4	80.1170	0.6007
4	+0+	20	45	4	84.3537	0.4683
5	000	15	45	3	82.9932	0.5517
6	−0+	10	45	4	81.8594	0.6840
7	0−−	15	25	2	75.9584	0.4553
8	000	15	45	3	82.9932	0.5517
9	−0−	10	45	2	82.6531	0.7007
10	+−0	20	25	3	73.6961	0.4020
11	−+0	10	65	3	78.9474	0.8177
12	+0−	20	45	2	76.1905	0.4870
13	000	15	45	3	82.9932	0.5517
14	0+−	15	65	2	73.0994	0.6967
15	−−0	10	25	3	70.8902	0.5650

B. Optimization Antioxidant Recovery by RSM

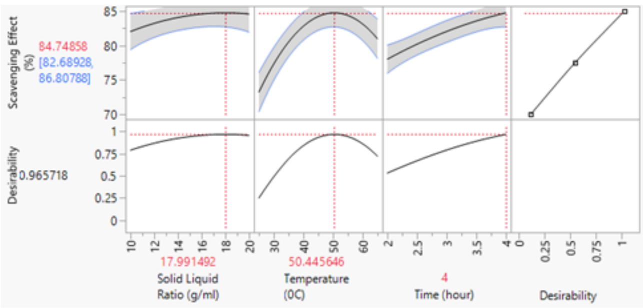
RSM experimental design with response data is shown in Table II. Which includes three parameters: solid to liquid ratio (X1), temperature (X2), and extraction time (X3) as well as two responses including DPPH and FRAP. Table III shown the regression coefficients of the intercept, linear, quadratic and interaction parameters of all models are fitted (P<0.05).

TABLE III: REGRESSION COEFFICIENT OF EXPERIMENTAL RESULT

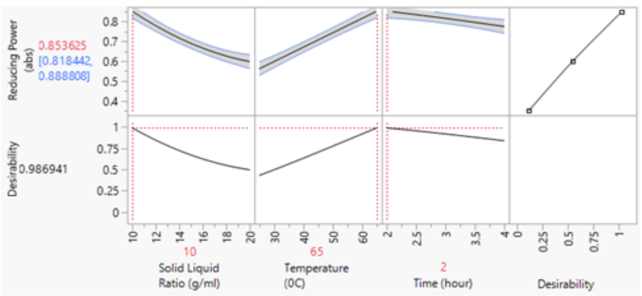
Term	Estimate	Std Error	*t* Ratio	Prob>|t|
a) DPPH				
Intercept	82.9932	0.670543	123.77	<0.0001*
X_1	−0.551412	0.410622	−1.34	0.237
X_2	1.955963	0.410622	4.76	0.0050*
X_3	1.262325	0.410622	3.07	0.0277*
X_1X_2	−1.5137	0.580707	−2.61	0.0479*
X_1X_3	2.239225	0.580707	3.86	0.0119*
X_2X_3	2.826525	0.580707	4.87	0.0046*
X_1^2	−1.066075	0.604419	−1.76	0.1380
X_2^2	−7.119075	0.604419	−11.78	<0.0001*
X_3^2	−0.66295	0.604419	−1.10	0.3227
b) FRAP				
Intercept	0.5517	0.006688	82.49	<0.0001*
X_1	−0.105675	0.004096	−25.8	<0.0001*
X_2	0.10135	0.004096	24.74	<0.0001*
X_3	−0.0175	0.004096	−4.27	0.0079*
X_1X_2	−0.0225	0.005792	−3.88	0.0116*
X_1X_3	−0.0005	0.005792	−0.09	0.9346
X_2X_3	−0.02185	0.005792	−3.77	0.0130*
X_1^2	0.0354	0.006029	5.87	0.0020*
X_2^2	0.00025	0.006029	0.04	0.9685
X_3^2	−0.0021	0.006029	−0.35	0.7418

Table IIIa has shown that DPPH radical scavenging effect was significantly affected by two linear (X_2, X_3) parameters, all interactions (X_1X_2, X_1X_3, X_2X_3), and one quadratic parameters X_2^2 (P<0.05). all linear parameters (X_1, X_2, X_3), as well as two interactions with temperature (X_1X_2, X_2X_3), and one quadratic parameters (X_1^2) significantly (P<0.05) affect FRAP value. The optimal values for DPPH and FRAP assay of optimized mangosteen pericarp extract can be obtained from the final predictive quadratic equations through the multiple regression analysis.

The maximum desirability DPPH was obtained by using 17.99 ml/g solid-to-liquid ratio, 50.44 °C and 4-hour extraction time (Fig. 1(A)). The final predictive quadratic extractions generated DPPH 84.75%. Fig. 1(A) expressed the DPPH with maximum desirability of predicted model and highest DPPH from experimental values were in good agreement and fitted the model ($p < 0.05$). The DPPH in 20 times dilution sample were at range 70.89 % to 84.35% presented in all run Table II. In other ways this result also proved MPE have high antioxidant value in term of DPPH even after 20 times dilution. Fig. 1(B) expressed the maximum desirability of FRAP 84.7486 abs when 10 ml/g solid to liquid ratio, 65 °C, and 2 h extraction time. The FRAP with maximum desirability of predicted model and the highest FRAP from experimental values were in good agreement and fitted the model ($P < 0.05$) as shown in Fig. 1(B).



(A)



(B)

Fig. 1. Prediction profile for DPPH (A) and FRAP (B) with maximum desirability.

IV. SUMMARY

The results from response surface methodology have shown that all independent variables affected the responses of DPPH and FRAP significantly. The optimal DPPH and FRAP values were obtained by following water extraction conditions; 1:10 solid-to-liquid ratio, 59.74 °C, temperature and 2.87 h extraction time. The predicted values for DPPH and FRAP were as followed; 81.01% and 0.789 abs, respectively. For industrial application, the obtained models can be the basis for pilot-scale in operating water extraction as green, low cost, and safer extraction technology for the extraction of antioxidant compounds from mangosteen pericarp waste. Moreover, the high antioxidant properties could be more applicable in cosmetic industry for making formulation ingredients. For future research, the effect of dilution factor for DPPH values needs to be evaluated and the attachment effect with another technology should be considered in achieving high antioxidant properties from mangosteen pericarp extract with water extraction.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Prof. Tsair-Wang Chung planned the research objectives and research priorities, and at the same time supervised the experiment and the data discussion; Mr. Irwan Saleh Kurniawan conducted the experimental runs based on the experimental design methodology and assisted in collating experimental data, making tables and charts, and participated in the discussion of experimental results; all authors had approved the final version.

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