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EVALUATION OF ANTIOXIDANT ACTIVITY OF SOME TROPICAL FRUIT PEEL EXTRACTS: EXTRACTION CONDITIONS OPTIMIZATION OF RAMBUTAN PEEL EXTRACT

Nor Helya Iman Kamaludin[1], Lau Sin Mun[1] and Rubiatul Adawiah Sa'adi[2]
[1]Department of Chemical Engineering Technology, Faculty of Engineering Technology, Universiti Malaysia Perlis (UniMAP), Perlis, Malaysia
[2]School of Bioprocess Engineering, Universiti Malaysia Perlis (UniMAP), Jejawi 3 Engineering Complex, Arau, Perlis, Malaysia
E-Mail: helya@unimap.edu.my

ABSTRACT

This work reports a study on evaluation of antioxidant activity of some tropical fruit peel extracts and optimization of extraction conditions for recovery of antioxidants from the selected fruit peel. Due to the extensively growing of many fruit processing industries, the fruit peels are often removed as a waste residue. Hence, the research has shift to focus on fruit residues as potential source of natural antioxidant replacing the synthetic one which may poses several side effects. Extraction was done on selected tropical fruits peels including rambutan, banana, mangosteen, logan to evaluate their antioxidant activity. Rambutan with ethanol extract possesses highest antioxidant activity which is 77.21 ± 0.17 % as compared to longan (73.24 ± 0.11%), mangosteen (46.97 ± 0.29) and banana (41.65 ± 0.22) for ethanolic extraction. Thus, rambutan was chosen to be continued with screening and optimization process. Single factor experiment using the one factor at a time (OFAT) method was done to study the effect of solvent to solid ratio (300:10 to 300:50 mL/g), extraction temperature (78 to 85^{o}C) and extraction time (120 to 360 minutes). Next, central composite rotatable design (CCRD) coupled with Response Surface Methodology (RSM) was applied to optimize the extraction conditions on the antioxidant activity of rambutan peels. From the result, the highest antioxidant activity of about 96.12 ± 0.013 % was found at optimum conditions of solvent to solid ratio, 300:33 (mL/g); extraction temperature, 81oC and extraction time, 262.95 minutes. Based on statistical analysis, the extraction temperature was the most significant ($p<0.0001$) parameter condition affecting antioxidant activity and R2 value of 0.9810 denoted that the model developed was adequate in optimizing the extraction conditions of antioxidant properties from rambutan peels.

Keywords: extraction condition, antioxidant activity, rambutan peel, response surface methodology (RSM).

INTRODUCTION

Tropical fruit is the fruit that grown in the tropical climates. Most of the biologically active substances, such as antioxidant are present in fruit (Alothman *et al.*, 2009). The common type of antioxidant found in fruit are carotenoids, phenolic, vitamin A, B, C and E. Fruit which is rich in natural antioxidants gained increasing interest among consumers and the scientific community because it is vital in reducing the occurrence of degenerative diseases, like cardiovascular diseases, inflammation, ageing and cancer (Thaipong *et al.*, 2006). Since fruit residues are by-product of many fruit processing industry, research has shift to focus on fruit residues as potential source of antioxidant. Despite stem bark, leaves, pulp and other part of the fruit, peels are preferable as it is the major by-product that causing environmental problem. The discarded fruit peels are easily available and mostly contain potential antioxidants.

Rambutan, which is one of the most popular tropical fruits, followed by banana, mangosteen, logan were studied in term of antioxidant activity, in which the bioactive compounds defense against a variety of stresses, usually caused by pathogen or unfavorable environmental conditions. However, each fruit usually consists of different phenolic content causing each of them exhibit distinct antioxidant activity. The growing interest in natural antioxidants has restricted the use of synthetic antioxidant as it may result in toxicity, carcinogenicity or hepatotoxicity in human body (Nantitanon et al., 2010). Since synthetic antioxidants including butylated hydroxytoluene (BHT) may cause side effect to human health, it is presumed unsafe to be used for food and medical purpose. Thus, through this research, the fruit peels can be reused as an important source of natural antioxidant meanwhile minimize the environmental impact.

In this research, antioxidants are recovered from fruit residuals using extraction. Extraction is a process that recovers crude extract of bioactive compounds from fruit waste. Soxhlet extraction method is chosen to be used in this case of study. Soxhlet method extracts antioxidant compounds by contact the fruit peels with the suitable extraction solvent for a period of time and followed by separation of the solvent from the extract. 1,1- diphenyl-2-picrylhydrzyl (DPPH) scavenging method is also studied in fruit peels extract for evaluation of antioxidant activity (Ajila *et al.*, 2007). The optimal extraction condition is developed by using Response Surface Methodology (RSM). The effects of parameter conditions, such as solvent to solid ratio, extraction temperature and extraction time on the antioxidant activity are studied in

order to recover maximum yield of antioxidant from selected fruit peels. Therefore, this study aims to develop the optimal process conditions for antioxidant activity of selected tropical fruit peels extract using RSM.

MATERIALS AND METHODS

Plant materials

The fruit samples used in this case of study includes banana, rambutan, mangosteen, longan. Fresh fruit of banana and longan were purchased from the commercial fruit market in Perlis. The fruits of uniform shape and colour were selected whereas diseased and blemished fruits were excluded. The fruit samples of rambutan and mangosteen were taken from a fruit farm after harvesting. Similarly, the rambutan and mangosteen were chosen based on its uniformity in shape, size and length.

Chemical and reagents

The solvents and chemicals used was analytical grade. The chemicals that were applied throughout the study include Folin- Ciocalteu's reagent, sodium carbonate anhydrous, gallic acids and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). The solvent that was used in this case of study includes methanol, ethanol, acetone and aqueous. Most of the chemicals were purchased from Merck Chemicals unless otherwise specified.

Preparation of fruit peel extracts

The fruits were washed under tap water in order to get rid of adhered impurities on the surface. The thick layer of skin and thorns were peeled off from the fruits followed by separating the peels from the pulp manually. The banana peels were sliced into a thickness of 2 mm while the other fruit peels were cut into pieces of about 1 cm^2. They were dried overnight in the convection oven at 50^oC. The dried peels were ground into powder form using a commercial blender (Chan *et al*., 2009). Then, the peel samples were stored in dark bags in order to keep in dry environment and prevent the oxidation of bioactive compounds when expose to light prior to the experiment.

Soxhlet extraction

The dried samples were weighed and placed inside the thimble of the soxhlet extractor. The distillation flask attached to the bottom of soxhlet extractor was filled with solvent. The soxhlet extractor was then equipped with a condenser. During extraction, the solvent was heated to form vapour and condensed into the chamber housing the thimble of dried sample. After many cycles of extraction, the solvent that running back down to the distillation flask was collected. The extracted solvent was eliminated by evaporation using a rotary evaporator (Yoswathana and Eshtiaghi, 2013). The extracted sample was used for the determination of antioxidant activity.

Experimental design

The experiment was started with the screening of fruit peels and type of solvents on antioxidant activity using solvent extraction method. Each of the fruit peel samples was extracted with ethanol, methanol, acetone, and aqueous respectively at room temperature for 24 hours. After extraction, the peels extract was filtered through filter paper and the filtrate was collected in small reagent bottle for determination of antioxidant activity. The fruit peels extracted using the best solvent type was chosen according to the highest value of antioxidant activity.

The first part was single factor experiment, which was used for screening. It was performed by manipulating one factor at one time whereas fixing the other factors constant in order to find the best range of conditions for extraction, including solvent to solid ratio, extraction temperature and extraction time. The second part was Response Surface Methodology (RSM). It was conducted to optimize the extraction conditions using Central Composite Rotatable Design (CCRD).

Screening by single factor experiment

a) Effect of solvent to solid ratio

The influence of solvent to solid ratio on antioxidant activity was determined by using the best solvent type. The fruit peel sample was extracted using solvent to solid ratio of 300:10 to 300:50 (mL/g) (300:10, 300:15, 300:20, 300:30 and 300:50) (Silva *et al*., 2007). By keeping the extraction temperature and extraction time fixed at 80^oC and 180 minutes, respectively. The best solvent to solid ratio was selected based on the highest value of DPPH scavenging activity (%).

b) Effect of extraction temperature

The influence of extraction temperature on antioxidant activity was determined by using the best solvent type and the best solvent to solid ratio chosen in (a). The same extraction procedures were repeated by varying the temperature ranged from 78 to 85 ^oC (78, 79, 80, 82 and 85 ^oC) while fixing the extraction time at 180 minutes (Silva *et al.*, 2007). The best extraction temperature was chosen based on the highest value of DPPH scavenging activity (%).

c) Effect of extraction time

The influence of extraction time on extraction was determined by using the optimum solvent to solid ratio and extraction temperature selected in (a) and (b). The fruit peel sample was extracted at time ranged 120 to 360 minutes (120, 180, 240, 300 and 360 minutes) at the best solvent type (Chew *et al.*, 2011). The best extraction temperature was selected based on the highest value of DPPH scavenging activity (%).

Optimization by Response Surface Methodology (RSM)

Design Expert (Version 7.1.5) statistical software was employed to conduct the Central Composite Rotatable Design (CCRD) using the range of independent variables and to evaluate the values collected from experiment in RSM. The CCRD including the factors, their levels and the experimental data are used to optimize the extraction conditions from fruit peels extract. Then, regression coefficients were created by model. The correlation between the three factors and the two responses were described by the generalized second-order polynomial model in equation (1):

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i}^{k-1} \sum_{j}^{k} \beta_{ij} X_i X_j \quad (1)$$

where β_0, β_i, β_{ii}, β_{ij} represent regression coefficients for intercept, linear, quadratic and interaction terms respectively. X_i and X_j represent coded value of the factors whereas k equals to the number of the factors (k=3).

Determination of antioxidant activity

The antioxidant activity of the plant extracts was determined using the DPPH free radical scavenging method with some modifications (Jamal *et al.*, 2011). 0.025 mL of the extracts solution was added with 0.075 mL of distilled water and followed by 0.1 mL methanol. Subsequently, 0.025 mL of 1 mM DPPH in methanol solution was transferred to the solution. The solution mixture was then mixed thoroughly and incubated at room temperature for 30 minutes in dark. The mixture is diluted with distilled water and the antioxidant activity was obtained by absorbance measurement at 517 nm. The percentage of DPPH radical scavenging activity was then determined based on the following equation (2) *(Huang et al.*, 2012).

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100\% \quad (2)$$

A_{sample} represent the Absorbance of peels extract.
$A_{control}$ represent the Absorbance of DPPH solution without extracts.

Statistical analysis

Analysis of variance (ANOVA) was carried out to define the significant level using $p<0.05$. Differences at $p<0.05$ were deliberated statistically significant.

Validation of antioxidant activity

The optimal experimental conditions used to obtain the maximum antioxidant activity from fruit peels were achieved using Response Surface Methodology (RSM). The predicted and experimental values of antioxidant activity were determined to validate the model. The series of solutions suggested by the model were used to verify the antioxidant activity of fruit peels extract at its optimized extraction conditions. The verification was conducted in triplicate under the selected extraction conditions.

RESULTS AND DISCUSSIONS

Screening of fruit peels and type of solvents on antioxidant properties

The fruit peel extract was selected based on its highest antioxidant activity obtained from extraction using different solvents, mainly methanol, ethanol, acetone and aqueous. Based on Table-1, rambutan peels extracted using ethanol showed the highest antioxidant activity. Similarly, rambutan peel extract obtained from solvent extraction in previous research showed the highest antioxidant activity of 98.19 % (Samuagam et al., 2013). The recovery of antioxidants from fruit peels is influenced by the solvent type, solvent polarity and the phenolic solubility in the particular solvent (Dent *et al.*, 2013). Since rambutan peel extracted using ethanol exhibited the highest antioxidant activity, it was preferred to be used for the screening experiment.

Table-1. The experimental data of antioxidant activity of fruit peels extracts.

Fruit peels	Extract	Antioxidant activity (%)
Rambutan	Methanol	56.60± 0.57
	Ethanol	77.21± 0.17
	Acetone	74.63± 0.32
	Aqueous	72.00± 0.69
Mangosteen	Methanol	18.81± 1.44
	Ethanol	46.97± 0.29
	Acetone	9.19± 1.77
	Aqueous	67.45± 1.05
Banana	Methanol	13.54± 1.39
	Ethanol	41.65± 0.22
	Acetone	9.71± 1.38
	Aqueous	68.01± 0.42
Longan	Methanol	67.31± 2.21
	Ethanol	73.24± 0.11
	Acetone	35.40± 0.84
	Aqueous	61.85± 0.57

represent selected fruit peels extract

Screening of the extraction condition using single factor experiment

a) Effect of solvent to solid ratio

The effects of solvent to solid ratio on the antioxidant activity of rambutan peels extract were shown in Figure-1. The maximum antioxidant activity was achieved at solvent to solid ratio of 300:30 (mL/g). The antioxidant activity increased up to 80.87 ± 1.03 % with increment of solvent to solid ratio followed by a small reduction with further increase in ratio to 300:50 (mL/g). The increases in antioxidant activity were due to the increase amount of solid sample being used, thus allow more bioactive compounds to be extracted from fruit peels to achieve the maximum antioxidant activity. The antioxidant activity gained at 300:50 (mL/g) was 76.95 ± 2.81 %, which is slightly lower than the maximum antioxidant activity. The decrease in antioxidant activity is resulted from the reduction of surface area of solid matrix that available for the solvent to pass through and thus reduce the ability to solubilize and dissolve the bioactive compounds while maintaining a constant volume of solvent used for extraction (Ballard *et al.*, 2010). This could result a reduction in antioxidant activity of rambutan peels extract. Thus, the solvent to solid ratio of 300:27, 300:30 and 300:33 (mL/g) were preferred to be used as the lower, middle and upper level respectively as the variable for RSM optimization.

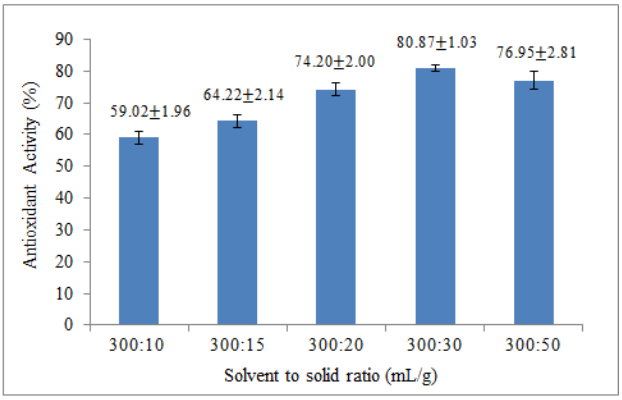

Figure-1. The effect of solvent to solid ratio on antioxidant activity. A significant difference ($p<0.05$) was obtained at solvent to solid ratio of 300:10 to 300:50 (mL/g).

b) Effect of extraction temperature

Extraction temperature also affects the antioxidant activity of rambutan peels extract. As shown in Figure-2, the antioxidant activity increases significantly to maximum of 80.32 ± 1.30 % when the extraction temperature reached 80 °C. The increase in temperature to 80 °C improved the diffusion coefficient, solubility and mass transfer of the bioactive compounds in the solvent. The intense heat from the solvent at elevated temperature also causes the breakdown of the cellular constituent of plant materials, thus enhancing the release of bounded and

cell wall bioactive compounds (Chew *et al.*, 2011). However, the antioxidant capacity of rambutan peels extract were started to decrease drastically when the extraction temperature beyond 80°C. This may due to the possible thermal decomposition of the bioactive compounds after maximum extraction temperature had been reached. Thus, the extraction temperature of 79, 80 and 81°C were preferred to be used as the lower, middle and upper level respectively as the variable for RSM optimization.

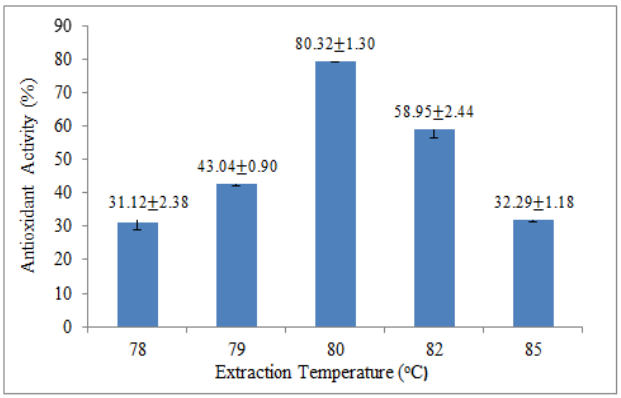

Figure-2. Effect of extraction temperature on antioxidant activity. A significant difference ($p<0.05$) was obtained at extraction temperature of 78 to 85 ℃.

c) Effect of extraction time

The effects of extraction time on the antioxidant activity of rambutan peels extract were reflected in Figure-3. The antioxidant activity increased as the extraction time increased from 120 to 240 minutes. The maximum antioxidant activity of 82.07 ± 0.43 % was achieved at 240 minutes because an increase in extraction time increased the amount of bioactive compounds extracted from the rambutan peels. After 240 minutes, further increase in extraction time caused the antioxidant activity decreased. This showed that the prolonged extraction time do not significantly enhanced the antioxidant activity of extracts. This finding was related to the Fick's second law of diffusion, which indicated that the solute concentration in the plant matrix will achieve final equilibrium with the bulk solution after a certain period of time (Chan *et al.*, 2009). Furthermore, excessive process duration will cause degradation of phenolic compounds due to light and oxygen exposure leading to the phenolic oxidation (Chew *et al.*, 2011). Thus, the extraction time of 210, 240 and 270 minutes were preferred as variable representing lower, middle and upper levels respectively in RSM optimization.

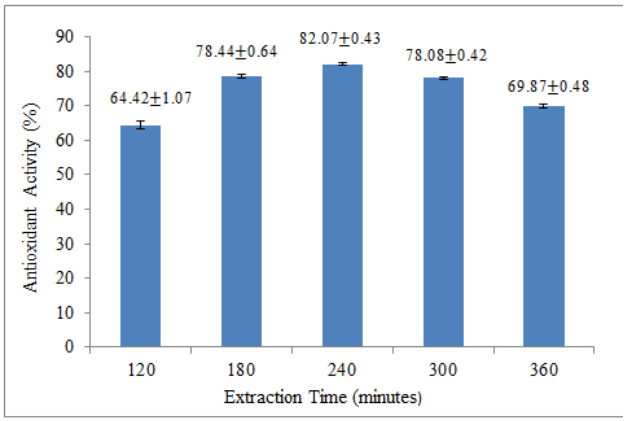

Figure-3. Effect of extraction time on antioxidant activity. A significant difference ($p<0.05$) was obtained at extraction time of 120 to 360 minutes.

Optimization by Response Surface Methodology (RSM)

a) Model fitting

Based on the range of independent variables, includes solvent to solid ratio, extraction temperature and extraction time obtained from Single Factor Experiment, a set of experiments was suggested by the Design Expert software for optimization of extraction conditions from rambutan peels extract. The software applied Central Composite Rotatable Design (CCRD) to develop 20 experimental points with 6 replicates of centre point in addition to the upper and lower values for each of the independent variables were set at +alpha and –alpha, which alpha represent value of 1.682. The model fitness was defined by evaluating the coefficient of determination (R^2) and the Fisher test value (F-value) obtained from the analysis of variance (ANOVA) (Ashutosh et al., 2011). The significance of the model, model parameters and lack of fit was determined at 95% confidence ($p<0.05$) level. Through multiple regression analysis, the empirical relationship between the antioxidant activity and the selected three factors was integrated into regression equation (3):

$$Y= 92.01 + 4.08X_1 + 16.85X_2 + 7.22X_3 - 2.29X_1^2 - 8.31X_2^2 – 9.45X_3^2 – 5.22X_1X_2 + 1.16X_1X_3 -2.38X_2X_3 \quad (3)$$

The multiple regression equations (3) were created by associating the response variable to coded levels of three independent variables. Besides that, the linear, quadratic and interaction effect was analysed using ANOVA on the solvent to solid ratio, extraction temperature and extraction time as well as their regression coefficients on response variables as tabulated in Table-2.

Based on the Table-2, the model was highly significant because the probability value is very low in which $p<0.0001$ with an F value of 57.31. The P value indicated the significance of the variables while F value is

defined from the replicated at design centre, where it is the ratio of the mean square error to the pure error (Khan *et al*., 2010). Furthermore, the lack of fit was determined to investigate whether the predicted response surface correspond to the actual shape of the surface. The lack of fit was not significant ($p>0.05$) as showed in Table-2, implied that the model was adequately fit and the regression equation was well explained the responses. Thus, it can be deduced that the ANOVA was statistically significant because the variability of the responses was well described by the model. In addition, the result denoted that quadratic model generated could be applied to predict the effect of the three factors, which were solvent to solid ratio, extraction temperature and extraction time on the antioxidant activity.

Table-2. Analysis of variance (ANOVA) for antioxidant activity of rambutan peels extract.

Regression	Sum of square	Degree of freedom	Mean square	F value	P value Prob > F
Model	7174.02	9	797.11	57.31	<0.0001
Linear	2458.27	11	223.48	28.98	0.0008
Quadratic	2083.45	3	694.48	49.93	<0.0001
Lack of fit	100.53	5	20.11	2.61	0.1582
Pure error	38.55	5	7.71		
Residue error	139.08	10	13.91		

Table-3 showed the multiple regression analysis and the significance of each of the regression coefficients for the antioxidant activity model. From the table, both the linear and quadratic terms of all parameters, includes solvent to solid ratio, extraction temperature and extraction time were significant with at least $p<0.05$ on the antioxidant activity. However, interactions did not give a significant effect on the antioxidant activity for each of the parameters. The antioxidant activity was only significantly affected by the interaction between the solvent to solid ratio and extraction temperature ($p<0.05$). Therefore, it could be stated that the linear and quadratic effects of parameters were the main terms that produce significant effect to the response of antioxidant activity.

In fact, the extraction temperature was the most significant parameter influencing the antioxidant activity as shown in Table-3. This is because the extraction temperature displayed significant linear and quadratic effect ($p<0.0001$) and significant interaction effect with the solvent to solid ratio ($p<0.05$). In term of linear effect, extraction temperature also indicated the largest positive regression coefficient. Hence, extraction temperature had the most critical effect on the antioxidant activity of rambutan peels extract.

Table-3. Predicted regression coefficient for antioxidant activity of rambutan peels extract.

Model parameter	Regression coefficient
	Antioxidant activity (%)
Intercept X_0	92.01
Linear X_1 **– Solvent to Solid Ratio** X_2 **– Extraction Temperature** X_3 **– Extraction Time**	4.08^{*} 16.85^{**} 7.22^{**}
Quadratic X_1^2 X_2^2 X_3^2	-2.29^{*} -8.31^{**} -9.45^{**}
Interaction X_1X_2 X_1X_3 X_2X_3	-5.22^{*} 1.16 -2.38

b) Effect of parameters studied on optimization of antioxidant activity

The design of response surface is used to evaluate the interaction of three independent variables and its effect on the response. The relationship between independent variables was illustrated by three dimensional response surface plots by fixing one variable constant at its optimal level (Ballard *et al.*, 2010). Thus, each of the response surface plots showed only the effect of the two selected variables on the antioxidant activity.

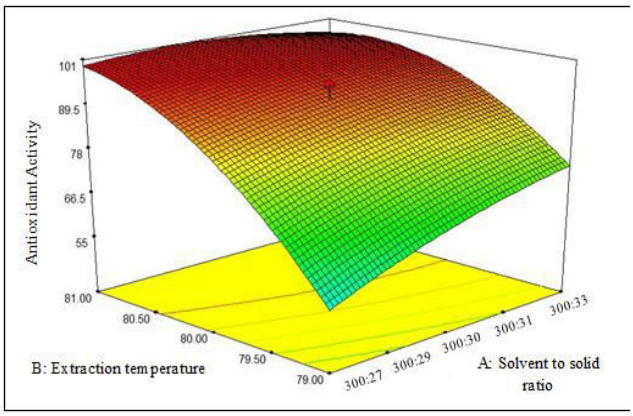

Figure-4. Response surface plot of the effect of solvent to solid ratio and extraction temperature on antioxidant activity. The extraction time was kept constant at 240 minutes.

Figure-4 revealed the predicted response surface of the effect of solvent to solid ratio and extraction temperature on antioxidant activity at constant extraction time of 240 minutes. The figure depicted almost 96% of antioxidant activity obtained in the region at solvent to solid ratio between 300:29 and 300:30 (mL/g) and extraction temperature between 80.5 and 81.0°C. The antioxidant activity gradually mounted up with the increase in solvent to solid ratio and extraction temperature to achieve an optimal level at about 300:29.25 (mL/g) and 80.75°C respectively, before a slight decrease afterward.

However, the contour gradient of solvent to solid ratio was less than that of the contour gradient of extraction temperature in each of its coordinate direction as reflected in Figure-4. Hence, extraction temperature is considered more important parameters affecting the antioxidant activity compared to solvent to solid ratio. Generally, increase the extraction temperature improves extraction by increasing the solubility of bioactive compounds in the solvent, thus producing higher antioxidant activity (Liyanapathirana and Shahidi, 2005). Therefore, it could be observed that in Figure-4, the antioxidant activity is much higher at 80.5 °C and further increase in temperature might cause thermal decomposition of the bioactive compounds, resulting in decrease of antioxidant activity (Silva *et al.*, 2007).

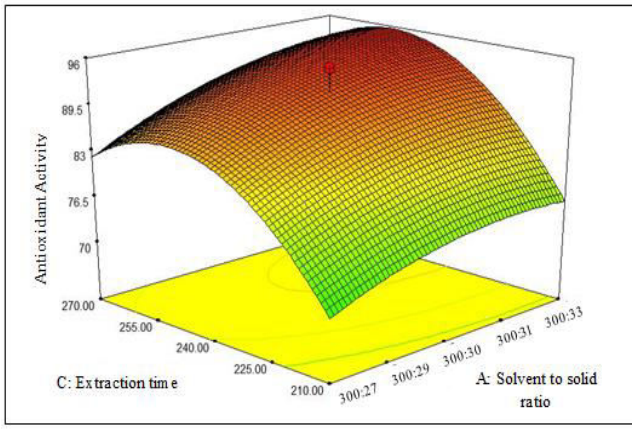

Figure-5. Response surface plot of the effect of solvent to solid ratio and extraction time on antioxidant activity. The extraction temperature was kept constant at 80°C.

Besides that, the relationship between the solvent to solid ratio and extraction time on antioxidant activity at constant extraction temperature of 80°C was revealed in Figure-5. The antioxidant activity was observed to be positively influenced by the synergism between the solvent to solid ratio and extraction time. Figure-5 indicated that in the region at solvent to solid ratio between 300:29 and 300:30 (mL/g) and extraction time between 255 and 270 minutes, around a maximum of 96% of antioxidant activity was achieved.

Other than that, the figure implied that the antioxidant activity decreases with the prolonged extraction time. The optimal extraction time was around 260 minutes. In term of parameter of solvent to solid ratio, increase in solid fraction relative to solvent affected the ability of the solvent to penetrate the solid, thus reduce the antioxidant activity obtained from extract (Ballard et al., 2010). The optimal solvent to solid ratio was achieved at around 300:29.25 (mL/g). Therefore, it can be stated that shorter extraction time in the presence of lower solvent to solid ratio favor the higher antioxidant activity and it is more economical and less time consuming.

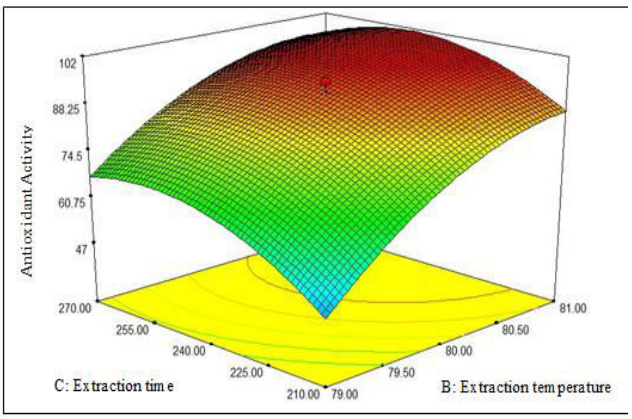

Figure-6. Response surface plot of the effect of extraction temperature and extraction time on antioxidant activity. The solvent to solid ratio was kept constant a 300:30 (mL/g).

Furthermore, the effect of extraction temperature and extraction time on antioxidant activity was illustrated in Figure-6 with constant solvent to solid ratio of 300:30 (mL/g). The antioxidant activity went up correspondingly with the increase of extraction temperature and time up to optimum level. The results revealed that almost 96 % of antioxidant activity obtained in the region at extraction temperature between 80.5 and 81.0^{o}C and extraction time between 255 and 270 minutes.

With regard to extraction time, the antioxidant activity increased readily when the extraction time increase up to around 260 minutes and afterward started to decline. This also revealed that the antioxidant activity obtained from rambutan peels increased as the extraction time increased but further increase in extraction time will cause heat degradation of the thermo- sensitive bioactive compounds, thus reducing the antioxidant activity *(Silva et al.*, 2007). This can be observed in Figure-6 that the optimum extraction time of 260 minutes was at 80.75 ^{o}C. In case of longer extraction time with higher extraction temperature, the antioxidant activity obtained was low due to the risk that the bioactive compounds in the extracting solvent undergo thermal degradation (Dent *et al*., 2013).

By combining all the three response surface plots presented in Figure 4, 5 and 6, it could be deduced that extraction temperature most significantly affected the antioxidant activity of rambutan peels extract followed by solvent to solid ratio and extraction temperature. Based on the results, the optimal extraction temperature at around 80.75^{o}C produced the highest antioxidant activity of around 96 % with respect to the solvent to solid ratio and extraction time at approximate 300:29.25 (mL/g) and 260 minutes respectively. However, based on the research carried out on the effect of extraction conditions from rambutan peels (Samuagam *et al*., 2013), solvent extraction required only extraction time of 1 hour and extraction temperature of 25^{o}C to obtain the highest antioxidant activity of 98.19%. Besides extraction temperature, solvent to solid ratio and extraction time were also important factors to be optimized in purpose to reduce the cost and time of the extraction process. Extraction process that carried out at moderate temperature for shorter time using adequate rambutan peels sample were sufficient to saturate the solvent with bioactive compounds (Chan *et al.*, 2009), producing highest antioxidant activity and at the same time minimize the possible impact of light or heat induced degradation and oxidation on plant bioactive compounds.

CONCLUSIONS

The present study demonstrated that the extraction conditions for antioxidant from the selected fruit (rambutan, banana, mangosteen, longan) peels was successfully optimized. Among them, rambutan peels was chosen to be used in this case of study because it showed the highest antioxidant activity when extracted using ethanol. The best solvent to solid ratio, extraction temperature and extraction time obtained from Single Factor Experiment were 300:30 (mL/g), 80 ^{o}C and 4 hours respectively. The lower, middle and upper levels of each of the parameters were selected to be employed in Response Surface Methodology (RSM) for optimization. Analysis in variance (ANOVA) indicated that the regression model was highly significant ($p< 0.001$). Extraction temperature ($p<0.0001$) was the most significant factor contributed to the antioxidant activity in rambutan peel extracts, followed by solvent to solid ratio and extraction time with at least $p<0.05$. On the other hand, the predicted optimum conditions suggested by RSM were solvent to solid ratio, 300:33 (mL/g); extraction temperature, 81 ^{o}C; and extraction time, 262.95 minutes. Under these extraction conditions, the antioxidant activity obtained was 96.12 $\pm$ 0.013 %, which is accordance to the predicted value established by RSM. Hence, the regression model developed was satisfactory in explaining and predicting the effect of three parameters on the antioxidant activity of rambutan peels extract. RSM had successfully applied to access the interaction effects among the extraction factors in term of reducing the usage of raw materials and extraction time at moderate extraction temperature to achieve the highest antioxidant activity.

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