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Ultrasound-Assisted Extraction of GAC Peel: An Optimization of Extraction Conditions for Recovering Carotenoids and Antioxidant Capacity

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Abstract: The peel of Gac fruit (*Momordica cochinchinensis* Spreng.), which is considered as waste of Gac processing, has been found to possess high levels of carotenoids and other antioxidants. This study aimed at determining the optimal conditions of an ultrasound-assisted extraction for recovering carotenoids and antioxidant capacity from Gac peel. A response surface methodology using the Box–Behnken design was employed to investigate the impact of extraction time, temperature and ultrasonic power on the recovery of total carotenoid and antioxidant capacity. The results showed that an extraction time of 76 min, temperature of 50 °C and ultrasonic power of 250 W were the optimal conditions for the extraction. The experimental carotenoid yield and antioxidant capacity obtained under the optimal extraction conditions were validated as 269 mg/100 g DW (dry weight) and 822 μM TE (Trolox equivalent)/100 g DW, respectively. These values were not significantly different from the values predicted by the models. The HPLC analysis for carotenoid composition showed that β-carotene, lycopene and lutein were the principal carotenoids of the extract, which constitute 86% of the total carotenoid content. Based on the obtained results, the ultrasound-assisted extraction using ethyl acetate under the above optimal conditions is suggested for the simultaneous recovery of carotenoids and antioxidant capacity from Gac peel.

Keywords: Gac peel; *Momordica cochinchinensis* Spreng.; ultrasound; extraction; carotenoid; antioxidant capacity

1. Introduction

Gac (*Momordica cochinchinensis* Spreng.) is a tropical vine popularly grown in Southeast Asia, China and India. Gac fruit has been reported as one of the richest natural sources of carotenoids [1,2]. In the processing of Gac fruit, only the aril (seed membrane) is used to produce commercial products like Gac oil and Gac powder while other parts of the fruit such as seeds, pulp and peel are discarded as wastes [3]. Gac seed has traditionally been used in Chinese medicine due to its beneficial bioactivities for human health [4]. The pulp (yellow fruit meat) and the peel (spiny red skin) of Gac fruit have also been found to contain a significant amount of bioactive compounds and have significant antioxidant ability [5,6].

The peel of Gac fruit has been reported to have a comparable carotenoid content as the well-known carotenoid-rich sources like tomatoes and carrots [7–9]. Thus, if carotenoids from Gac peel can be

efficiently recovered, this may be a good source of these compounds instead of being regarded as a by-product of Gac processing. In order to recover carotenoids and other bioactive compounds from the peel, drying and extraction techniques have been recently developed. For example, hot-air drying combined with ascorbic acid pre-treatment was found as the most suitable method for preserving content of carotenoids in the dehydrated Gac peel in comparison to other drying methods such as heat pump drying, vacuum drying and freeze drying [7]. For the recovery of carotenoids and antioxidant capacity from dried Gac peel, the use of ethyl acetate as the solvent with the liquid–solid ratio of 80:1 (mL/g) at temperature of 40.7 °C and the extraction time 150 min were reported as optimal conditions of the extraction process [10].

Although conventional extraction is the most popular method for recovering bioactive compounds, it requires a large solvent amount, high energy consumption and long extraction time to achieve an efficient extraction yield. Therefore, many advanced extraction techniques such as microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction and pressurized liquid extraction have been developed in order to overcome the disadvantages of the conventional extraction method [11,12]. Among the recently developed extraction techniques, ultrasound-assisted extraction (UAE) is considered as one of the most practical extraction methods for recovering bioactive compounds from plant sources because of its high efficiency and the popularity of the ultrasonic equipment [13]. UAE can increase the rate of mass transfer of the extraction based on the cavitation generated within the material. The cell wall of the material is destructed when the cavitation bubbles are produced and collapsed by ultrasound and thereby the release of the soluble compounds from material into the liquid phase is promoted [14].

The literature has shown that the application of UAE in for extracting carotenoids can enhance the recovered carotenoid yield, reduce amount of solvent and shorten extraction time in comparison to the classical solvent extraction. For instance, Nowacka and Wedzik (2016) reported an increase up to 50% in carotenoid extractability from the ultrasound-treated carrots compared to the untreated carrots [8]. The use of UAE have also led to higher extraction yield of carotenoids including lycopene and β-carotene from plant sources with shorter time, lower temperature and smaller solvent volume compared to the conventional extraction process [15–17]. In our previous studies, the ultrasound-assisted extraction of Gac peel using ethyl acetate as the solvent for 80 min resulted in a comparable carotenoid yield and a higher antioxidant capacity compared to those obtained from the conventional extraction of Gac peel for 150 min which used the same amount of the solvent [10,18].

Although the advantages of UAE have been proven and this technique has been widely applied for recovering carotenoids from plant sources, its application in carotenoid extraction from Gac fruit and particularly Gac peel is limited. Beside the investigation into the influences of extraction conditions to the extraction yield, the application of modeling techniques to exactly determine optimal conditions to achieve the maximum carotenoid yield from the material is necessary. Response surface methodology (RSM) is one of the most efficient techniques used for investigating both the impacts of single parameters and their interactive effects on the dependent responses [19]. In comparison to the classical single variable optimizing method, RSM shows a number of advantages such as the lower number of experiments and the clear expression of the interactive impacts of the parameters on the responses via 2D contour as well as 3D surface profilers [19–21].

In this study, the response surface methodology (RSM) using the Box–Behnken design was applied to investigate the effects of extraction time, extraction temperature and ultrasonic power on the recovery of carotenoids and antioxidant capacity from Gac peel. The optimal values of these extraction parameters were also determined to maximize the recovery of total carotenoid content and antioxidant capacity from Gac peel.

2. Materials and Methods

2.1. Chemicals

Analytical graded ethyl acetate and HPLC graded acetonitrile, dichloromethane and methanol were purchased from Merck Millipore (Bayswater, VIC, Australia). ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate, trolox ((*S*)-(-)-6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid, 98%) and β-carotene, lycopene and lutein standards were purchased from Sigma-Aldrich Pty Ltd. (Castle Hill, NSW, Australia).

2.2. Materials

Fully ripe Gac fruits (full-red surface) were harvested from Wootton, NSW, Australia and then transported to the laboratories of the University of Newcastle, Australia (Central Coast campus). Gac peel was separated from other parts of the fruit and then freeze dried to the moisture content below 4%. The dried peel was then ground, well mixed and sieved to obtain powder with particle size ranging from 250–500 μm. The peel powder was stored in a freezer at −18 °C until being used for the experiments.

2.3. Ultrasound-Assisted Extraction of GAC Peel

Of ethyl acetate 80 mL and 1 g of the dried Gac peel powder were added into a conical flask. After that, the flask was covered by glass fiber and placed in the Soniclean 1000 HD ultrasonic bath (Soniclean Pty Ltd., Thebarton, SA, Australia). The extraction was then carried out in a fume hood at different ultrasonic powers (150–250 W, 43.2 kHz of ultrasonic frequency) and different temperatures (30–50 °C) for different periods of time (60–100 min). After each extraction experiment, the liquid phase was collected, filtered through a 0.45 μm syringe filter and analyzed for the content of total carotenoid and antioxidant activity.

2.4. Experimental Design Using Response Surface Methodology (RSM)

Preliminary experiments were carried out to determine the likely ranges of the single parameters. The suitable ranges of extraction time (X_1, min), extraction temperature (X_2, °C) and ultrasonic power (X_3, W) for the extraction of total carotenoid and antioxidant capacity from Gac peel were 60–100 min, 30–50 °C and 150–250 W, respectively.

A RSM using Box-Behnken design was applied to investigate the effects of the extraction parameters on the total carotenoid extraction yield and antioxidant capacity of the extracts and determine the optimal values of the variables. The coded levels for each variable were selected according to the results of the preliminary experiments and presented in Table 1. The experimental design is shown in Table 2, which consisted of 15 experimental runs (12 factorial points and three central points) and the combinations of the variables in each run.

Table 1. Independent parameters and their coded levels.

Coded Variable Levels	Independent Variables		
	X_1 (min)	X_2 (°C)	X_3 (W)
−1	60	30	150
0	80	40	200
+1	100	50	250

Table 2. Actual and predicted values of total carotenoid (Y_1) and antioxidant activity (Y_2) extraction yields from Gac peel.

Run	Pattern *	X_1 (min)	X_2 (°C)	X_3 (W)	Y_1 (mg/100 g DW)		Y_2 (μM TE/100 g DW)	
					Experimental	Predicted	Experimental	Predicted
1	−−0	60	30	200	257.4	257.0	689.2	684.0
2	−0−	60	40	150	258.6	258.8	715.3	707.4
3	−0+	60	40	250	262.8	264.7	742.3	748.4
4	−+0	60	50	200	274.7	272.9	762.3	769.4
5	0−−	80	30	150	266.6	266.8	718.6	731.7
6	0−+	80	30	250	272.4	270.9	730.4	729.6
7	000	80	40	200	262.0	264.4	760.0	777.0
8	000	80	40	200	268.7	264.4	785.6	777.0
9	000	80	40	200	262.6	264.4	785.4	777.0
10	0+−	80	50	150	274.9	276.4	807.8	808.6
11	0++	80	50	250	278.6	278.4	855.6	842.5
12	+−0	100	30	200	272.0	273.8	739.7	732.6
13	+0+	100	40	250	271.5	271.3	773.3	781.3
14	+0−	100	40	150	272.9	271.0	796.7	790.6
15	++0	100	50	200	274.5	274.9	831.7	836.9

X_1: extraction time (min); X_2: extraction temperature (°C) and X_3: ultrasonic power (W). * The Pattern describes the combination of independent variables at different coded levels generated by Box–Behnken design: "−", "0" and "+" represented for the coded levels "−1", "0" and "+1", respectively.

The experimental results are expressed as mean values (n = 3).

A second-order polynomial was used to express the extraction yield of total carotenoid and antioxidant activity of the extracts as dependent responses of the independent variables as follows:

$$Y_i = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2,$$

where Y_i is the dependent response, X_i is the independent parameter and β_o, β_i, β_{ij} and β_{ii} are the regression coefficients of the intercept, linear, interaction and quadratic terms, respectively.

2.5. Measurement of Total Carotenoid Content

To determine total carotenoid content of Gac peel extract, the filtered extract was diluted by ethyl acetate using a volumetric flask to obtain an absorbance ranging from 0.2 to 1.0 at 450 nm.

A standard curve of β-carotene in ethyl acetate was established by measuring the absorbance of standard solutions at 450 nm using a spectrophotometer (Cary 50 Bio UV-Visible, Varian Australia Pty. Ltd., Mulgrave, VIC, Australia).

The total carotenoid content of the extract was calculated and expressed as mg β-carotene equivalent/100 g dry weight (DW) based on the standard curve of β-carotene in ethyl acetate.

2.6. HPLC Analysis of Carotenoid Composition

The composition of carotenoids of the extract from Gac peel obtained under optimal conditions was analyzed based on the method developed and validated by Kha et al. (2013) [22]. A Kinetex C18 (150 mm × 4.6 mm i.d; 5 μm) column (Phenomenex, Lane Cove, NSW, Australia) was used for the HPLC analysis in combination with a 10A VP HPLC system (Shimadzu Corp., Kyoto, Japan). An isocratic elution was used with the mobile phase composition of acetonitrile (50%), dichloromethane (40%) and methanol (10%). The injection volume of 20 μL and the column temperature of 20 °C were used. The carotenoids were detected by an UV-vis detector at the wavelength of 450 nm. Chromatograms of individual carotenoids in the standard solution and Gac peel extract obtained under optimal extraction conditions are shown in Figure 1. The retention times and the standard curves of the external standard carotenoids were used to calculate contents of individual carotenoids in the extracts.

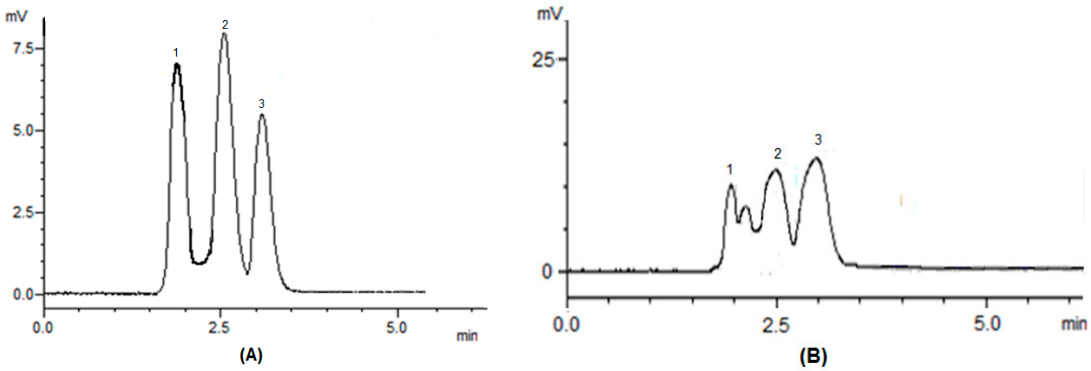

Figure 1. HPLC chromatograms of lutein (1), lycopene (2) and β-carotene (3) in the standard solution (**A**) and Gac peel extract (**B**).

2.7. Determination of Antioxidant Activity

The literature has shown that the estimation of total antioxidant capacity of a sample requires different antioxidant assays because an individual compound or an extract shows different antioxidant abilities on different assays [23]. Consistently, our previous studies have showed that the carotenoid-rich extracts from Gac peel do not exhibit DPPH antioxidant activity and have insignificant FRAP activities (ferric reducing antioxidant power). The carotenoid-rich extracts from Gac peel only showed strong ABTS antioxidant activity, which was highly correlated with their carotenoid content [6,7]. Therefore, the ABTS was selected as the assay in this study to evaluate the antioxidant activity of carotenoid-rich extracts from Gac peel.

The method for determination of ABTS antioxidant assay described by Thaipong et al. (2006) [23] was used for Gac peel extracts. The ABTS stock solution was obtained by the reaction of 7.4 mM ABTS solution with 2.6 mM potassium persulfate at a ratio of 1:1 (*v*/*v*) for 12–16 h at 20 °C in the dark. The ABTS working solution with an absorbance of 1.1 ± 0.02 at 734 nm was achieved by diluting the ABTS stock solution with methanol.

To determine ABTS antioxidant activity of an extract, 0.15 mL of extract and 2.85 mL of ABTS working solution were mixed in a test tube and left for reacting in the dark for 2 h. This reacted solution was then measured for absorbance at 734 nm using the above mentioned spectrophotometer. A standard curve of Trolox solutions was established to calculate antioxidant activity of the extracts from Gac peel. The ABTS was expressed as μmole Trolox equivalents (TE) per 100 g dry weight (g DW) of Gac peel.

2.8. Statistical Analysis

JMP 13.0 software (SAS, Cary, NC, USA) was used for establishing the experimental design of the optimization process. The experimental runs and the validated extractions at the predicted optimal conditions were carried out in triplicates.

The results are expressed as the mean values along with standard deviations. Multiples range test and LSD (least significant differences) were used for the comparisons of the mean values. A confidence interval of 95% ($p < 0.05$) selected for all the statistical tests.

3. Results and Discussion

3.1. Fitting the Model for the Prediction of Total Carotenoid Content and Antioxidant Capacity

The actual results for the extraction yield of total carotenoid (Y_1), antioxidant activity of the extracts (Y_2) and the corresponding predicted values generated by the statistical models are shown in Table 2. Very high correlations between the experimental values and predicted values for both total carotenoid yield ($R^2 = 0.92$) and antioxidant activity ($R^2 = 0.96$) were observed (Figure 2 and Table 3).

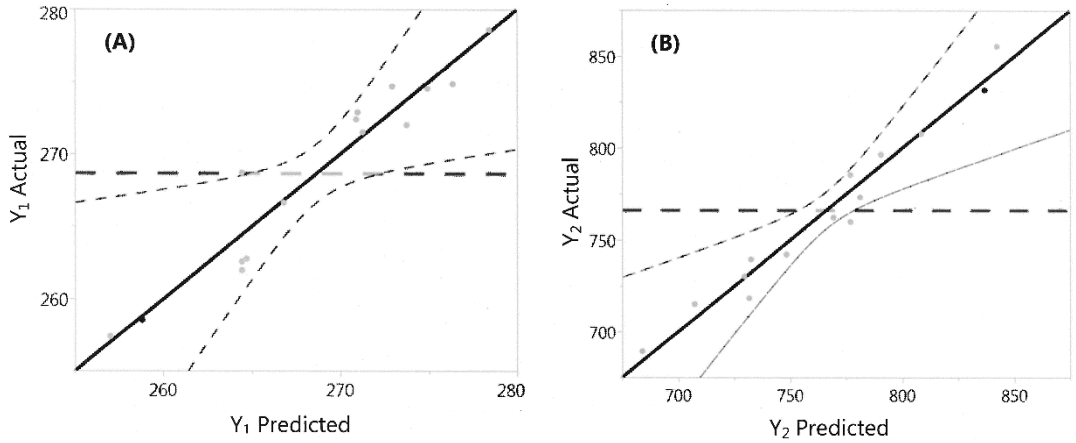

Figure 2. Correlation between the predicted and experimental values: (**A**) carotenoid yield (mg/100 g dry weight (DW)) and (**B**) antioxidant capacity (μM TE/100 g DW).

Table 3. Analysis of variance for determining model fitting.

Statistical Parameters	Total Carotenoid	Antioxidant Capacity
R^2	0.92	0.96
P of model	0.024	0.005
P of lack of fit	0.746	0.513

Results in Table 3 show that the p values for the lack of fit of both predicted models were much higher than 0.05 (0.75 and 0.51). These results indicate that the established models can be adequately employed for reflecting impacts of the input parameters on the independent responses and predicting the optimal conditions to obtain the maximum values of total carotenoid yield and antioxidant activity.

3.2. Effects of Extraction Conditions on the Total Carotenoid Extraction Yield

The results presented in Table 4 describe the analysis of the regression coefficients (β_i) of the model for the extraction yield of total carotenoid from Gac peel. The plus and minus values of a regression coefficient describe positive and negative correlations between the corresponding input variable and output response, respectively. The linear coefficients indicated that the yield of carotenoid was positively correlated with all the investigated extraction conditions ($\beta_i > 0$). However, only extraction time and temperature significantly influenced the carotenoid yield ($p = 0.0071$ and $p = 0.0103$, respectively) while the individual effect of the ultrasound power on the yield of carotenoid was not significant ($p = 0.2084$). Conversely, the interactive coefficients showed negative interactive effects of the extraction conditions on the carotenoid yield ($\beta_i < 0$) but all of the interactive impacts were not significant ($p > 0.05$). For the quadratic coefficients, only extraction temperature exhibited a significant positive effect on the carotenoid yield while the quadratic effects of the others were not significant. In addition to the analysis of the regression coefficients, the linear and the interactive effects of the parameters on the yield of total carotenoid are presented in Figure 3. The results show that when the extraction was conducted at low temperatures and ultrasound powers, the carotenoid yield significantly increased with the extended extraction times (Figure 3B). However, when high temperatures and powers were employed, the increase in extraction time did not lead to any significant increase and even caused a decrease in the carotenoid yield.

Table 4. Estimated regression coefficients and the statistical analysis.

Regression Coefficient	Total Carotenoid		Antioxidant Capacity	
	Estimated Value	Prob > |*t*|	Estimated Value	Prob > |*t*|
Intercept				
β_o	264.4 *	<0.0001	777.0 *	<0.0001
Linear				
β_1	4.689 *	0.0071	29.031 *	0.0028
β_2	4.277 *	0.0103	47.433 *	0.0003
β_3	1.544	0.2084	7.928	0.1971
Interaction				
β_{12}	−3.690	0.0586	4.728	0.5581
β_{13}	−1.404	0.3958	−12.586	0.1559
β_{23}	−0.510	0.7496	8.994	0.2864
Quadratic				
β_{11}	−0.734	0.6604	−21.222 *	0.0425
β_{22}	5.956 *	0.0128	−0.015	0.9986
β_{33}	2.734	0.1429	1.146	0.8896

* Significance at $p < 0.05$.

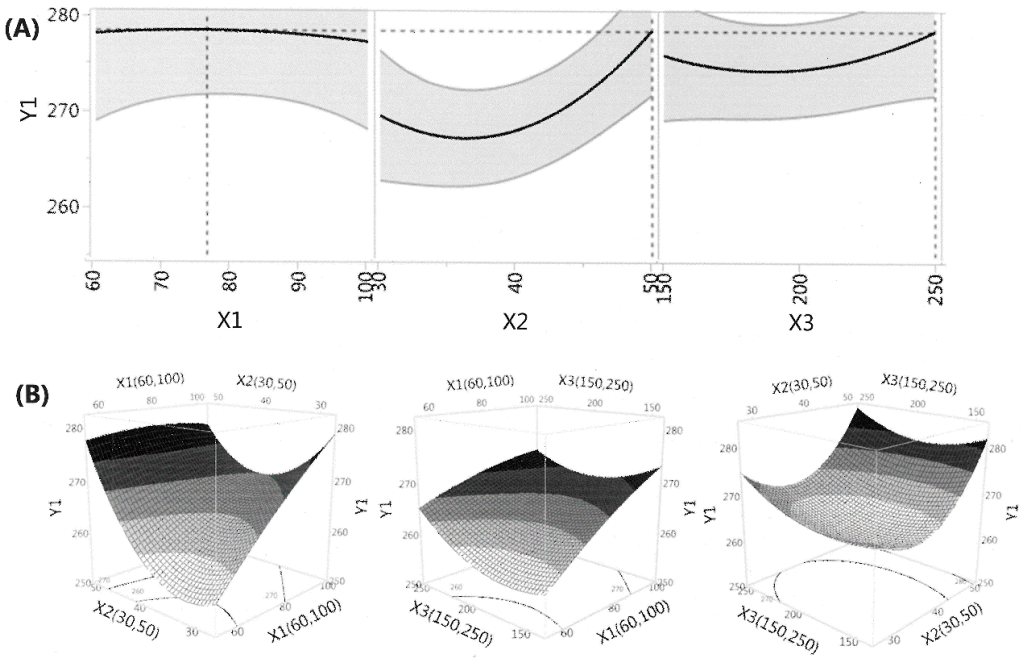

Figure 3. Linear effects (**A**) and interactive effects (**B**) of extraction time (X_1, min), temperature (X_2, °C) and ultrasound power (X_3, W) on total carotenoid extraction yield (Y_1, mg/100 g DW).

The converse correlations of carotenoid extraction yield with the extraction time at low and high levels of temperature and power may be due to the stability of carotenoids, which are very sensitive to the destructive effects of heat and ultrasonic waves. β-carotene, lycopene and lutein, the major carotenoids in Gac peel, are known to be thermally sensitive [6,16]. Treatment with ultrasound has also been reported to result in significant losses of carotenoids in other materials [24,25]. Thus, the extraction of Gac peel at high temperature and high ultrasonic power may cause a degradation of

carotenoids, which was greater than the amount released into the solvent, thereby resulted in the lower carotenoid extraction yield at the extended extraction period.

3.3. Effects of Extraction Conditions on Antioxidant Capacity of the Extracts

The regression coefficients of the model for antioxidant activity of Gac peel extracts show that the antioxidant ability of the obtained extracts was positively associated with the individual extraction parameters ($\beta_i > 0$; Table 4). The extraction temperature exhibited the highest linear effect on the antioxidant capacity followed by the extraction time. However, the linear effect of the ultrasound power on this response was significant ($p > 0.05$; Table 4). The extraction time was the only parameter having a significant quadratic effect on the antioxidant capacity ($p < 0.05$, Table 4). In consistence with the model for carotenoid extraction yield, the interactive effects of the variables on the antioxidant activity of the extracts were also insignificant ($p > 0.05$, Table 4).

Figure 4A,B describes the linear and interactive influences of the extraction time, temperature and ultrasound power on the antioxidant capacity of the extracts. The antioxidant capacity was predicted to be improved with the increases of all three variables. However the improvement in antioxidant capacity could be achieved for a period of time before reaching the plateau value and then declined. This phenomenon could be clearly observed in the interactive effect of time and high ultrasound power (Figure 4B).

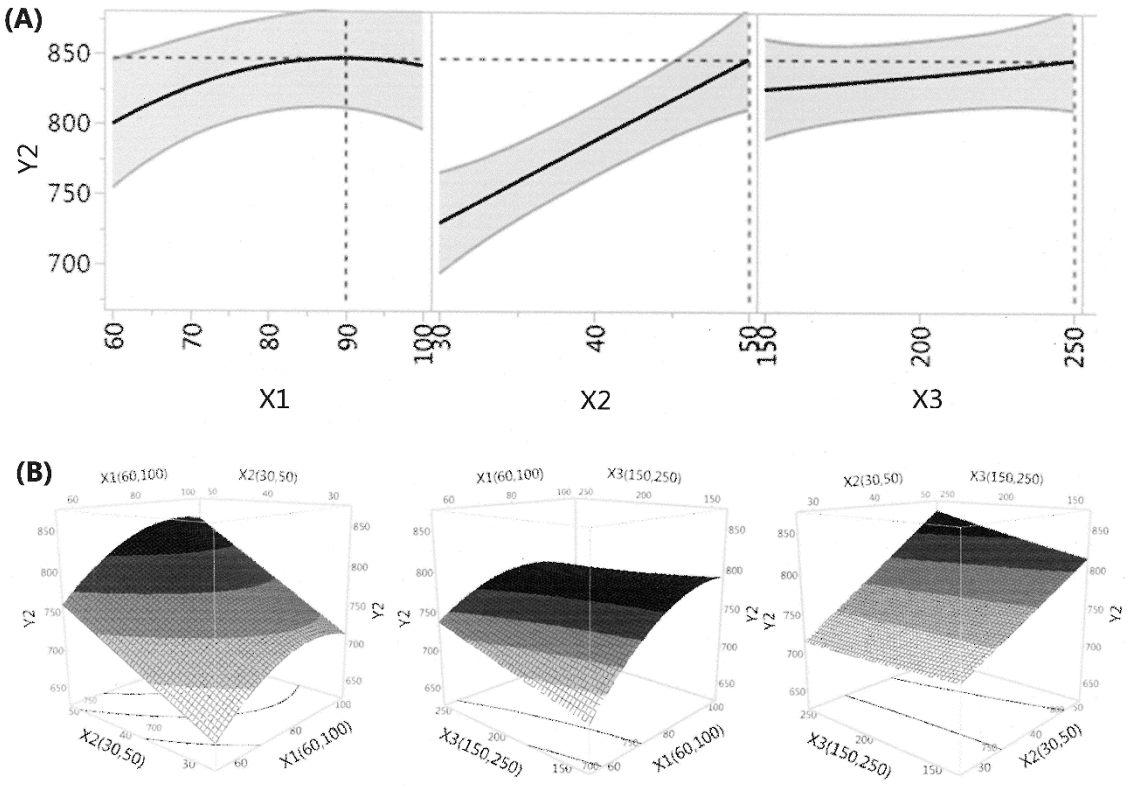

Figure 4. Linear effects (**A**) and interactive effects (**B**) of extraction time (X_1, min), temperature (X_2, °C) and ultrasound power (X_3, W) on antioxidant capacity of the extracts (Y_2, μM TE/100 g DW).

The predicted optimal extraction time for the recovery of antioxidant capacity was 90 min, which was significantly longer than that for the carotenoid yield (76 min). This difference may be due to the additional release of other bioactive compounds from Gac peel, which also contributed to the antioxidant capacity into the liquid phase at the extended time. Kubola and Siriamornpun (2011) [5] reported that in addition to the high levels of carotenoids, Gac peel also contains significant levels of

phenolic acids and flavonoids, which required a longer extraction time to reach their highest extraction yield compared to the time to achieve the highest carotenoid yield from Gac peel [26].

3.4. Optimal Extraction Conditions and Validation of the Model

A maximum carotenoid extraction yield of 278.5 mg/100 g DW was predicted by the second-order polynomial model for the carotenoid extraction. This value could be achieved from an ultrasound-assisted extraction at 50 °C with 250 W of ultrasonic power for 76 min (Figure 3A). The optimal temperature and optimal ultrasound power for recovering antioxidant capacity were also predicted as 50 °C and 250 W, respectively, but the predicted optimal extraction time for obtaining the maximum antioxidant capacity (847.7 μM TE/100 g DW) was longer than that for carotenoid yield (90 min, Figure 3A). The polynomial model for recovering antioxidant capacity also predicted that a recovery of 837.4 μM TE /100 g DW of antioxidant capacity could be achieved after 76 min of extraction (the optimal time for carotenoid yield). Thus, if the extraction time was reduced by 15.6%, only 1.2% of recovered antioxidant capacity would be lost compared to the maximum value. Consequently, the extraction time of 76 min, the temperature of 50 °C and the ultrasound power of 250 W were nominated as the optimal parameters for the simultaneously recovery of carotenoids and antioxidant activity from Gac peel.

To validate the predicted values of the variables, the actual experiment of an ultrasound-assisted extraction was carried out under the predicted optimal conditions (50 °C, 250 W and 76 min). Results of the validated experiment showed that the actual carotenoid yield and antioxidant capacity were not significantly different from those predicted by the models (Table 5). This similarity indicates that the established models are adequate for optimizing the recovery of carotenoids and antioxidant activity from Gac peel.

Table 5. Validation of the predicted results and a comparison with the conventional extraction of Gac peel.

Responses	Predicted Value	Actual Result	Conventional Extraction
Extraction time (min)	76	76	150 *
Temperature (°C)	50	50	40.7 *
Carotenoid yield (mg/100 g DW)	278.5 ± 6.8 ^{a}	269.1 ± 12.2 ^{a}	271.1 ± 8.5 ^{a}*
Antioxidant capacity (μM TE/100 g DW)	837.4 ± 36.0 ^{b}	822.3 ± 32.2 ^{b}	737.3 ± 23.8 ^{c}*

* Chuyen et al. (2017) [10]. The results are expressed as mean values ± standard deviations (n = 3). Different superscripts show significantly differences among values within each row ($p < 0.05$).

In comparison to the results from our previous study on a conventional extraction of Gac peel using the same solvent and solid-liquid ratio (1:80) [10], the maximum carotenoid yield obtained from the ultrasound-assisted extraction in this study was comparable. However, the maximum recovered antioxidant capacity from the method in the present study was significantly higher than that of the conventional extraction while the extraction time was reduced by approximately a half (Table 5).

3.5. Carotenoid Composition of the Extract Obtained under Optimal Conditions

The composition of carotenoids in Gac peel extract obtained under the optimal conditions was analyzed and calculated according to the retention times and standard curves of the standard carotenoids using a HPLC system. The results showed that the principal carotenoids were β-carotene, lycopene and lutein, which constitute 86% of the total amount of carotenoids in the extract (Table 6). The percentage of β-carotene was the highest (46%) followed by lycopene (28%) and lutein (12%). This result is in agreement with our previous studies, which showed that β-carotene, lycopene and lutein contributed to approximately 90% of total carotenoid content in the peel of Gac fruit [6,7]. Ethyl acetate was also found as the most suitable solvent for a simultaneous extraction of those principal carotenoids from Gac peel compared to other solvents such as hexane, acetone and ethanol [26].

Table 6. HPLC analysis of carotenoid composition in Gac peel extract.

Individual Carotenoid	Retention Time (min)	Percentage (%)
β-carotene	1.89	46.1
Lycopene	2.55	28.1
Lutein	3.12	11.8

Previous studies on biological activities of Gac fruit showed that carotenoids from this fruit possess a variety of beneficial effects to human health. For example, β-carotene from Gac fruit, a precursor to vitamin A, has been efficiently used to treat the deficiency of vitamin A in children living in the poor communities in the North of Vietnam via the addition of Gac aril into their diet [27]. Lycopene from Gac fruit has been found to highly correlate with the antioxidant capacity of Gac products, which may contribute to the positive effects in the treatment of diabetes, cancers or cardiovascular diseases [5,28]. Lutein and β-carotene have also been widely used to improve visual ability and treat eye diseases due to their macular-protective activities [27,29]. These results suggest that carotenoids extracted from Gac peel may have a potential to be used as natural bioactive compounds for food, cosmetic and medicinal products.

4. Conclusions

The response surface methodology using the Box–Behnken design was successfully applied for optimizing extraction time, extraction temperature and ultrasound power of an ultrasound-assisted extraction to recover carotenoids and antioxidant activity from Gac peel. The carotenoid yield of 269 mg/100 g DW and antioxidant capacity of 822 μM TE/100 g DW were validated as maximum values which obtained from an extraction at 50 °C and 250 W of ultrasound power for 76 min. Although the ultrasound-assisted extraction did not result in a higher carotenoid yield compared to the conventional extraction, the antioxidant activity of the extract was significantly improved and the extraction time was much shorter. Thus, the ultrasound-assisted extraction using the above optimal conditions is recommended for recovery of carotenoids and antioxidant activity from Gac peel.

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