Yield of Carotenoids, Phenolic Compounds and Antioxidant Capacity of Extracts from Gac Peel as Affected by Different Solvents and Extraction Conditions

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***Abstract*—Gac fruit (*Momordica cochinchinensis* Spreng.) is a rich source of bioactive compounds especially carotenoids. Currently, only the aril of the Gac fruit is processed and the peel is discarded although it contains high levels of carotenoids and phenolic compounds, which could be extracted for commercial use. In the present study, solvent type, ratio of solvent to material, extraction time and temperature were investigated for the extraction yields of carotenoids and phenolic compounds, the antioxidant activity of the extract from Gac peel. Ethyl acetate extract showed the highest extraction yield of carotenoids, phenolics and ABTS antioxidant capacity. The highest levels of carotenoid yield and antioxidant capacity were obtained at 2 hours of extraction with the ratio of solvent to material of 20:1 (ml/g). Phenolic extraction yield reached the highest level after 2.5 hours of extraction and rose with the increase in extraction temperature while the highest extraction yield of carotenoids was obtained at 40 to 50 ℃ and declined at higher temperature. The extraction using ethyl acetate with the ratio of 20:1 (ml solvent/g Gac peel) for 2 hours at 50 ℃ is suggested for extraction of bioactives from Gac peel.**

***Index Terms*—Gac peel, extraction, carotenoid, phenolic compound, antioxidant**

I. Introduction

Gac fruit (*Momordica cochinchinensis* Spreng.) has been reported as a rich source of bioactive compounds including carotenoids, phenolic and flavonoid compounds [1]-[3]. Several studies have focused on the aril of the fruit because of its extremely high levels of lycopene and β-carotene compared to other natural sources [4]-[6]. Gac aril has been manufactured into powder, oil and capsules for food, cosmetic and pharmaceutical uses [2], [7], [8]. Whereas, the peel which constitutes a significant bulk (up to 15% w/w) of the fruit and contains a significant amount of bioactives, is usually discarded as waste [2], [3].

β-carotene has been well-known as a pro-vitamin A compound and lutein has also been used widely in the treatment of eye diseases [7], [9] while lycopene has been found to have a variety of biological functions such as cardioprotective and anticancer activities [10]. Phenolic compounds have been regarded as natural bioactives having significant positive effects on the treatments of many chronic diseases including diabetes, cancers and cardiovascular diseases [11]. Therefore, if these compounds can be extracted effectively, the peel might become a potential source of natural bioactive compounds instead of causing environmental issues as waste of Gac processing.

There are a number of available extraction techniques for extraction of bioactives from natural plant materials. However, the extraction efficiency is varied widely due to the target bioactive compounds and the type of material [12]. For example, phenolic compounds are extracted more effectively with high-polarized solvents while non-polar or low-polarized solvents showed a higher extraction yield for lipophilic compounds like carotenoids [13], [14]. The extraction conditions including time, temperature and amount of solvent are also important parameters affecting the extraction yield of the bioactive compounds and antioxidant capacity of the extracts [15], [16].

Finding extraction techniques having high extraction efficiencies on bioactive compounds from Gac peel is one of the key factors to validate it as a potential source of the compounds. For this reason, this study aimed to investigate the effects of different organic solvents and extraction conditions: ratio of solvent to material, extraction time and temperature on the yield of carotenoids and phenolic compounds as well as the antioxidant capacity of the obtained extracts.

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II. Materials and Methods

A. Chemicals

Acetone, ethanol, hexane, methanol and ethyl acetate were purchased from Merck Millipore (Bayswater, VIC, Australia). β-carotene, Folin-ciocalteu's phenol regent, gallic acid, sodium carbonate, potassium persulfate, ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium) were purchased from Sigma-Aldrich Pty Ltd. (Castle Hill, NSW, Australia).

B. Gac Material

Gac fruits were harvested at full ripen stage from the greenhouses of the NSW Department of Primary Industries, Ourimbah, NSW, Australia (151° 22'E, 33° 21'S). The peel of the fruits was separated and dried using a hot-air oven at 70 ℃ to the moisture content of 4±0.2%. The dried peel was then ground, sieved to obtain the particle size of 0.25-0.5mm and well mixed into one uniform lot. The ground dried Gac peel was stored in vacuum sealed bags in a freezer at -18 ℃ in the dark for the extraction of bioactive compounds.

C. Experimental Design

Extraction with different solvents: Each one gram of the dried Gac peel was extracted by 20mL of organic solvents: hexane, acetone, ethyl acetate and ethanol in a beaker at 20 ℃ for 120 minutes with agitation. The extraction mixture was then filtered using a 0.45 μm filter paper to obtain extract for the analysis of total carotenoid content, total phenolic content and antioxidant activity.

Extraction with different ratios of solvent to material: The chosen solvent (ethyl acetate) that resulted in highest yield of carotenoid, phenolics and antioxidant capacity was examined for the extraction efficiency at the ratios of 5:1, 10:1, 20:1, 40:1 and 80:1 (ml/g of solvent : Gac peel) at 20 ℃ for 120 minutes. The extraction mixtures were then filtered using a 0.45 μm filter paper to obtain extracts for further analysis

Extraction with different time: One gram of the dried Gac peel was extracted by 20mL of ethyl acetate in a beaker at 20 ℃ for 30, 60, 90, 120, 150, 180, 210 and 240 minutes. The extraction mixtures were then filtered using a 0.45 μm filter paper to obtain extracts for further analysis.

Extraction at different temperature: One gram of the dried Gac peel was extracted by 20mL of ethyl acetate in a beaker placed in a water bath set at different temperatures: 20, 30, 40, 50, 60^{o}C. After 120 minutes, the extraction mixtures were quickly cooled down to room temperature using an ice water bath and filtered using a 0.45 μm filter paper to obtain extracts for further analysis.

D. Determination of Total Carotenoid Content

The absorbance at 450nm of the extracts from Gac peel or standard solutions was determined using a Cary 50 Bio UV-Visible spectrophotometer (Varian Australia Pty. Ltd., Mulgrave, VIC, Australia). The total carotenoid content of the extracts was expressed as mg β-carotene equivalent /100g dry weight (DW) based on the standard curves of β-carotene in hexane, ethanol, acetone and ethyl acetate.

E. Determination of Total Phenolic Content

Total phenolic content of the extracts was measured as described by Vuong *et al.* [16] with some modifications. 0.5ml of extract and 2.5mL of 10% (v/v) Folin-Ciocalteu reagent were added into a test tube and left at room temperature for 5 minutes before adding 2ml of 7.5% (w/v) Na_2CO_3. The mixture was mixed and left for reacting in the dark at room temperature for 1 hour and then measured for the absorbance at 765nm using the same spectrophotometer described above. The total phenolic content of the extracts was expressed as mg gallic acid equivalent (mg GAE) based on the standard curve of gallic acid solutions.

F. Determination of Antioxidant Activity

The ABTS antioxidant assay of Gac peel extracts was carried out based on the methods described by Thaipong *et al.* [17]. The ABTS stock solution (7.4mM) and the potassium persulfate stock solution (2.6mM) were mixed with a ratio of 1:1 and left to react for 12-16 hours in a dark room. The ABTS working solution was then made by diluting the reacted solution with methanol to obtain an absorbance of 1.1±0.02 units at 734nm using the spectrophotometer described previously.

A volume of 2.85mL of the ABTS working solution and 0.15mL of extract from Gac peel or 0.15mL of standard Trolox solution were transferred into a test tube and the mixture reacted for 2 hours in a dark room. The absorbance of this reacted solution was then determined at 734nm using the spectrophotometer. The ABTS antioxidant activity of the Gac peel extracts was expressed as μmole Trolox equivalents (TE) based on the standard curve of the Trolox solutions.

G. Statistical Analysis

All the extraction experiments were repeated in triplicate and the results were expressed as the mean value ± standard deviation. The overall statistical significance for each experiment was determined using the analysis of variance test (ANOVA) and the LSD post-hoc test was used for comparisons amongst the mean values if the ANOVA was significant. Differences were considered to be significant at $p<0.05$

III. Results and Discussion

A. Effect of Solvents on the Extraction Yield of Carotenoid and Phenolic Compounds and Antioxidant Activity of Extract from Gac Peel

The use of different solvents showed a significant variation in the extraction yield total carotenoid, total phenolic and the total antioxidant capacity of the obtained extracts (Fig. 1). Ethanol resulted in the highest yield of phenolic extraction and second highest antioxidant capacity but its carotenoid yield was the lowest compared to the other solvents. In contrast, although hexane extract showed the second high yield of carotenoid, the phenolic extraction yield and antioxidant capacity of hexane were

the lowest. Among the investigated solvents, ethyl acetate exhibited the highest carotenoid extraction yield and antioxidant activity and a comparable extracted phenolic content as ethanol.

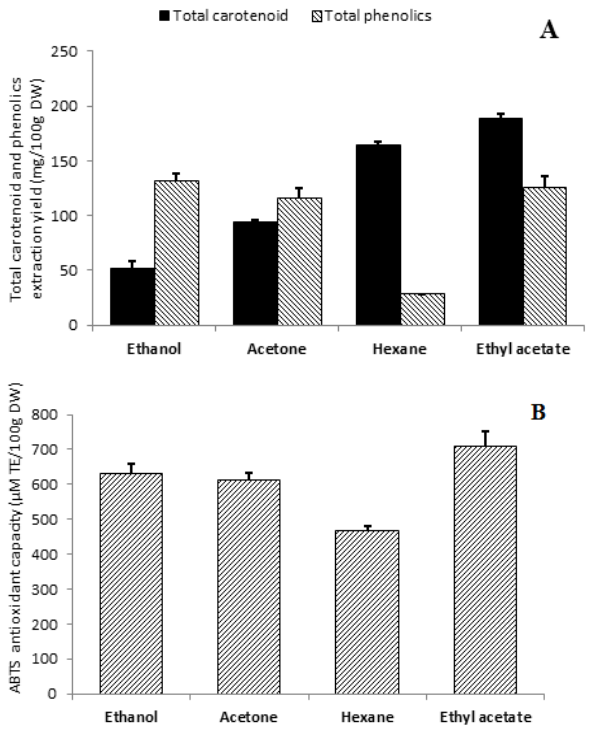

Figure 1. Extraction yields of carotenoids and phenolics (A) and ABTS antioxidant capacity (B) of extracts using different solvents (n=3, error bar=SD).

The achieved yields of phenolics and carotenoids in this study are in agreement with previous studies which reported that phenolic compounds are easier to be extracted by high-polarized solvents such as water, methanol and ethanol because of their hydrophilic characteristic [13], [16], [18]. Conversely, the preferable solvents for carotenoid extraction are non- or weak-polarized solvents including hexane, chloroform and ethyl acetate [14]. Ethyl acetate, which resulted in a high level of carotenoid, phenolic and antioxidant capacity in the present study, has also been found as a suitable solvent for extraction of both phenolic and carotenoid compounds from other plant materials [19], [20].

B. Effects of Extraction Time on the Yield of Carotenoid and Phenolic Compounds and Antioxidant Activity of Extract from Gac Peel

The influence of extraction time on the extraction of carotenoids, phenolic compounds and antioxidant capacity of extracts from Gac peel using ethyl acetate is shown in Fig. 2. The total extracted carotenoid and antioxidant capacity increased with the extraction time and reached the highest levels (189 mg/100g DW and 709 µM TE/100g DW, respectively) at 2 hours of extraction before being reduced gradually. The phenolic content obtained the peak of 148mg/100g DW after 2.5 hours and then fluctuated until the end of the extraction (4 hours).

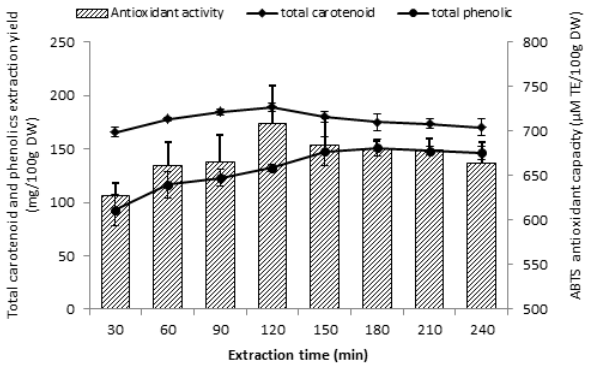

Figure 2. Extraction yields of carotenoids and phenolics and ABTS antioxidant capacity of Gac peel extracts at different extraction time (n=3, error bar=SD).

Many studies on carotenoid extraction suggest that the highest yield of carotenoid extracted from fruits and vegetables of suitable particle sizes can be achieved after a short period (usually less than 2 hours) and remains unchanged or declines over longer periods due to degradation caused by oxygen, light and temperature [15], [21], [22]. Similarly, the peak of carotenoid yield was obtained after 2 hours of the extraction and decreased when the extraction time was expanded to 4 hours. The extraction yield of phenolic compounds was also reported to reach the maximum value after a certain extraction time depending on materials and used solvents and fluctuated at high level for longer [23], [24]. The decrease in antioxidant capacity of the extract after 2 hours of extraction might be due to the loss in carotenoids, which significantly contributes to the antioxidant capacity of Gac fruit [23], while the phenolic content increased slightly or was unchanged during this period.

C. Effects of the Ratio of Solvent to Material on the Yield of Carotenoid and Phenolic Compounds and Antioxidant Activity of Extract from Gac Peel

Several studies on extraction of bioactive compounds have stated that the amount of solvent significantly affected the extraction efficiency of the desired compounds [16], [25]. Fig. 3 indicates that the change in ratio of solvent to Gac peel weight also influenced the total extracted carotenoid and phenolic amounts as well as the antioxidant capacity. The increase in the volume of ethyl acetate exhibited greater effects on extracted phenolic yield and antioxidant capacity compared to the carotenoid yield. The total extracted carotenoid amount was very high at the lowest volume of solvent (168mg/100g DW at the ratio of 5:1 (ml/g)) and increased slightly with the higher ratios of solvent to material. Both phenolic yield and antioxidant capacity increased nearly twofold when the ratio of solvent to material increased from 5:1 to 20:1 (ml/g). However, no significant improvement in all extracted carotenoid, phenolic and antioxidant capacity was found when the solvent volume increased from the ratio of 40:1 to 80:1 (ml/g).

Theoretically, the increase in ratio of solvent to material results in a higher extraction yield of the soluble compounds because a greater amount of compounds from material can be extracted into the bigger solvent volume

[26]. However, practically, the extraction yield of the compounds cannot be grown infinitely but it reaches the maximum value at a certain ratio and fluctuates around this value or increases insignificantly [25], [27]. In the present study, the highest yield of carotenoid and phenolic and antioxidant capacity was found at the ratio of 20:1 (ml/g) of ethyl acetate to Gac peel and no significant increase was observed at higher ratios (40:1 and 80:1 (ml/g)).

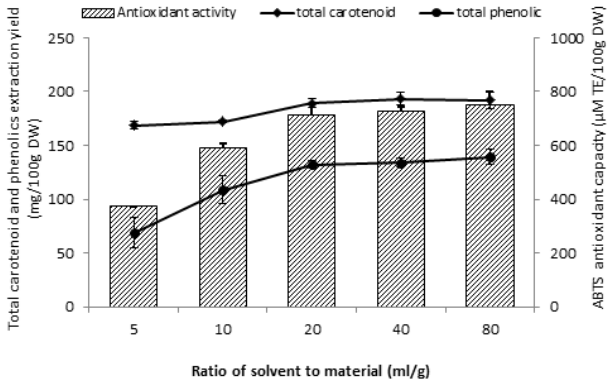

Figure 3. Extraction yields of carotenoids and phenolics and ABTS antioxidant capacity of Gac peel extracts using different ratios of solvent to material (n=3, error bar=SD).

D. Effects of Extraction Temperature on the Yield of Carotenoid and Phenolic Compounds and Antioxidant Activity of Extract from Gac Peel

The extraction temperature showed an obvious influence on the extraction yield of phenolic compounds and antioxidant capacity while the carotenoid extraction yield was slightly affected by this parameter (Fig. 4). When the temperature increased from room temperature (20 ℃) to 60 ℃ the extraction yield of total phenolic and the antioxidant capacity were improved 29% (from 132 to 170mg/100g DW) and 33% (from 640 to 852 μM TE/100g DW). Only an increase of 4% in total extracted carotenoid was achieved by the increase of temperature to 40-50 ℃ and the obtained carotenoid amount was reduced at higher extraction temperature.

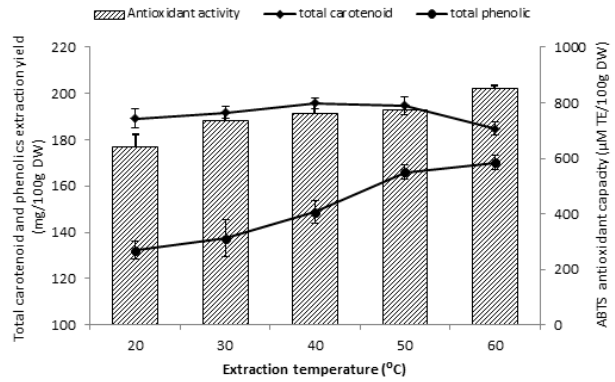

Figure 4. Extraction yields of carotenoids and phenolics and ABTS antioxidant capacity of Gac peel extracts at different extraction temperatures (n=3, error bar=SD).

Several studies have shown that phenolic extraction yield and total antioxidant capacity of extracts increase along with an increase in extraction temperature and highest levels are obtained at high temperatures (70-80 ℃) [16], [28]. However, the extraction of carotenoids is recommended at medium temperatures (less than 70 ℃) to obtain the highest extraction yield because carotenoids are extremely sensitive to high temperatures [25], [29], [30]. In this study, the antioxidant activity and phenolic content of extracts Gac peel increased steadily from 20 to 60 ℃ while the highest extracted carotenoid content achieved at 40-50 ℃.

A high correlation between antioxidant capacity and phenolic extraction yield of Gac peel extracts was shown (R^2=0.8). The correlation between the antioxidant capacity and carotenoid content of Gac peel extracts was less marked (R^2=0.62). This suggests that phenolic compounds have a greater contribution to antioxidant capacity of Gac peel extract than carotenoids.

IV. Conclusion

The investigated solvents, solvent amounts, extraction time and extraction temperatures significantly influenced total carotenoid, total phenolic extraction yields and antioxidant capacity of Gac peel. While the solvent type had a strong effect on carotenoid yield, the ratio of solvent to material and extraction temperature exhibited greater effects on phenolic yield and antioxidant capacity of Gac peel extracts. The extraction using ethyl acetate with the ratio of solvent to Gac peel of 20:1 (ml/g) at 60 ℃ for 2 hours is suggested for the extraction of carotenoids and phenolic compounds from Gac peel. However, more studies on other extraction techniques such as enzyme-assisted, microwave-assisted and ultrasound-assisted extractions are recommended for the improvement of extraction yield of bioactive compounds and antioxidant capacity from Gac peel.

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