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Effects of extraction conditions on antioxidant properties of sapodilla fruit (*Manilkara zapota*)

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Abstract

This study investigated the effects of different percentages of ethanol (0 - 100%), extraction times (1 - 5 h) and temperatures (25 - 60°C) on total phenolic content (TPC) and antioxidant activity (AA) of sapodilla pulp and peel. TPC was determined by Folin-Ciocalteu reagent method, while AA was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and β-carotene bleaching (BCB) assay. Based on the optimal extraction conditions used, sapodilla pulp extract had TPC of 3.89 mg GAE/g, 63.20% of DPPH scavenging activity, 4.30% of ABTS scavenging activity, 19.17% of BCB activity, and FRAP value of 15.24 mg TE/g; while its peel extract had TPC of 9.23 mg GAE/g, 92.95% of DPPH scavenging activity, 5.36% of ABTS scavenging activity, 8.14% of BCB activity, and 27.85 mg TE/g (FRAP value). Using the optimal extraction conditions for sapodilla pulp (40% ethanol as extraction solvent that extracted at 60°C for 4 h) and sapodilla peel (80% ethanol and 2 h extraction time at 40°C), highest antioxidants can be extracted from the pulp and peel.

Introduction

Antioxidants are present in lower concentration than oxidants, and are capable of delaying or inhibiting oxidation (Ahmadi *et al*., 2007). Antioxidants have been shown to reduce risks of oxidative stress related diseases (Liu, 2003). Recently, researchers, manufacturers and consumers are more interested in natural antioxidants due to their safety level. Synthetic antioxidants such as butylated hydroxylanisole (BHA) and tertiary butyl hydroquinone (TBHQ) are toxic and carcinogenic (Yu *et al*., 2000). As reported by Suja *et al*. (2005), the use of TBHQ in food has been banned in Japan and Europe. Against this health risk backdrop, numerous studies had been done on herb and plant extracts for their "natural" antioxidant potential to replace their synthetic counterparts. Some promising antioxidative potential has been identified, for example in bitter melon (*Momordica charantia*) (Budrat and Shotipruk, 2008). Leong and Shui (2002) also reported that Sapodilla fruit had the highest antioxidant activity that mainly contributed by polyphenolic compounds.

Sapodilla fruit (*Manilkara zapota*) is known as "ciku" in Malaysia. The genus of sapodilla fruit is Manilkara and it is from the Sapotacaea family. The tree is a slow-growing, long lived, ever-green, also known as "chicle" tree. It grows up to 20 m high (Lim, 2013). Skin of the fruit is rusty and brown in colour, while its pulp is brownish-yellow or red in colour. The fruit is very sweet, sandy and tastes caramel like. Broad cultivation of sapodilla fruit has occurred in India and Philippines has led to the development of several cultivars (Balerdi *et al.*, 2008). There are four common cultivars known as Brown sugar, Prolific, Russel and Tikal.

Extraction conditions were optimised to achieve highest level of antioxidant from fruit sample. Although optimisation of extraction parameters for sapodilla pulp and peel using ethanol-water binary solvent has yet reported, it is favourable to determine optimum extraction conditions for sapodilla pulp and peel using this binary solvent system. Besides, some studies that comparing fruit pulp and peel have shown that the peel usually possesses higher antioxidant activity (AA) and total phenolic content (TPC). For instance, AA of red guava and Gala apple peels were approximately two times higher than the pulp (Hassimotto *et al*., 2009). Fruit peels are often found to have higher AA or TPC which may be associated with their protective role (Wijngaard *et al*., 2009). Thus, comparisons of TPC and AA in sapodilla pulp

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and peel are crucial for better understanding on health benefits of the fruit.

Sapodilla fruit (ciku) was used for this study because it has the highest antioxidant capacity among tropical fruits (Leong and Shui, 2002). The sapodilla fruit has special sweet taste and juicy compared to most of the tropical fruits. In this study, an experiment with one-at-a-time design was performed to determine the optimal parameters for extraction of antioxidants in sapodilla and its peel. The parameters include the percentage of ethanol/water in the binary solvent, and times and temperatures for extraction. The optimal extraction conditions for sapodilla pulp and peel were investigated based on TPC and AA.

Materials and Methods

Sample preparation and extraction

Sapodilla fruit was purchased from a fruit farm in Pulau Gadong, Malacca, Malaysia. The Sapodilla fruit has been registered with the Department of Agriculture, Malaysia (No. C62: Ciku Jantung) (Plant Variety Protection Malaysia, DOA). Exactly 2.85 kg of the fruit was washed under the running tap water and wiped with tissue paper. The peel of the fruit was separated from the pulp and both the pulp and peel were cut into small pieces. In the present study no sterilisation of the samples was performed with the purpose of determining the antioxidant activity at their raw state.

The samples were then separately oven dried at 45°C for 24 h using convection oven (UNE-200-800, Memmert, Germany), and further ground into powder using electronic grinder (MX-AC210, Panasonic, Malaysia) under room temperature (25°C) in Food Science Laboratory of UCSI University. The powdered samples were vacuum packed in laminated vacuum packaging bag using vacuum packager (DZQ 400/500, Clarity, China), wrapped in aluminium foil and stored at room temperature in the dark until further analysis.

The first experiment started by using five different percentages of ethanol (0%, 20%, 40%, 60%, 80%, and 100%) for extraction. Exactly 50 ml of each ethanol-water binary solvent was added to 5.0 g of each dried powder. The mixture was shaken using an orbital incubator shaker (Model S1500, Stuart, UK) at 150 rpm, 25°C for 1 h. The samples were then centrifuged at 3000 rpm, 25°C for 10 min using centrifuge (Universal 320R, Hettich Zentrifugen, UK), and the supernatants were filtered. All sample residues were re-extracted once. The filtrates were combined and rotary evaporated at 40°C to remove the solvent using rotary evaporator (Rotavapor R-200, Buchi, Switzerland). The extract was then freeze dried at –50°C for 48 h using freeze dryer (Alpha 1-4 plus, Martin Christ Gefrietrocknungsanlagen, Germany). The extraction process was carried out under dark environment and the extracts obtained were stored in freezer (-20°C) (E388LW, Fisher & Paykel, USA) prior to antioxidant analysis.

The optimum concentration of ethanol-water binary solvent for sapodilla pulp and peel was identified by comparing the TPC and AA (DPPH and BCB assays). The second experiment, using the optimum concentration of ethanol-water binary solvent for sapodilla pulp and peel and following the same protocol, applied five different extraction times, namely 1, 2, 3, 4, and 5 h. For the third experiment, using the optimum concentration of ethanol-water binary solvent and the optimum extraction time, applied 5 different temperatures, namely 25, 30, 40 50 and 60°C.

Total phenolic content

Total phenolic content (TPC) assay was performed using Folin-Ciocalteu reagent (FCR) method (Hassimotto *et al*., 2009). A 1.0 ml of diluted sample or distilled water (control) was mixed with 4.0 ml of FCR (previously diluted 10-fold). After 3 min, 5.0 ml of sodium carbonate solution (7.5%, w/v) was added. Absorbance was taken using UV-Vis Spectrophotometer (UviLine 9400, Secoman, France) against blank at 725 nm after incubation for 30 min. Measurements were calibrated using a gallic acid standard curve (0.2 to 25 µg/ml), with y = 0.0165x–0.0003 (R^2 = 0.9972). The results were expressed as mg gallic acid equivalent (GAE) /g of lyophilised extract.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH assay was performed according to a modified method from Reddy *et al*. (2010) and Sun *et al.* (2009). Briefly, 1.0 ml of DPPH reagent was added with 1.0 ml of diluted sample. The absorbance was read at 517 nm against blank after the mixture was incubated for 30 min in dark. DPPH radical scavenging activity of the extract was calculated using the following equation:

$$\text{DPPH radical scavenging activity (SA, \%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

EC_{50}, which is the effective concentration of extract to scavenge 50% of DPPH radicals, was calculated from equation generated from graph of SA (%) or

%SA against sample concentrations.

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity

ABTS assay was performed as described by Almeida *et al.* (2011). ABTS reagent was prepared by addition of 5.0 ml of 7 mM ABTS to 88.0 µl of 140 mM of $K_2S_2O_8$ solution, and allowed to stand in dark for 16 h. After incubation, the ABTS reagent was prepared by adding 95% ethanol so that an absorbance of 0.7 ± 0.05 was achieved. Briefly, an exactly 10.0 µl of sample extracts or distilled water (control) was mixed with 1.0 ml of ABTS reagent. Absorbance was taken against blank at 734 nm after incubated for 6 min. ABTS radical scavenging activity was calculated:

$$\text{ABTS radical scavenging activity (SA\%)} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

Graph of SA (%) or %SA against dried extract concentrations was plotted. IC_{50}, which is the sample concentration to quench 50% of the initial ABTS radical, was obtained from the graph.

Ferric-reducing antioxidant power assay

Ferric-reducing antioxidant power (FRAP) assay was carried out based on a method described by Thaipong *et al.* (2006). The FRAP reagent was freshly prepared by mixing 300 mM of acetate buffer of pH 3.6, 10 mM tripyridyltriazine (TPTZ) solution, and 20 mM $FeCl_3$ in a ratio of 10:1:1 in a beaker. It was incubated in water bath at 37°C prior to analysis. Exactly 3.0 ml of FRAP reagent was pipetted into 0.1 ml of diluted sample or distilled water (control). The mixtures were incubated at 37°C for 4 min before absorbance measured at 593 nm. The measurements were calibrated to a Trolox standard curve (5 - 400 µg/ml), with y = 0.0057x–0.0214 (R^2 = 0.9984). The results were expressed in mg Trolox equivalent (TE)/g of lyophilised extract.

β-Carotene bleaching assay

Inhibition activities of sample extracts were evaluated using β-carotene bleaching (BCB) assay, as described by Ahmadi *et al.* (2007). Exactly 1.0 ml of β-carotene solution (2.0 mg of β-carotene powder per ml of chloroform) was mixed with 0.02 ml of linoleic acid and 0.2 ml of Tween 40. Chloroform was removed at 30°C using a rotary evaporator for 10 min. The mixture was added with 100 ml of distilled water and shaken vigorously to form emulsion. Exactly 100 µl of the dried extractor distilled water (control) was mixed with 1.0 ml of the emulsion. Absorbance of the mixture was read at 470 nm from t = 0 min until t = 120 min, with 20 min interval. BCB rate (R) and inhibition activity (%) were calculated:

$$R = \ln\left(\frac{A_0}{A_t}\right) \times \frac{1}{t}$$

Where, ln indicates the natural logarithm, Ao indicates the absorbance at 0 min and A_t represents absorbance reading at 20, 40, 60, 80, 100, and 120 min; t represents the time (min) afore-mentioned. The inhibition rate of β-carotene was further calculated as the percentage of inhibition activity based on the following equation:

$$\text{Inhibition activity (\%)} = \frac{(R_{control} - R_{sample})}{R_{control}} \times 100$$

Where, $R_{control}$ and R_{sample} are the bleaching rates of β-carotene in the emulsion without antioxidant and with sample extract, respectively.

Statistical analysis

All analyses were conducted in triplicate and expressed as mean ± standard deviation. The data were analysed using SPSS 18.0 and subjected to one-way ANOVA or T-test. Tukey's test was used to determine the significant differences among means at $p < 0.05$.

Results and Discussion

Yield of antioxidants and phenolics from natural sources was strongly associated with various extraction parameters, such as solvent concentration, extraction time and temperature (Wang *et al.*, 2008). Thus, initial part of this experiment was to identify optimised conditions for these three extraction parameters. For each extraction parameter, the extracts were subjected to three assays, which were DPPH assay, TPC assay and BCB assay, as markers to identify the optimal condition for the extraction parameter, and DPPH assay was the main marker, since it is highly reproducible, accurate and easy to perform (Thaipong *et al*., 2006). Consequently, the optimal conditions were selected based on DPPH assay.

Effects of ethanol concentration on TPC and AA

Mean values of TPC, EC_{50} (DPPH assay), and %IA (BCB assay) in sapodilla pulp and peel were significantly different ($p < 0.05$) when different percentages of ethanol were used (Figure 1), except

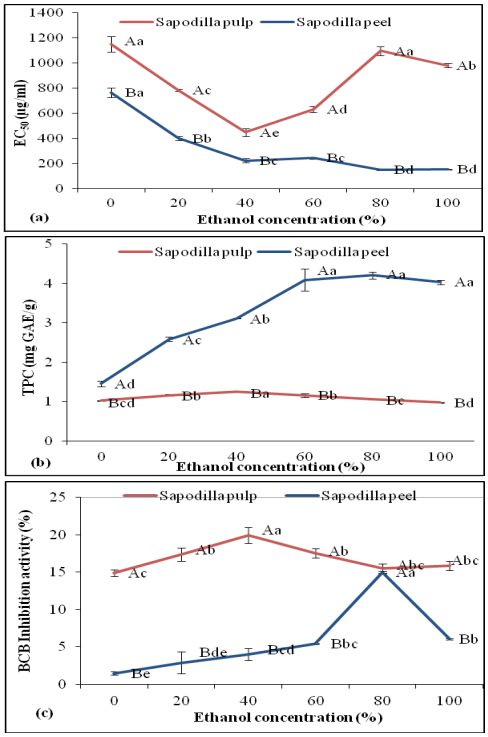

Figure 1. Effect of ethanol concentration on (a) EC_{50}, (b) TPC, and (c) IA%. Similar upper case letters (between sapodilla pulp and peel); and similar lower case letter (sapodilla pulp and peel, respectively) indicates no significant difference ($p \geq 0.05$).

for %IA at 80% ethanol. Results showed that among the different percentages of ethanol used to extract antioxidants from sapodilla pulp, 40% ethanol yielded the lowest EC_{50} value (446.45 ± 30.78 µg/ml), with the highest levels of TPC (1.26 ± 0.01 mg GAE/g of lyophilised extract) and %IA (19.95 ± 1.09%). Similarly, sapodilla peel that was extracted by 80% ethanol showed the lowest EC_{50} (146.85 ± 1.01 µg/ml), with the highest levels of TPC (4.21 ± 0.08 mg GAE/g of lyophilised extract) and %IA (14.99 ± 0.15%).

The finding of 40% ethanol as the optimal percentage solvent for extraction of sapodilla pulp suggested that most of the antioxidants yielded are semi-polar. It is in agreement with the study on mengkudu (*Morinda citrifolia*) that was extracted using 40% ethanol and showed the highest percentage of DPPH scavenging activity (Thaipong *et al.*, 2006). Besides, the use of 40 - 50% ethanol for extraction of polyphenols in longan pericarp was proven to be more effective than 100% ethanol (Prasad *et al.*, 2009). However, a maximum level of total phenolics in black currant was yielded from RSM optimization using 60% ethanol as extraction solvent (Cacace and Mazza, 2003). For sapodilla peel, similar finding was reported for extraction of dried sage using 80% ethanol, which resulted in highest TPC (Durling *et al.*, 2007).

Sapodilla pulp and peel that were extracted using water (0% ethanol) showed relatively low TPC and AA. Similar observation was found by Lapornik *et al.* (2005) that TPC in alcoholic extracts of red and black currants as well as grape were higher than the water extracts. This might be due to the extraction using 0% ethanol releases has led to the present of water soluble impurities such as polysaccharides that could reduce the AA in the extracts. Besides, sapodilla pulp that was extracted with 100% ethanol had relative low TPC and AA. Therefore it can be concluded that binary solvent is more effective than a single solvent in extraction of antioxidants. Pure ethanol is not able to extract most of the water soluble polyphenols in fruit, and might denature or destroy the polyphenols (Thoo *et al.*, 2010).

Extracted using different percentages of ethanol, sapodilla peel had TPC higher than the pulp. It is in agreement with Wijngaard *et al.* (2009) that grapefruit peel consists of higher amounts of phenolic compounds than the pulp. The high content of phenolic compounds for example naringenin in fruit peel is due to the protective effect of the peel to its fruit against extreme environment stress (Wu *et al.*, 2007). Fruit peel also rich in other bioactive compounds, which may act as effective antioxidants. Sapodilla peel had the lowest EC_{50} value as compared to its pulp, which corresponded to the highest level of scavenging effect, measured by DPPH assay. Inversely, sapodilla pulp that was extracted using different percentages of ethanol had %IA higher than its peel. It may due to BCB assay involved in different antioxidant pathway besides DPPH scavenging assay. Basically, DPPH and TPC assays are based on single-electron transfer mechanism, while BCB assay is based of hydrogen-atom transfer mechanism (Huang *et al.*, 2005).

Effects of extraction time on TPC and AA

Mean values of TPC, EC_{50} (DPPH assay), and IA% (BCB assay) for different extraction times applied were significantly different ($p < 0.05$) between sapodilla pulp and peel (Figure 2). The results show that antioxidants that extracted from sapodilla pulp with 4 h extraction time possessed the strongest scavenging effect (lowest EC_{50}, 280.08 ± 8.23 µg/ml) and IA% (16.98 ± 2.60%), while TPC of sapodilla pulp was highest at 3 h extraction (1.46 ± 0.03 mg GAE/g extract). However, significant difference was not found for the TPC of sapodilla pulp between 3 h and 4 h extraction. Sapodilla peel extracted based on 2 h extraction time had the highest TPC (10.70 ± 0.20 mg GAE/g extract), scavenging effect (lowest EC_{50},

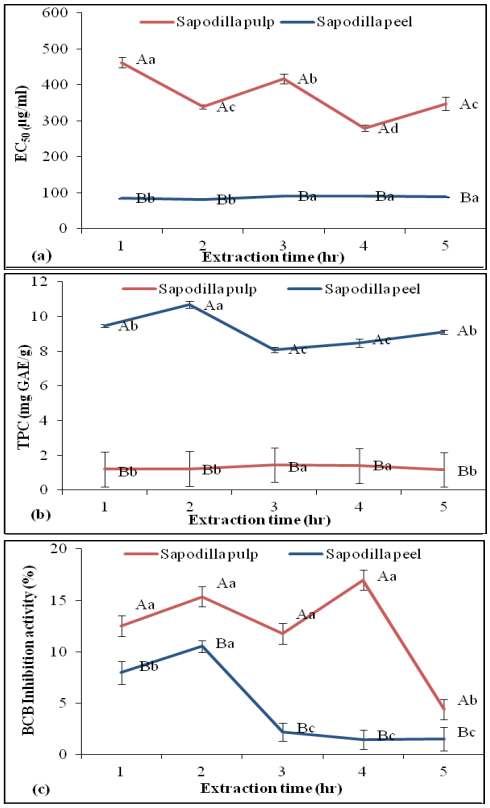

Figure 2. Effect of extraction time on (a) EC_{50}, (b) TPC, and (c) IA%. Similar upper case letters (between sapodilla pulp and peel); and similar lower case letter (sapodilla pulp and peel, respectively) indicates no significant difference ($p \geq 0.05$).

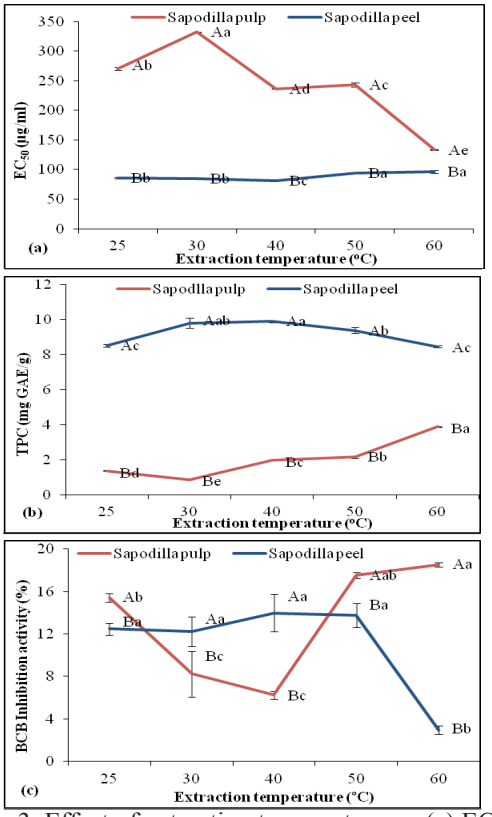

Figure 3. Effect of extraction temperature on (a) EC_{50}, (b) TPC, and (c) IA%. Similar upper case letters (between sapodilla pulp and peel); and similar lower case letter (sapodilla pulp and peel, respectively) indicates no significant difference ($p \geq 0.05$).

81.73 ± 2.31 µg/ml) and IA% (10.52 ± 0.69 %).

TPC and scavenging activity (DPPH assay) of sapodilla peel that extracted based on different extraction times were higher than its pulp. A study by Sousa *et al.* (2007) found the wastes of Irish fruits and vegetables (core, peel and seed) possessed stronger scavenging activity than the edible flesh. Inversely, the percentages of inhibition for sapodilla pulp that extracted at different extraction times were higher than its peel. High level of sugars the fruit pulp might inhibit the bleaching of β-carotene, thus resulted in inaccuracy of the data obtained. No increasing or decreasing trend was found for the TPC and AA of sapodilla pulp and peel that extracted based on different extraction times. Further increment in extraction time does not yield more antioxidant. Besides, prolonged extraction (> 2 h) led to degradation of antioxidants, which resulted in lower scavenging activity.

The used of the best extraction time for extraction of phenolic compounds in fruit has been studied previously (Ng *et al.*, 2012). The time needed to extract phenolic compounds from fruits is depending on the type and matrices of the fruits. It is related to various degree of binding of antioxidants to the fruits' matrices. Prolonged extraction may allow extend exposure of antioxidants to light and oxygen, which lead to rapid degradation of the antioxidants.

Effects of extraction temperature on TPC and AA

Mean values of TPC, EC_{50} (DPPH assay), and IA% (BCB assay) for different extraction temperature chosen were significantly different ($p < 0.05$) between sapodilla pulp and peel (Figure 3). The result showed that TPC in sapodilla peel that extracted based on different extraction temperatures were significantly higher than the pulp. The best extraction temperatures determined for sapodilla pulp and peel were 60°C and 40°C, respectively.

For sapodilla pulp, increment of extraction temperatures from 30°C to 60°C showed an increase in TPC. This is due to weaken of viscosity and surface of plant cell wall as extraction temperature increases, resulted in solvent easily reach the sample matrices (Liu, 2003). Similar observation was found for apple pomace that increased in extraction temperature from has resulted a gradual increased in total phenolic content extracted (Çam and Aaby, 2010). Besides, sapodilla pulp that extracted at room temperature (25–30°C) had the lowest TPC. This might be due to low extraction temperature is not able give enough kinetic energy for the solvent to free the bonded-phenolics and further activate the enzymes.

Increase in extraction temperature may somehow

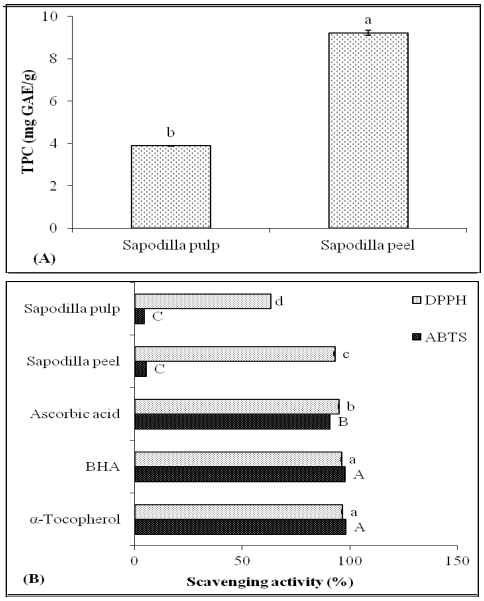

Figure 4. Effect of optimised extraction conditions on (a) TPC and (b) DPPH & ABTS. Similar upper/lower case letter indicates no significant difference ($p \geq 0.05$).

enhance solubility of antioxidants in the aqueous solvent. At higher temperature, oxidation of certain thermal sensitive antioxidants in the sample could be happened (Dai and Mumper, 2010). As observed from the data obtained, antioxidants in sapodilla peel were mainly thermal sensitive as higher extraction temperature used shown a decrease in TPC. As compared between pulp and peel, the result obtained is supported by Prakash *et al.* (2011) that the peels of *Citrus sinensis* and *Mangifera indica* had significantly higher level of total phenolics than the pulps.

In this study, we have observed that increasing in extraction temperature have reduced in both TPC and AA of the sapodilla pulp and peel. Although the increment of extraction temperature had increased the TPC extracted, a reduction of AA was observed for the sapodilla peel after 40°C. The used of 40°C to 60°C of extraction temperature showed a gradual reduction in TPC, thus decreased in AA for both DPPH and BCB assays. Based on this observation, the antioxidants in sapodilla peel are heat sensitive. Besides, Ku and Mun (2008) showed that at longer extraction time, increased in extraction temperature had a gradual degradation of total anthocyanins.

TPC and AA determined based on optimal extraction conditions

Results showed that TPC of sapodilla peel (9.23 ± 0.13 mg GAE/g extract) was significantly higher than its pulp (3.89 ± 0.01 mg GAE/g extract) (Figure 4A). Different extract concentrations used have shown a high linearity for TPC with $R^2 > 0.99$. Analysed using Folin-Ciocalteu reagent assay, TPC estimated in most of the fruit pulp were significantly lower than the peel (Arazo *et al*., 2011). As compared to other Malaysian fruits, the TPC determined in the sapodilla fruit were relatively low (Ikram et al., 2009). Fruits' maturity is one of the factors that have caused variation in TPC for Malaysian fruits. TPC in fruit has also been known to be decreased upon ripening due to polyphenol oxidase activity (Ma *et al*., 2006). Besides, different geographical origin, fruit's cultivar, and climates are the other factors that have contributed to variation in TPC of fruit sample.

The percentages of scavenging activity (DPPH assay) for sapodilla pulp (63.20 ± 0.06%) and peel (92.95 ± 0.33%) were significantly lower than the commercial antioxidants (94.8 - 96.4%). SA% (ABTS assay) for sapodilla pulp (4.30 ± 0.16%) and peel (5.36 ± 0.66%) were extremely lower than the commercial antioxidants tested (90.7 - 97.9%) (Figure 4B). Besides, Trolox equivalent (TE) values of FRAP assay for sapodilla pulp (15.24 ± 0.19 mg TE/g extract) and sapodilla peel (27.85 ± 0.29 mg TE/g extract) were extremely lower than the commercial antioxidants tested (143.6 - 363.2 mg TE/g) (Figure 5A).

IA% for sapodilla pulp (8.14 ± 1.16%) and peel (19.17 ± 0.64%) were relatively lower as compared to commercial antioxidants (Figure 5B). The IA% of sapodilla pulp is seemed comparable to ascorbic acid. However, BHA and α-tocopherol had IA% (> 90%) far higher than the IA% of sapodilla pulp and peel. It may due to the antioxidants extracted from sapodilla fruit are mainly hydrophilic. It is in agreement with Ma *et al.* (2006) that sapodilla fruit has high levels of ascorbic acid, water soluble tannins, catechin and epicatechin. Besides, sapodilla fruit has relatively low levels of lipophilic antioxidants, such as β-carotene and vitamin E (Charoensiri *et al*., 2008).

Although the SA% and TE of sapodilla pulp and peel were low, the extracts are still potential sources of antioxidants. As the SA% for DPPH assay are high for both sapodilla pulp and peel, the low antioxidant activities assessed using ABTS and FRAP assay may not show that the sapodilla pulp and peel are not potent antioxidants. As the mechanism of reaction for DPPH assay is different from FRAP and ABTS assays (Huang *et al*., 2005), low antioxidant activity for the samples is not questionable. Sapodilla pulp and peel also potential sources of functional food as they are natural antioxidants and their safety level is not an issue. The extracts of sapodilla pulp and peel can be used as natural reducing agents too. Based on the result obtained from DPPH assay, the scavenging activity of sapodilla peel was comparable to the commercial antioxidants. Therefore sapodilla peel is potential source of antioxidant with strong scavenging

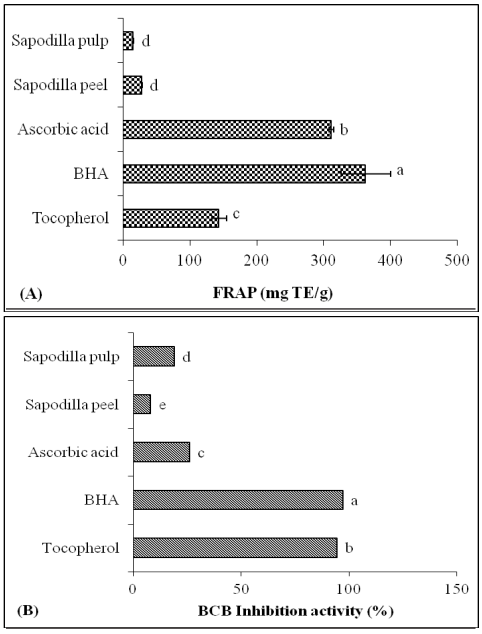

Figure 5. Effect of optimised extraction conditions on (a) FRAP and (b) BCB (IA%). Similar upper/lower case letter indicates no significant difference ($p \geq 0.05$).

activity. Its extract can be used to replace BHA in food industry in the near future since the maximum permitted level of BHA in food is 0.02%.

Conclusions

The optimal extraction conditions for sapodilla pulp and peel were 4 h extraction time at 60°C using 40% ethanol and 2 h extraction at 40°C using 80% ethanol, respectively. Sapodilla peel that contained high phenolic compounds requires higher percentage of ethanol for extraction as compared to the pulp. The optimized extraction conditions for Sapodilla peel have yielded TPC and AA higher than the pulp, except for IA%. In conclusion, sapodilla pulp and peel have the potential as natural antioxidant sources for food industry applications.

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