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INFLUENCE OF DIFFERENT EXTRACTION CONDITIONS ON ANTIOXIDANT PROPERTIES OF SOURSOP PEEL

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ABSTRACT

Background. Soursop is a healthy fruit. Peels form about 20% of the soursop fruit and are usually discarded as waste product. With a view to utilizing soursop peel as a source of valuable compounds, this study aimed to investigate the influence of different extraction conditions on total phenolic content (TPC) and antioxidant capacity (AC) of soursop (*Annona muricata* L.) peel.

Material and methods. Different ethanol concentrations (20–100%, *v*/*v*), extraction temperatures (25–60°C), and extraction time (1–5 h) were tested. Extracts were prepared on the basis of the best optimal extraction conditions (20% ethanol, 40°C the extraction temperature, and 4 h of extraction time), an optimal TPC and AC was determined for the soursop peel using DPPH, ABTS, FRAP and β-carotene bleaching (BCB) assays. The different extraction conditions tested at best optimum conditions have significantly affected the TPC and AC of the soursop peel.

Results. Soursop peel extract extracted in the best optimal extraction conditions had moderate levels of TPC (52.2 μg GAE/ml), and FRAP value (58.9 μg TE/ml extract). The extract demonstrated high BCB inhibitory activity (80.08%). The EC_{50} values of the extract were high, 1179.96 and 145.12 μg/ml, as assessed using DPPH and ABTS assays, respectively. The TPC was positively and highly correlated with the AC of soursop peel assessed by ABTS, FRAP, and BCB assay, but it was moderately correlated with DPPH radical scavenging activity. A moderate correlation of TPC with DPPH suggested that polyphenols in the extracts were partially responsible for the AC.

Conclusions. By-products of soursop such as its peel could be an inexpensive source of good natural antioxidants with nutraceutical potential in the functional food industry.

Key words: antioxidant capacity, *Annona muricata*, extraction parameters, soursop peel, total phenolic content, waste products

INTRODUCTION

Consumption of fruits and vegetables is one of the dietary recommendations that helps to maintain human health (Halliwell, 2012). This is because in the human diet, fruits and vegetables in particular provide a wide range of plant-derived phenolic compounds with antioxidant activity, which helps our human body to cope with oxidative stress (Hertog et al., 1993). Soursop (*Annona muricata* L.) is native to tropical

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America and belongs to the Annonaceae family. There are about 100 species of shrubs in the family of Annonaceae, and soursop is one of the tropical fruits. Generally, soursop is produced in an irregular shape or sometimes in an oval or heart shape. The fruit has white pulpy flesh, shiny dark brown seeds, and dark-green skin with a thorny and reticulated leathery look. Each fruit weighs around 0.5 to 4 kg and the soursop fruit ranges around 15–23 cm in diameter. In trerms of its composition, the soursop fruit consists of 67.5% edible pulp, 20% peel, 8.5% seeds and 4% core by weight (Ayit, 2009).

Various studies have found that soursop is highly nutritious and contains high levels of vitamins (especially vitamin C) and minerals, as well as being low in fat (Enweani et al., 2004; Onimawo, 2002). The epicarp, mesocarp and juice of soursop contain potassium, sodium, iron, magnesium, calcium, chloride and bicarbonate (Dembitsky et al., 2011). The peel of the soursop is known to have antileishmanial activity (Jaramillo et al., 2000). Other studies also reported that the seeds of the soursop demonstrate strong anticancer and anti-tumor properties (Li et al., 2001; Liaw et al., 2002). Due to the higher nutritional values of soursop peel, this peel should be investigated for its antioxidant properties rather than the seeds. As a by-product of the soursop juice industry, utilization of soursop peel as a potential source of nutraceutical ingredients will help to reduce waste material from the industry for nutritional and functional purposes.

Extraction conditions such as solvent type, solvent concentration, extraction temperature, extraction time and solid-solvent ratio are the major concerns with regard to enhancing the efficiency in order to obtain the highest yield of antioxidative compounds from natural resources (Pinelo et al., 2005; Prior et al., 2005; Spigno et al., 2007). So far, no specific or appropriate extraction solvent is recommended for optimal recovery of phenolic compounds for most of the plants tested previously. This is due to the diverse chemical structures of phenolic compounds, which might affect their solubility in the solvent used (Prior et al., 2005). Studies determining the optimized extraction conditions using various plant extracts, such as *Inga edulis* (Silva et al., 2007), *Morinda citrifolia* (Thoo et al., 2010), flaxseed (Anwar and Przybylski, 2012), passion fruit peel (Wong et al., 2014) and banana peel (Toh et al., 2016) have increased considerably in number in recent years. To the best of our knowledge, studies on optimum extraction conditions of antioxidants from natural sources are still scarce. Hence, the purpose of this study is to investigate the influence of different extraction parameters (solvent concentration, extraction temperature, and extraction time) on the total phenolic content and antioxidant capacity (AC) of soursop peel. The selection of the solvent and concentrations applied in this study was based on the reported efficiency in extracting phenolic compounds and other antioxidant components by previous studies (Toh et al., 2016; Wong et al., 2014; Yim et al., 2009).

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents were of analytical grade. Ethanol, hexane, gallic acid, linoleic acid, Trolox, Tween 40, sodium carbonate anhydrous, chloroform, iron(III) chloride anhydrous, and potassium persulfate were purchased from Fisher Scientific Co. (Fisher Scientific, Loughborough, UK). Acetone, methanol, Folin-Ciocalteu's reagent, and 2,2'-azino-di[3-ethyl-benzthiazoline sulfonate] (ABTS) were from Merck KGaA (Lichrosolv, Darmstadt, Germany), while 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, butylated hydroxy anisole (BHA), acetic acid, and sodium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Distilled water was obtained from a Milli-Q water purification unit (Millipore, Milford, MA, USA).

Sample preparation and extraction

Ten whole fruits of soursop (*Annona muricata* L.) with yellowish-green colored peel (slightly matured) ranging from 0.5–2.0 kg were randomly selected from an orchard in Pahang, Malaysia. The fruit was registered with the Department of Agriculture, Malaysia (No. DB1: *Durian Belanda*). The fruits were washed and cut into few pieces, and the peel separated from the flesh. This peel was cut into uniform pieces of 1 cm^3 and oven-dried using a hot air oven (UFB 500, Memmert, Schwabach, Germany) at 45°C for 24 h until a constant weight was obtained. The soursop peel powder (5 g) was extracted with 50 ml ethanol (100%, v/v) and subjected to agitation using a shaking

incubator at 150 rpm at room temperature (25°C) for an hour. The residues were collected, re-extracted with ethanol and centrifuged at 4500 rpm for 15 min using a benchtop centrifuge Mikro 200 (Hettich, Tuttlingen, Germany). The supernatant was concentrated using a rotary evaporator (BUCHI, Switzerland) at 40°C and freeze-dried as soursop peel powder. The powder was stored in –20°C for further analysis. Triplicate extractions were performed for each extraction conditions.

Experimental design

A single-factor experiment was used to determine the optimum extraction conditions for soursop peel. The influence of extraction parameters, namely percentages of ethanol, extraction times, and extraction temperatures were studied (Toh et al., 2016; Wong et al., 2014). Different ethanol concentrations (0, 20, 40, 60, 80 and 100%, v/v) were first applied with other variables and remained constant at room temperature (25°C) for 60 min. Then different extraction times of 60, 120, 180, 240, and 300 min at room temperature were applied using the best ethanol concentration determined. Finally, extraction was carried out based on different extraction temperatures (25, 30, 40, 50 and 60°C) applying the best ethanol concentration and extraction time. The best ethanol concentration, extraction time and extraction temperature were determined based on the highest total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity obtained from soursop peel. Based on the screening tests, 500 μg/ml extract yielded the highest DPPH radical scavenging activity compared to other extract concentrations (100, 200, and 300 μg/ml). Therefore, 500 μg/ml of the ethanolic extract was applied in this study.

Total phenolic content

Total phenolic content was measured spectrophotometrically based on a modified method (Thaipong et al., 2006). The ethanolic extract (1 ml) was mixed with 1.0 ml of Folin-Ciocalteu reagent (FCR) (diluted 10×) and agitated using a vortex mixer (BPECO, Germany) for a few seconds. The mixture was left to stand for 3 min in the dark. Then, 1.0 ml of sodium carbonate (7.5 g/100 ml) solution was added to the mixture and topped up to 10 ml with distilled water. The absorbance was measured at 725 nm using a UV–VIS spectrophotometer (PRIM, Secomam, France) against a blank. The calibration equation of the gallic acid standard curve was $y = 0.0106\ x + 0.0066$ ($R^2 = 0.9993$). Total phenolic content was expressed as mg gallic acid equivalents (GAE) per ml extract in triplicate independent analyses.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the extracts was measured based on the method by Xu and Chang (2007) with slight modifications. First, the ethanolic extract (1 ml) was added to 500 μl of ethanolic DPPH solution (final concentration of 0.2 mM) in a test tube, and the mixture was shaken vigorously using the vortex mixer, and then kept in the dark for 30 min. After incubation, absorbance of the mixture was measured at 517 nm using a UV–VIS spectrophotometer (PRIM, Secomam, France) against an ethanol blank. Distilled water was used as a negative control. Butylated hydroxy anisole (BHA) and α-tocopherol were used for comparative purposes. The AC of the extract was calculated based on the following equation:

$$\text{DPPH radical scavenging ability, \%} = \left(1 - \frac{Abs_{\text{sample}}}{Abs_{\text{control}}}\right) \times 100 \quad (1)$$

The calibration for the percentage of scavenging activity was plotted against a logarithm (Log) of extract concentration to calculate EC_{50} that defined as the concentration of the extract to reduce the initial DPPH concentration by 50%, where EC_{50} was obtained from a linear regression equation.

b-Carotene linoleate bleaching assay

A β-carotene bleaching (BCB) assay was conducted using the method developed by Nsimba et al. (2008) with slight modifications. To prepare the working reagent, 0.2 mg of β-carotene was dissolved in 1 ml of chloroform, and then mixed with 0.02 ml of linoleic acid and 0.2 ml of Tween 40. Then, 1 ml of β-carotene solution was transferred into a round-bottomed flask, where the chloroform was removed under a vacuum at 40°C. The remaining solution was then diluted with 50 ml of oxygenated water, forming an emulsion mixture. The assay was initiated by adding 4.0 ml of the emulsion mixture to 0.5 ml of the sample ethanolic extract. The mixture was shaken vigorously until liposomes were formed. The mixture was immediately

placed into a 50°C water bath (WB/OB 7–45, Germany) for 2 h after absorbance at 470 nm was taken at 0 min. BHA and α-tocopherol were used for comparative purposes. The absorbance of the reacting mixture was taken at 20 min interval until 120 min of incubation. The BCB rate of the sample was calculated based on the following equation:

$$\text{β-carotene bleaching rate, R} = = \frac{Abs_{t=0} - Abs_{t=120\ \text{min}}}{120} \quad (2)$$

where:

ln – the natural log,

$t = 0$, $t = 120$ – the initial absorbance at time 0 and at 120 min.

Antioxidant capacity (AC) was calculated as a percentage of inhibition relative to control using the equation below:

$$\text{Percentage of inhibition, \%} = = \frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}} \times 100 \quad (3)$$

where:

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

Ferric-reducing antioxidant power (FRAP) assay

A FRAP assay was determined based on the reduction of Fe^{3+}-TPTZ to a blue colored Fe^{2+}-TPTZ according to Thaipong et al.'s method (2006) with slight modifications. The FRAP reagent was prepared freshly by adding 10 mM 2,4,6-tri(2 pyridyl)-s-triazine (TPTZ) in 40 mM HCl with 20 mM ferric trichloridehexahydrate ($FeCl_3 \cdot 6H_2O$) and 0.3 M acetate buffer (pH 3.6) at a ratio of 10:1:1 (*v*/*v*/*v*). The straw-colored solution was kept in a water bath at 37°C. Subsequently, the freshly warmed FRAP reagent (1.5 ml) was added with 50 µl ethanolic extract, and the mixture was shaken and incubated for 4 min. Absorbance was determined at 593 nm relative to the FRAP reagent blank. BHA and α-tocopherol were used for comparative purposes. The result was calculated using the equation obtained from a calibration curve plotted against various Trolox concentrations (10–400 $\mu g \cdot ml^{-1}$), and expressed as Trolox equivalent antioxidant capacity (TEAC, $\mu g \cdot ml^{-1}$) with the calibration equation of $y = 0.0057x - 0.0214$ ($R^2 = 0.9984$).

ABTS radical inhibition activity

ABTS radical inhibition activity was performed as described by Vasco et al. (2008) with some modifications. First, 5 ml of 7 mM ABTS mixture was kept in the dark for 16–18 h at room temperature to allow the complete generation of ABTS radical cation ($ABTS^{\cdot+}$) before use. The ABTS stock solution (1 ml) was diluted with 70 ml of ethanol in order to obtain an absorbance of 0.70 ±0.05 measured using a spectrophotometer at the wavelength of 734 nm. An aliquot of 100 µL ethanolic extract was added to 1 ml of ABTS reagent and mixed thoroughly. Absorbance of the reaction mixture was then measured at 734 nm against an ethanol blank after 6 min. BHA and α-tocopherol were used for comparative purposes. The inhibition activity was calculated based on the following equation:

$$\text{ABTS radical inhibition ability, \%} = = \left(1 - \frac{Abs_{\text{sample}}}{Abs_{\text{control}}}\right) \times 100 \quad (4)$$

Statistical analysis

All data were expressed as mean ±standard deviation in triplicate independent analyses, and were analyzed using SPSS statistical software version 21.0 (SPSS Inc., Chicago, Illinois, USA), where the results were expressed as mean ±standard deviation. One-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test was used for comparing the experimental means. Pearson correlation was used to determine the correlation between TPC and AC (DPPH, FRAP, BCB, and ABTS). The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Influence of ethanol concentrations on TPC and AC

Figure 1 demonstrates the effect of different solvent concentrations on the TPC and AC determined by DPPH assay. The use of different percentages of ethanol demonstrated significant effects ($p < 0.05$) on both TPC and AC. The TPC increased with increasing percentages of ethanol until 80% ethanol, and the TPC was significantly reduced when applying 100% ethanol ($p < 0.05$) (Fig. 1A). The highest AC was obtained using 100% ethanol, while 0% ethanol yielded the lowest AC (Fig. 1B). The results indicated that

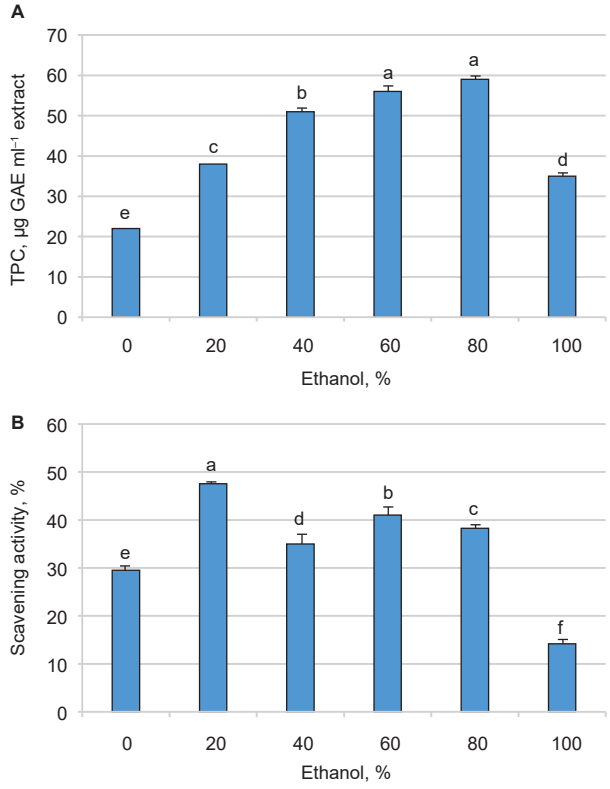

Fig. 1. Effect of different percentages of ethanol on (A) TPC and (B) AC (DPPH assay) of soursop peel extract. The values were presented as mean ±standard deviation of triplicate analyses. Different lower case letters (a–f) denote that they are significantly different ($p < 0.05$)

higher amount of TPC were obtained with increasing solvent polarity and the use of 80% ethanol showed the highest TPC. A previous study also demonstrated that 80% ethanol was the most effective solvent for the extraction of phenolic compounds as compared to 100% ethanol (Yoo et al., 2008).

The result was consistent with the findings reported by some studies that the binary solvent system, particularly aqueous alcohol, was more effective than the use of alcohol alone for extraction of antioxidants (Spigno et al., 2007; Thoo et al., 2010; Wong et al., 2014). The antioxidants extracted from soursop peel were somehow non-polar. It is because the highest TPC was obtained from 80% ethanol. Although a high TPC was obtained, the AC was low (Fig. 1B). The use of 20% ethanol gave the highest AC, but the TPC was lower compared to the 80% ethanol used. Thoo et al. (2010) also reported that antioxidant activity depended on the synergistic effects of the compounds present. Moreover, no single type of solvent was able to recover all the phenolic compounds from a particular sample. Solvents with intermediate polarity are often used for extraction compared with a non-polar or highly polar solvent. Ethanol as one of the solvents with intermediate polarity has been used for extraction of phenolic compounds such as flavanoids, catechols, and tannins from plants materials (Naczk and Shahidi, 2004; Shahidi and Ambigaipalan, 2015; Spigno et al., 2007). For example, ethanol has been used for the extraction of phenolic compounds in mango peels (Kim et al., 2010) and cocoa beans (Othman et al., 2007). This is because ethanolic solvent helped to maximize and enhance the interaction of DPPH radicals with the antioxidants present in the sample. Moreover, ethanol is also a more polar solvent which tend to highly solubilize hydroxylated aglycone forms of phenolic compounds (Arts and Hollman, 1998). By considering the moderate TPC and the highest AC obtained, 20% ethanol was chosen as the best extraction solvent for subsequent extraction of antioxidants using different extraction time and temperatures.

Influence of extraction time on TPC and AC

As shown in Figure 2, the extraction time significantly affected ($p < 0.05$) the TPC and AC of the soursop peel extract. The TPC increased from 1 h to 2 h of extraction time, then reduced when extraction time was increased. (Fig. 2A). The trend observed might be explained by Fick's second law of diffusion (Silva et al., 2007), where the final equilibrium among solute concentrations in the solid matrix and in the bulk solution was estimated to be achieved after a period of time. However, TPC dropped at 5 h extraction and could be due to the prolonged extraction time that leads to oxidation of phenolic compounds (Naczk and Shahidi, 2004), as well as the degradation of antioxidants by enzymatic reactions in plant tissue (Kuljarachanan et al., 2009; Toh et al., 2016). Conversely, the highest DPPH radical scavenging activity was obtained for the 4 h extraction time, while the lowest scavenging activity was obtained for the 5 h extraction time (Fig. 2B). This could be due to DPPH radical scavenging activity, which was not dependent only on the phenolic compounds extracted from the fruit peel, but on

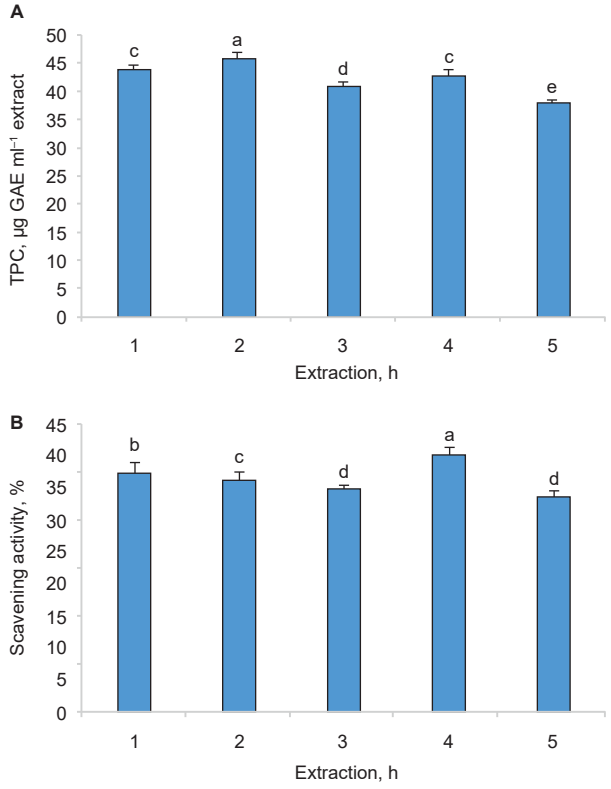

Fig. 2. Effect of different extraction time on (A) TPC and (B) AC (DPPH assay) of soursop peel extract. The values were presented as mean ±standard deviation of triplicate analyses. Different lower case letters (a–e) denote that they are significantly different ($p < 0.05$)

a combination of various antioxidants extracted during the prolonged extraction time (Prior et al., 2005).

A longer extraction time demonstrated little difference or was even not helpful in increasing the level of total phenolic as compared to a shorter extraction time. The use of a longer extraction time (4 h) to extract phenolic compounds may be due to the varying degrees of phenolic polymerization, solubility of the phenolic compounds, and interaction between phenolic compounds and the sample matrix (Silva et al., 2007; Wong et al., 2014). The final equilibrium of diffusion between solvent and solid was also attained at an optimal extraction time. After considering the cost for extraction and its practicality based on the optimal level of the phenolic compounds extracted and AC, 4 h was selected as the best extraction time for this study.

Influence of extraction temperature on TPC and AC

TPC and AC of the soursop peel extracted using different extraction temperatures are shown in Figure 3. The use of different extraction temperature has significantly affected ($p < 0.05$) the TPC, but no significant difference was found for the AC among the different extraction temperatures ($p > 0.05$). The increase in extraction temperature has yielded a linear increase in TPC (Fig. 3A). As reported by Shui and Leong (2006) and Yim et al. (2009), increasing the extraction temperature from 30°C to 75°C and from 25°C to 60°C respectively leads to an increase in TPC. The possible explanation for the results obtained could be due to the heat applied (due to the higher kinetic energy) causing the breakdown of cellular constituents, which

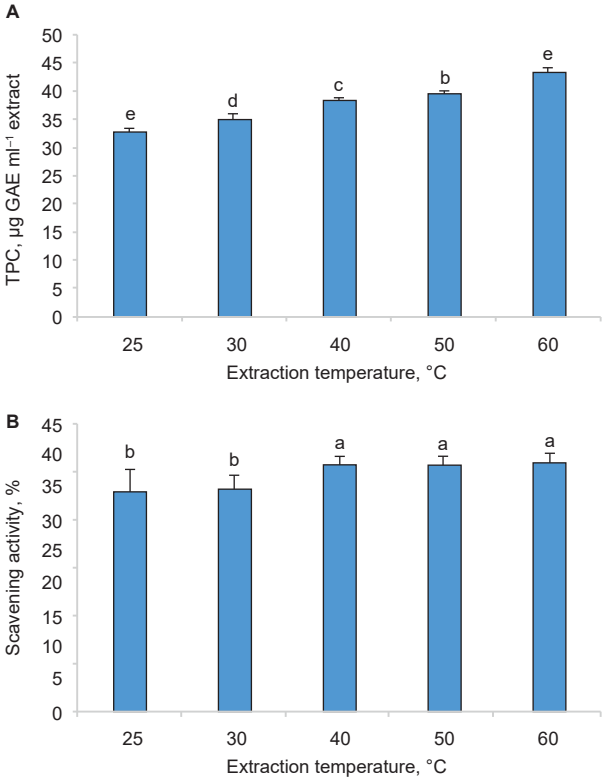

Fig. 3. Effect of different extraction temperatures on (A) TPC and (B) AC (DPPH assay) of soursop peel extract. The values were presented as mean ±standard deviation of triplicate analyses. Different lower case letters (a–e) denote that they are significantly different ($p < 0.05$)

subsequently enhance the release of bound phenolic compounds to the extraction solvent (Toor and Savage, 2006). It also increases the solubility of phenolic compounds in the extraction solvent (Tabart et al., 2007). The selected range of extraction temperatures (25–60°C) for this study was based on the results reported previously (Toh et al., 2016; Wong et al., 2014; Zhang et al., 2007), where the highest extraction temperature should not exceed the boiling point of the solvent used.

The highest DPPH scavenging activity was obtained at 60°C while the lowest activity was at 25°C (Fig. 3B). The results also showed that an increase in the extraction temperature from 40°C to 60°C did not reveal any significant difference in the scavenging activities. This could be due to the presence of thermosensitive phenolic compounds beyond 40°C. Moreover, certain phenolic compounds could be degraded at a high temperature, causing the loss of AC in the plant extract (Liyana-Pathirana and Shahidi, 2005). Since a high amount of thermosensitive vitamin C (20.6 mg/100 g dry weight) has been reported in soursop fruit (Ayit, 2009), the vitamin C content in the soursop peel might contribute to the overestimation of TPC as determined using Folin-Ciocalteu reagent. Taking into consideration the industrial point of view, where higher extraction temperatures increase solvent consumption and the cost of extraction (Toh et al., 2016; Wong et al., 2014), the selection of a moderately high extraction temperature (40°C) would be the best extraction temperature for extraction of antioxidants from soursop peel.

TPC and AC of soursop peel based on the best extraction condition

TPC and AC of the soursop peel extract were determined based on the best extraction conditions of 20% ethanol at an extraction temperature of 40°C for 4 h. The AC of the soursop peel extract was determined using DPPH, ABTS, FRAP and BCB assays. The TPC of the soursop peel extract was a 52.15 mg GAE/ml extract. As shown in Table 1, the soursop peel extract had high EC_{50} values for both of the DPPH and ABTS assays compared to the antioxidant standards. The results showed that the antioxidant compounds in the soursop peel extract were weak reducing agents. It might be due to the fact that the antioxidants of soursop peel did not reduce the DPPH and ABTS radical cations strongly.

The EC_{50} value of the soursop peel extract obtained from ABTS assay was 9 times lower than the EC_{50} value obtained from DPPH (Table 1). The actual reason is unknown. As both of the assays involve electron-transfer reaction pathways (Huang et al., 2005), the reducing effects of antioxidants extracted from the soursop peel should be similar for these two assays. Similarly, Zieliński and Kozłowska (2000) reported higher ABTS radical scavenging activity by different

Table 1. Total phenolic content (TPC) and antioxidant activities of soursop peel extracts at optimized extraction conditions (oven-dried, 20% ethanol, 40°C and 4 hr)

Sample	TPC (GAE) µg/ml	DPPH radical scavenging EC_{50} µg/ml	FRAP (TEAC) µg/ml	BCB (antioxidant activity) %	ABTS (inhibition activity) %
Soursop peel	52.2 ±0.4	1180 ±937.6	58.9 ±1.5^{c}	80.1 ±4.8^{b}	97.1 ±2.0^{a}
BHA	NA	<0.01	866.7 ±0.00^{a}	98.9 ±0.3^{a}	97.9 ±0.2^{a}
α-tocopherol	NA	<0.01	352.2 ±26.9^{b}	96.4 ±0.2^{a}	98.0 ±0.1^{a}

Each value was expressed as mean ±standard deviation of triplicate analyses.
NA – not applicable.
Different superscripts within the column (FRAP, BCB and FRAP) denote that they are significantly different ($p < 0.05$).
EC_{50} was calculated by interpolation of linear regression analysis (based on concentration-dependent result – data not shown). It was defined as an effective concentration that was able to scavenge 50% of the total DPPH radicals.

Table 2. Correlation between TPC and DPPH, FRAP, BCB and ABTS assays at optimized extraction conditions (oven-dried, 20% ethanol, 40°C and 4 hr)

TPC	DPPH	FRAP	BCB	ABTS
Soursop peel	0.69	0.99	0.85	0.93

All values were significant at $p < 0.01$.

hydrophilic cereal grains than lipophilic extracts (Gao et al., 2000). More importantly, the ABTS result obtained from this was higher than the DPPH scavenging activity. The FRAP value of the soursop peel extract was significantly lower ($p < 0.05$) compared to the antioxidant standards. Although the antioxidant activities of the soursop peel extract as assessed by DPPH, ABTS, and FRAP assays were low, these assays involved electron-transfer reaction pathways (Huang et al., 2005).

Conversely, the soursop peel extract had high BCB inhibitory activity (80.08%), where high BCB inhibitory activity was reported in a previous study (Hassimotto et al., 2005). Although the BCB inhibitory activity of the soursop peel extract was significantly lower ($p < 0.05$) than the antioxidant standards (Table 1), the high BCB inhibitory activity shows that soursop peel has a protective effect against oxidative stress. Due to the different reaction pathway involved in the BCB inhibitory assay (hydrogen atom transfer pathway) as compared to the other assays tested, there is a need to perform more than one type of measurement of antioxidant activity for plant extracts. This also takes into account the various mechanisms of antioxidant assay involved and the limitations of each method (Huang et al., 2005). Hence, different antioxidant methods might measure the different types of antioxidants present in soursop peel.

Pearson correlation analysis

The results showed a high correlation coefficient (r) between TPC and AC for all the antioxidant assays except for the DPPH assay. According to the Guildford rule of thumb, a value of 0.9 or higher was considered as a very high correlation. For FRAP ($r = 0.998$) and ABTS ($r = 0.928$) assays very high positive correlations were found between TPC and these assays, while the correlation between TPC and DPPH radical scavenging assay was considered as moderately positive ($r = 0.693$). A high correlation ($r = 0.849$) was also found between TPC and BCB assay. DPPH radical scavenging activity was moderately correlated with the TPC in the soursop peel extract and might be due to the poor interaction of the polar phenolic compounds in the soursop peel extracts with the DPPH powder that was dissolved in 100% ethanol. Most antioxidants such as ascorbic acid and phenolic acids in any plant extracts are poorly soluble in 100% ethanol, thus the reducing activity of DPPH radical cation is disrupted. Moreover, DPPH assay has been demonstrated to work well with lipophilic antioxidants (Xie and Schaich, 2014). The antioxidant compounds from soursop peel could be hydrophilic in nature. The high correlations found for ABTS and FRAP assays could also be due to the good association of the polar antioxidants in the soursop peel extracted with 20% ethanol with the aqueous solution of ABTS and FRAP reagents that accelerate the reducing ability of the antioxidants.

Furthermore, high ACs of passion fruit peel have been reported to be highly correlated with their phenolic content (Wong et al., 2014). Thaipong et al. (2006) also reported that the TPC and antioxidant activity of guava extract were highly correlated ($r = 0.97$) between TPC and FRAP. A very high correlation ($r = 0.99$) was also demonstrated by another study (Ruan et al., 2008). A moderate correlation ($r = 0.56$) between TPC and DPPH radical scavenging activity was also found for guava leaf extract (Tachaktitirungrod et al., 2007). Due to the poor specificity of the Folin-Ciocalteu reagent assay, the reagent was unable to react selectively with phenolic compounds (Prior et al., 2005), and therefore gave rise to a lower correlation between TPC and AC. In fact, AC is not only contributed by phenolic compounds, other compounds such as ascorbic acid, tocopherols, carotenoids, reducing sugars, and terpenes, as well as the synergistic effects among the antioxidant compounds could also contribute to the total AC of a particular food sample (Shahidi and Ambigaipalan, 2015). The very high correlations between TPC and ACs (BCB, ABTS and FRAP) were not surprising due to the similarity of the redox reactions between these assays (Huang et al., 2005). Hence, further work is also required for the isolation and identification of individual phenolic compounds present in these soursop peels to identify the

bioactive compounds responsible for the AC obtained. Other unknown compounds in soursop peel might have contributed to the AC, thus more studies are required in order to determine the relationship between these unknown and their ACs.

CONCLUSION

The best extraction condition selected for extraction of antioxidants in soursop peel was 20% ethanol at 40°C for 4 h. The best extraction condition yielded optimal levels of TPC and AC. Applying different extraction parameters significantly influenced the TPC and AC of soursop peel. Based on the best extraction conditions, the soursop peel extract showed notable levels of TPC and AC that were assessed by DPPH, ABTS and FRAP assays. The high BCB inhibitory activity also demonstrates the protective effect of the soursop peel extract. Soursop peel, as a by-product of soursop juice industry, could be used as an inexpensive source for nutraceutical ingredients. Due to the nutraceutical potential of soursop peel, a further characterization of antioxidants present in the peel is suggested.

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