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Quantification of Total Phenolic compounds in Papaya fruit peel

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ABSTRACT

Phenolics are widely distributed in many plants and are known to play a major role in the plant and animal kingdom. Phenolics exhibit strong antioxidant properties and have been used as antitumor, anticarcinogenic, antiviral and hypotensive agents. Plant by-products contain a variety of these phenolic compounds and can therefore be used as an alternative source of phenolics due to their higher antioxidant capacity and low toxicity compared to those of synthetically derived phenolics. In this study, *Sekaki* papaya (*Carica papaya*) peel was used as an alternative source of phenolics. Response Surface Methodology (RSM) was employed to optimise process conditions to achieve the highest phenolic content from the fruit peel. Total Phenolic Content was analysed using the Folin-Ciocalteau method and the total phenolic content (TPC) was expressed as Gallic Acid Equivalent (GAE). The highest TPC i.e. 1735.1 mg/L GAE was obtained at a temperature of 1200C and a time of 5 h in a solid-solvent ratio of 1:40 g/mL while the lowest TPC of 616.57 mg/L GAE was obtained at a temperature of 900C and a time of 3 h at a solid-solvent ratio of 1:20 g/mL. With such a high phenolic content, *Sekaki* papaya (*Carica papaya*) peel can be used as a natural antioxidant and can protect the human body from various free-radical-associated diseases.

Keywords: phenolics, antioxidant, Total Phenolic Content (TPC), Folin-Ciocalteau method

INTRODUCTION

By-products derived from plants contain a variety of phytochemicals and phenolic compounds besides carotenoids such as flavonoids, phenolic acids, lignin, tannins and others. These compounds represent antioxidant as well as antiradical activities (Shahidi & Naczk, 2004) and possess

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anticarcinogenic, antimutagenic and antiproliferative properties (Yang et al., 2005; Alasalvar et al., 2006). The antioxidant activity of phenolics is mainly attributed to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

'*Sekaki*' papaya (*Carica papaya*) is a popular tropical fruit widely used for its fruit flesh as well as processing activities. Malaysia is a predominant producer as well as exporter of this fruit; two main varieties of papaya (*Carica papaya*) are grown in the country, with *Sekaki*, also known as 'Hong Kong' variety, being the second most cultivated variety after *Eksotika*. The *Sekaki* has red flesh and a bright yellow, even-coloured skin without freckles. Papaya (*Carica papaya*) peel is often considered waste and can pose an environmental threat in excessive production. It does not receive much attention in terms of utilisation or recycling and is usually discarded. This could result from a lack of application for commercial purposes (Soong & Barlow, 2004). Interestingly, by-products of fruit such as peel and seed fractions may contain many valuable compounds such as carotenoids, flavonoids and phenolics and like the fruit pulp itself, may exhibit strong antioxidant capacity (Jayaprakasha et al., 2001). Research has been conducted on many fruit products such as tomato pomace, the seeds and skin of which accounted for a high level of lycopene (Choudhari & Ananthanarayan, 2007); pomegranate peel, which exhibited higher antioxidant activity than its pulp (Li et al., 2006); grape seed, which is a stronger antioxidant than its pulp, contains a high level of proanthocyanidin and can scavenge various reactive oxygen free-radical species (Guo et al., 2003). While papaya (*Carica papaya*) fruit pulp has nature's most concentrated source of carotenoids, especially lycopene, which shows strong antioxidant activity (Jamal et al., 2016), the peel of the fruit also exhibits strong antioxidant as well as phenolic content.

Recently, there has been increasing interest in the antioxidant properties exhibited by phenolic compounds extracted from certain vegetables and fruit due to their strong antioxidant activity and low toxicity compared to synthetic phenolic antioxidants such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole) and propyl gallate. There are various methods to determine the total phenolic content of plants and their fruit extracts. Phenols can be assessed by a variety of methods; however, results obtained from each test are difficult to analyse as sometimes, only the phenolic content of the final extracts are reported and not the total yield. Previously, total phenolic content was determined colorimetrically and measured as grams of Gallic acid (Singleton & Rossi, 1965) but the Folin-Ciocalteau method is slightly modified (Sato et al., 2004). Total Phenolic Content of the antioxidants is expressed as the Gallic acid equivalent concentration (mg/L) (Tachakittirungrod et al., 2007).

Multiple reaction characteristics and mechanisms are responsible for strong antioxidant activity of plant extracts. Several

methods including FRAP assay, DPPH radical scavenging activity, ABTS or the Trolox equivalent test, ORAC, can be employed to give an estimation of a plant's antioxidant capacity. All these methods may be subjective to conditions and the various reagents used (Chandrasekara & Shahidi, 2011). Coupling DPPH and FRAP assay together helps provide a complete vindication of antioxidant capacity of the lycopene extract from papaya fruit peel (Sanchez -Moreno et al., 2003; Shi & Le Maguer, 2000; Liu et al., 2007; Dewanto et al., 2002). Also, phenolic compounds account for a major portion of the antioxidants in many fruit extracts (Duthie & Crozier, 2000; Gertenbach, 2001; Vuong et al., 2010). Plant-derived phenolics are reported to have better antioxidant activity than those of vitamin C (Wang et al., 1996) and elucidate their activity by several mechanisms in action such as free radical scavenging, lipid peroxidation and metal ion chelation (Nilar & Harrison, 2002). Notwithstanding the criticisms of the chemical assays, numerous papers have reported good to strong correlation between the total phenolic content and the antioxidant capacity of extracts; thus, these compounds are considered major contributors to the antioxidant activity of fruit extracts. Total phenolic content is generally determined using the Folin-Ciocalteau method which, as pointed out by Prior et al. (2005), has an oxidation/reduction reaction as its basic mechanism in determining the antioxidant activity of compounds.

Therefore, the objective of this study is to demonstrate the potentiality of a new source of antioxidant that can give a new dimension to the usage of *Sekaki* papaya (*Carica papaya*) peel. After suitable heat treatment, which aided in full extraction of the carotenoids from the fruit peel due to cell disruption and release of the desired compounds (Shi et al., 2000; Chang et al., 2006), the effects of reaction parameters (temperature, time and solid-solvent ratio) on Total Phenolic Content using the Folin-Ciocalteau method to optimise the highest phenolics using response surface methodology (RSM) were studied.

MATERIALS AND METHODS

Plant Materials

Papaya (*Carica papaya*) peel of the papaya variety *Sekaki* was collected from a local fruit shop located in the International Islamic University Malaysia (IIUM) and the separated peel was freeze-dried until a constant weight was obtained. The dried samples were then ground using a grinder machine to get a uniform size of sample. Samples were kept in airtight containers and stored at -20°C until further analysis. All the experimental procedures were carried out under dim light.

Plant Extraction

A sample (1 gm) of the *Sekaki* papaya (*Carica papaya*) peel was extracted using a mixture of solvents (hexane, acetone and alcohol in the ratio of 2:1:1) containing

0.05% (w/v) butylatedhydroxytoluene (BHT) (Shi & Le Maguer, 2000). The samples were subjected to heat treatment for set time periods (Honest et al., 2010; Kaur et al., 2007; Fish et al., 2002; Choudhari & Ananthanarayan, 2007). Antioxidants such as butylated hydroxytoluene (BHT) were employed in solvents used for extraction so as to avoid oxidation and isomerisation reactions. Cold distilled water (15 ml) was added to the mixture and the suspension was agitated at 200 rpm for 8 min. The solution was then allowed to stand at room temperature for 15 min for separation of polar and non-polar layers. The nonpolar supernatant hexane layer containing the desired carotenoid compounds as well as phenolics were separated and further analysed for quantification of its lycopene content (Jamal et al., 2016). The oleoresin was then subjected to saponification and purification to separate the carotenoids, mainly lycopene and β-carotene from the phenolics. The presence of phenols was then investigated using the Folin-Ciocalteau Method.

Chemicals

Ethanol (HmbG Chemicals), hexane (EMSURE, Merck KGaA, Germany) and acetone (Bendosen Lab Chemicals) were used to extract the antioxidant compounds from the fruit peel. For the total phenolic test hydrochloric acid, Gallic acid (Fluka), Folin-Ciocalteau's reagent (Merck) and sodium carbonate (HmbG Chemicals) were used.

Determination of the Total Phenolic Content: Folin-Ciocalteau Method

Total phenolic content (TPC) of all the extracts and fractions was determined following the Folin-Ciocalteau method as described by Khoo (2009) with slight modification. In a 100-mL volumetric flask, 0.5 grams of dry Gallic acid was dissolved in 10 mL of ethanol and diluted to volume with water. For the preparation of standard curve, 0, 1, 2, 3, 5 and 10 mL of phenol stock was taken into 100-mL volumetric flasks and diluted to the required volume with water. These solutions had concentrations of 0, 50, 100, 150, 250 and 500 mg/mL Gallic acid.

Total phenolic content (TPC) of the *Sekaki* papaya peel extracts was determined using the Folin-Ciocalteau colorimetric method. First, appropriate dilution of the extracts was prepared (1 mg/mL). Then, 20 µL of diluted crude extract was added to 1.58 mL of deionised water and 100 µL of Folin-Ciocalteau reagent (FCR) in a 15-mL test tube wrapped in aluminium foil. After 10 min, 300 µL of (20%) sodium carbonate (Na_2CO_3) was added into the test tube. The mouth of the test tube was then covered with parafilm and aluminium foil and the test tube was vortexed for about 10 to 15 s. The mixture was then incubated for 2 h at room temperature in a dark environment for colour development. The absorbance was measured at 765 nm against the blank reagent using a UV-VIS spectrophotometer. The measurements were done in triplicate for more accurate results. Gallic acid was used for calibration of a standard curve with different concentrations as shown in Figure

1 below and the results were expressed as mg/L Gallic Acid Equivalent (GAE) (Tachakittirungrod et al., 2007).

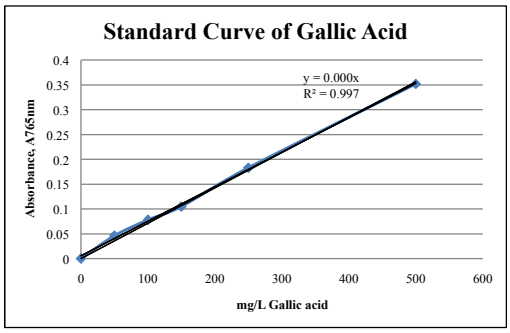

Figure 1. Standard curve for Folin-Ciocalteau test

Experimental Design

The process conditions for the total phenolic content were optimised using the Face-Centred Central Composite Design (FCCCD) under Response Surface Methodology (RSM) using the software, Design-Expert Version 8.0.8 (Stat-Ease Inc., Minneapolis, USA). Three factors were investigated in this study, namely X1 (temperature), X2 (time) and X3 (solid-solvent ratio). A total of 19 experiments were generated by FCCCD and all the experiments were replicated three times and the mean value was recorded.

The results for the phenolic content were analysed using regression analysis, where multiple equations were developed and followed by the analysis of these regression equations by statistical tools; ANOVA (analysis of variance) and p-test. The average yield of TPC was taken as the dependent variables of response (Y). A second order polynomial was developed to fit in the response as shown in Equation (1).

$$Y= \beta0+ \beta1A+ \beta2B + \beta3C + \beta11A2 + \beta22B2 + \beta33C2 + \beta12AB + \beta13AC + \beta23BC \quad (1)$$

where
A=Extraction Temperature (0C);
B=Extraction Time (h);
C=Solid to solvent ratio (g/mL);

RESULTS AND DISCUSSION

The experimental conditions allowed a fast, quantitative and maximum extraction of the antioxidant compounds and determination of the total phenolic content of the extract obtained. From the experiment results shown in Table 1, it is evident that the use of the statistical process condition optimisation approach and response surface methodology helped to locate the most significant conditions with minimum effort and time utilised. According to Toor and Savage (2006), phenolics from the extract are soluble in organic solvents such as hexane, light petroleum and methanol, and this can potentially account for some of the antioxidant activity of the extract. Therefore, as expected, different optimum operating conditions were found to yield maximum results for the assay (highlighted in Table 1).

Design of Experiment and Statistical Analysis

The polynomial regression model relating TPC (Y) with the independent variables, temperature (A), time (B) and solid-solvent ratio (C) is shown in Equation (2).

$$Y\ (mg/L\ GAE) = +1163.44+190.40*A+72.75*B+164.01*C+164.07*A*B+ 50.85\ *A*C-51.56*B*C+72.92*A2-25.72*B2-207.86*C2 \quad (2)$$

where,
A=Temperature (0C)
B=Time (h)
C=Solid to Solvent Ratio (g/mL)

The average OD of triplicate values obtained for each run was calculated as the observed TPC and summarised in Table 1 and the combination of the variables, observed and predicted are presented.

Table 1
Values of Observed and Predicted TPC Response

Run Order	Temperature (°C)	Time (h)	Solid to Solvent Ratio (g/mL)	TPC mg/L GAE	
				Observed	Predicted
1	90	3	1.20	616.57	738.9659
2	120	3	1.20	798.5	689.9425
3	90	5	1.20	780.87	659.4643
4	120	5	1.20	1135.71	1266.71
5	90	3	1.40	1218.82	1068.422
6	120	3	1.40	1120.78	1222.788
7	90	5	1.40	693.512	782.6717
8	120	5	1.40	1735.1	1593.306
9	90	4	1.30	985.71	1045.958
10	120	4	1.30	1409.42	1426.763
11	105	3	1.30	1030.42	1064.971
12	105	5	1.30	1167.44	1210.48
13	105	4	1.20	815	791.5674
14	105	4	1.40	1018.57	1119.594
15	105	4	1.30	1230	1163.442
16	105	4	1.30	1098.12	1163.442
17	105	4	1.30	1282.85	1163.442
18	105	4	1.30	1178.57	1163.442
19	105	4	1.30	1182.85	1163.442

The matching quality of the data obtained by the model proposed in Equation (1) was evaluated considering the correlation coefficient, R^2, between the experimental and modelled data. In this study, the determination coefficient (R^2=0.8734) indicated a higher correlation, 87.34%, between the observed values and the predicted values. This indicated the degree of precision with which the Total Phenolic Content was related to the three independent variables i.e. temperature, time and solid-solvent ratio.

The corresponding analysis of variance (ANOVA) is presented in Table 2. The ANOVA of the quadratic model demonstrated the significance of the model, as shown by Fisher's F-test with F-Value of 6.90 and relatively low probability (P_{model}>F)=0.0041. The P-value implied that this model was significant. It was identified that the linear term, B, was not significant. In this case A, C, AB, C^2 were significant model terms. Thus, temperature and amount of solvent were critical factors for phenol extraction of the samples.

Table 2
Analysis of Variance (ANOVA) of Response Surface Quadratic Model for TPC

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F	
Model	110774	9	123419.4	6.897574	0.0041	Significant
A-Temperature	362532.3	1	362532.3	20.26095	0.0015	
B-Time	52931.74	1	52931.74	2.958212	0.1196	
C-Solid-Solvent ratio	269003.3	1	269003.3	15.03387	0.0037	
AB	215344.5	1	215344.5	12.03502	0.0071	
AC	20683.54	1	20683.54	1.155947	0.3101	
BC	21269.33	1	21269.33	1.188685	0.3039	
A^2	14528.68	1	14528.68	0.811969	0.3910	
B^2	1806.951	1	1806.951	0.100986	0.7579	
C^2	118056.2	1	118056.2	6.597844	0.0303	

Analysis Using Response Surface Methodology (RSM)

The primary mode of action of various phenolic compounds is their radical scavenging activity; this assay was employed to measure that activity. Phenolic compounds are considered to be the major contributors of antioxidant activity and are determined according to the chemical structure that they possess. Antioxidant capacity generally increases when total phenolic content increases.

Figure 2 depicts that when temperature was kept constant, the TPC value varied considerably with the solvent ratio. Under certain conditions a maximum contour (1200 mg/L GAE) could be determined, meaning that the slightest change in the

amount of solvent used or time would not increase the TPC yield. These figures showed that the highest TPC was obtained at a time period of 4.33 h and a solid-solvent ratio of 1:33 g/mL while the temperature was set constant at 105°C.

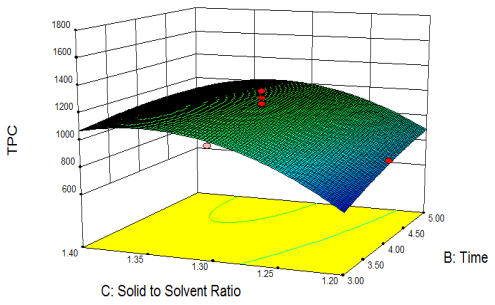

Figure 2. 3D surface plot showing the effect of Time (h) and Solid-Solvent Ratio (g/mL) to yield highest TPC value

of phenolic content, which leached into the water. Previous studies done by Lin and Tang (2007) quantified total phenolic content in powder form and lyophilised form; however, a liquid sample was used in our study.

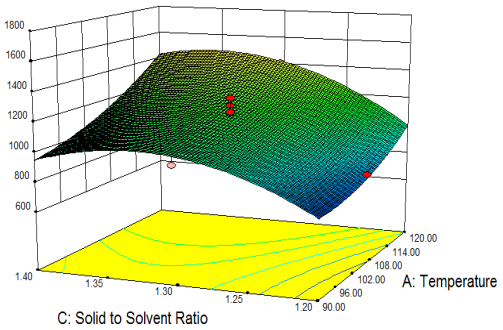

Figure 3. 3D surface plot showing the effect of Temperature (°C) and Solid to Solvent Ratio (g/mL) to yield highest TPC value

The surface plot in Figure 3 indicates that temperature was an important parameter for the TPC. However, as per a study done by Gertenbach (2001), while higher solvent temperatures typically increase mass transfer rates during extraction, in the case of polyphenols, elevated temperatures trigger competing processes such as decomposition and epimerisation (Gertenbach, 2001; Vuong et al., 2010). Temperature control during the extraction and isolation process for *Sekaki* papaya needs to be strictly controlled to minimise the loss of polyphenols as suggested by the regression model as well. According to Choo et al. (2014), the phenolic compounds in bitter gourd were sensitive to heat and the heat treatment caused a significant loss

While no previous studies regarding the optimisation of extraction conditions under heat treatment for phenolics from papaya (*Carica papaya*) plant peel were found, studies on other plant materials were found and they reported similar findings. The presence of phenolics as well as the antioxidant activity of papaya peel is highly dependent upon its maturity as stated by Prior et al. (2005). Rivera-Pastrana et al. (2010) identified ferulic acid, caffeic acid and rutin as the phenols present in the exocarp of *Maradol* papaya. Ranges of content of ferulic acid [1.33-1.62 g kg(-1) dry weight], caffeic acid [0.46-0.68 g kg(-1) dw] and rutin [0.10-0.16 g kg(-1) dw] were quantified and the presence of the phenols was found to be highly dependent

on storage temperature and ripening stage. *Sekaki* papaya peel used for this study was obtained at its maturity stage and stored at room temperature to maintain the phenolic profile of the exocarp.

The phenolic content of the heat-treated papaya peel was comparable to the study done by Lim et al. (2007) on dragonfruit (21±6 mg/100g), papaya (28±6 mg/100g), guava (138±31 mg/100g), starfruit (131±54 mg/100g) and orange (75±10 mg/100g). According to a study done by Normala and Suhaimi (2011), total phenolic compound content can be significantly influenced by the solvent and different vegetative parts. Nepote et al. (2005) reported that extraction time had a significant impact on extractability of phenolic compounds present in peanut skin, while Aludatt et al. (2011) found that with longer extraction time, both the overall yield of phenolic compounds and the antioxidant activity of the extracts from olive seeds improved. Xiaowei et al. (2011) did a polyphenolic extraction test on mango and found similar results in terms of temperature and solubility time. Also, according to Toor and Savage (2006), some phenolics are soluble in the nonpolar hexane layer of the extract and can contribute to antioxidant activity.

CONCLUSION

The distribution of phenolics was examined in this study. It gave an assessment of the diverse compounds that may be present in the *Sekaki* papaya (*Carica papaya*) peel extract. Total phenolic content was analysed using Folin-Ciocalteau's reagent and its concentration was expressed as Gallic Acid Equivalents (GAE). The results indicated that the highest amount was equal to 1735.1 mg/L GAE, which also contributed to antioxidant activity. The phenolics could be later identified. They can be utilised as potent antioxidants.

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