Sains Malaysiana 47(1)(2018): 99–107
http://dx.doi.org/10.17576/jsm-2018-4701-12

Effect of Extraction Solvents and Drying Conditions on Total Phenolic Content and Antioxidant Properties of Watermelon Rind Powder

(Kesan Pelarut Ekstrak dan Kaedah Pengeringan ke atas Jumlah Kandungan Fenol dan Sifat Antioksida pada Serbuk Kulit Tembikai)

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ABSTRACT

The objective of the present study was to determine the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant properties, i.e. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and ferric reducing antioxidant potential (FRAP) assay, of red- and yellow-fleshed watermelon rind powders prepared using different drying conditions (hot-air oven drying at 40 and 60°C and freeze drying). All the samples were subjected to four different solvent extract using water, methanol, ethanol and acetone prior analyses. Water extract from red- and yellow-fleshed watermelon rind powders presented highest value for TPC and TFC. However, methanol extract samples showed highest value for antioxidant properties (DPPH and FRAP) followed by acetone, ethanol and water extract. By comparing the drying conditions, all samples dried using hot-air dryer at 40 and 60°C had significantly higher ($p<0.05$) in TPC value than the samples dried using freeze dryer. However, samples dried using freeze dryer showed highest in DPPH and FRAP values. The present obtained results would be useful to the food and pharmaceutical industries for developing of functional ingredients.

Keywords: Antioxidant properties; drying condition; total flavonoid content; total phenolic content; watermelon rind

ABSTRAK

Objektif kajian ini adalah untuk menentukan jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC) dan sifat antioksidan, i.e. cerakin penghapus radikal bebas 2,2-difenil-1-picrylhydrazyl (DPPH) dan cerakin potensi antioksidan penurun ferik (FRAP), pada serbuk kulit tembikai berisi merah dan kuning yang disediakan dengan menggunakan kaedah pengeringan yang berbeza (pengeringan oven udara panas pada 40 dan 60°C dan pengeringan sejuk beku). Semua sampel dilarut menggunakan pelarut ekstrak yang berbeza, air, metanol, etanol dan aseton, sebelum analisis. Ekstrak air daripada serbuk kulit tembikai berisi merah dan kuning menunjukkan nilai tertinggi untuk TPC dan TFC. Walau bagaimanapun, sampel ekstrak metanol menunjukkan nilai sifat antioksidan yang tertinggi (DPPH dan FRAP) diikuti oleh aseton, etanol dan ekstrak air. Dengan membandingkan kaedah pengeringan, sampel (serbuk kulit tembikai berisi merah dan kuning) yang dikering dengan menggunakan kaedah pengeringan oven udara panas pada 40 dan 60°C mempunyai nilai TPC yang lebih tinggi secara signifikan ($p<0.05$) berbanding sampel yang dikering dengan menggunakan kaedah pengeringan sejuk beku. Walau bagaimanapun, sampel yang dikering dengan menggunakan kaedah pengeringan sejuk beku menunjukkan nilai DPPH dan FRAP yang tertinggi. Keputusan yang diperoleh akan memberi manfaat kepada industri makanan dan farmaseutik dalam pembangunan kandungan fungsian.

Kata kunci: Jumlah kandungan fenol; jumlah kandungan flavonoid; kaedah pengeringan; kulit tembikai; sifat antioksida

INTRODUCTION

Watermelon (*Citrullus lanatus*, family Cucurbitaceae) is a vine-like flowering plant originated from southern Africa. The pulp and juice of watermelon is used for human consumption while rind and seeds, represent 30% of the whole fruit are major solid wastes (Anonymous 2014). Usually, the watermelon rind is discarded, applied to feeds or fertilizer. However, in China, watermelon rinds are commonly consumed as vegetable by stewing or stir-frying (Fila et al. 2013). According to Larrosa et al. (2002), agricultural and industrial residues are attractive sources of natural antioxidants and dietary fibre.

Phenolic compounds play an important role in maintaining human body health due to its strong antioxidant potency. Antioxidant compounds in food play an important role as a health-protecting factor due to its function to reduce the risk for chronic disease (Hue et al. 2011) including cancer and heart disease. Several previous findings have reported the influence of factors such as drying techniques and solvent extraction on the phenolics and antioxidant activity of numerous plant materials (Anwar et al. 2013; Norra et al. 2016; Pham et al. 2015; Yi & Wetzstein 2011).

Drying is a vital process in sample preparation to reduce the moisture content less than 15% as such

condition can prevent the growth of microbial. Hot-air oven drying is the most common method used in food processing to extend the product shelf life, minimising packaging requirement and also reducing shipping weights (Hamrouni-Sellami et al. 2011). Freeze drying, also known as lyophilisation, is a drying method that significantly produces a more pronounced lightening of the product surface and lesser loss of green colour (Guiné & Barroca 2012). This method is usually restricted to delicate, heat-sensitive materials of high value. However, freeze-drying is a time-consuming and relatively costly process, which limits its use to products with high value added (Louka & Allaf 2002). According to Norra et al. (2016) and Pham et al. (2015), solvent used for extracting bioactive compounds from plant material has significant influence on the concentration of extracted bioactive compounds because these compounds have different properties as well as polarities. The most commonly used solvents for extracting bioactive compounds from fruits, vegetables, and other foodstuffs are water, methanol, ethanol, and acetone (Anwar et al. 2013; Norra et al. 2016; Pham et al. 2015; Yi & Wetzstein 2011). To this, it is necessary to identify the most suitable drying conditions and extraction solvent in order to obtain the highest concentration of phenolic compounds and antioxidant activity from watermelon rind powders.

Recently, it has taken into account that the watermelon by-products are important sources of protein, dietary fibres, and natural antioxidants, i.e. carotenoids and phenolics (Perkins-Veazie et al. 2007, 2002). To date, the effects of different drying conditions as well as types of extraction solvents on the total phenolic content and antioxidant properties of watermelon rind from red- and yellow-fleshed varieties has been rarely reported. Therefore, the present study aim to determine the effect of three various drying condition (hot-air oven drying at 40 and 60°C and freeze drying) and four different extraction solvents (water, methanol, ethanol and acetone) on total phenolic contents, total flavonoid content and antioxidant properties of red- and yellow-fleshed watermelon rind powders. The optimal drying conditions and the best extraction solvent drawn from the present findings could be useful for both food and pharmaceutical industries in preparing bioactive compounds extracts from watermelon rind powders.

Materials and Methods

Watermelon rind powders preparation

Ripe red- and yellow-fleshed watermelons were purchased from stall located at Tembila, Besut Terengganu. The watermelon (*Citrullus lanatus*) was selected according to the guidelines described by Sapii and Muda (2005), where ripe watermelons should have the characteristics such as yellowish-cream ground spot, shinning skin, dispersed stripes and produce hollow sound when flicked. Watermelons were washed under running tap water and the outer green rinds were removed using peeler. A sharp knife was used to cut these watermelons transversely between the bloom and stem ends, followed by removing the flesh from the white rind. The white rinds were then sliced into uniform pieces using an industrial fruit slicer (Santos, Lyon, France). Sliced watermelon rinds were then dried at three different drying conditions; hot-air oven drying at 40°C (RF40 and YF40 for red- and yellow-fleshed watermelon rind, respectively) and 60°C (RF60 and YF60 for red- and yellow-fleshed watermelon rind, respectively) for overnight and freeze dried (RFFD and YFFD for red- and yellow-fleshed watermelon rind, respectively) for 2 days. Dried watermelon rinds were ground using stainless steel grinder to obtain powder. Ground watermelon powders were kept in the airtight container at room temperature prior to extraction.

Sample extraction

Watermelon rind powder (0.5 g) was suspended into four different solvents (50 mL) separately; distilled water, methanol, ethanol and acetone to produce 10 g/L concentration of extracts. The mixture was then stirred on the hot plate stirrer at room temperature for 24 h before filtering to collect the filtrate for further analysis. The fresh extracts were used for determination of total phenolic content, total flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay, and ferric-reducing antioxidant potential (FRAP) assay.

Determination of total phenolic content (TPC)

The Folin-Ciocalteau method was referred for determination of TPC in watermelon rind powder (Alothman & Karim 2009). Sample extract (3 mL) was mixed with 4 mL of Na_2CO_3 (7.5%, w/v) and incubated for 5 min in dark condition. Then, mixture was added with 3 mL Folin-Ciocalteu reagent (pre-diluted, 10 times), vortex and incubated for 30 min. The absorbance was measured by using UV-VIS spectrophotometer (Shimadzu UV-1601PC, Japan) at 760 nm. TPC of the sample were expressed as gallic acid equivalent per 100 g of dry matter (mg GAE/100 g d.m.).

Determination of total flavonoid content (TFC)

The TFC of the watermelon rind powder was determined according to the method described by Alothman and Karim (2009). Sample extract (3 mL) was mixed with 0.3 mL of 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$ was added to the mixture and then after 6 min, 2 mL of 1 M NaOH were added. After being left at room temperature in the dark room for 30 min, the absorbance of the reaction mixture was measured at 510 nm by using UV-VIS spectrophotometer. Total flavonoid content was expressed as mg catechin equivalent per 100 g of dry matter (mg CEQ)/100 g of d.m.).

2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) FREE RADICAL-SCAVENGING ASSAY

The free radical-scavenging effect of all sample extracts on the DPPH radical was determined based on the method proposed by Mosquera et al. (2007). Sample extract (3 mL) was mixed with 3.9 mL of DPPH solution (0.025 g/L methanol). The mixture was thoroughly mixed with vortex mixed and incubated. After being left at room temperature in the dark room for 30 min, the absorbance of the reaction mixture was measured at 517 nm by using UV-VIS spectrophotometer. Results were expressed as percentage of ascorbic acid inhibition.

FERRIC REDUCING ANTIOXIDANT POTENTIAL (FRAP) ASSAY

Ferric reducing assay were performed according to the method described by Benzie and strain (1996). Sample extract (100 µL) were added to 3 mL of FRAP reagent (300 mM sodium acetate buffer at pH3.6, 20 mM iron chloride and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) dissolved in 40 mM HCl at a ratio of 10:1:1). The solution was mixed well and incubated at 37°C for 4 min. The reagent blank was a mixture of 100 µL distilled water and 3 mL of FRAP reagent incubated at 37°C for 1 h. The absorbance of the standard, sample extracts and reagent blank was measured against the blank (distilled water) at 593 nm by using UV-VIS spectrophotometer. The FRAP value of the sample was expressed as mg of ferrous (Fe^{2+}) equivalent per 100 g of dry matter (mg FE/100 g of d.m.).

STATISTICAL ANALYSIS

Two experiments with factorial design of type 3 × 4 was carried out to study the effect of two factors, the drying method (X_1) and type of solvent (X_2), on four different responses (TPC, TFC, DPPH and FRAP) of red- and yellow-fleshed watermelon rind powders. Three drying methods (hot-air drying at 40 and 60°C and freeze-drying) and four types of solvent (water, methanol, ethanol and acetone) were tested. Three replicates were tested for each sample and the total number of sample runs was 36.

Analysis of variance (ANOVA) and Bonferroni method for multiple comparisons were used for analysing the data. Values of $p<0.05$ were regarded as statistical significant level. SPSS (Version 18.0 for Windows, SPSS Inc., Chicago, IL) was used to complete the statistical tests.

RESULTS AND DISCUSSION

Past studies have shown that functional properties of a substance are influenced by the type of drying method and solvent used for extraction (Boeing et al. 2014; Hong et al. 2015; Jahangiri et al. 2011; Narmin et al. 2014). However, it is not known how these two factors would influence the functional properties of watermelon rind powders. Therefore, two experiments, one for red-fleshed watermelon rind powder and the other one for yellow-fleshed, were carried out to study the effects of two factors, the drying method (X_1) and the types of solvent used in extraction (X_2), on total phenolic content and total flavonoid content (TPC and TFC) and antioxidant properties (DPPH and FRAP) parameters of these powders. The results of ANOVA for both red- and yellow-fleshed watermelon rind powders showed that the effects of X_1 and X_2 were significant for all the independent variables (Tables 1 and 2 for red- and yellow-fleshed watermelon rind powders, respectively). The effect of interactions between the drying method and the types of solvent used in extraction was significant on the total phenolic content and antioxidant properties. This significant interaction indicated that these factors did not work independently and thus, changes in the total phenolic content and total flavonoid content and antioxidant properties were mainly due to interaction effects. The coefficients of determination (R^2) were high (approximately 1), indicating that the statistical model explained more than 99% of the total variation for all of the dependent variables.

TOTAL PHENOLIC CONTENT

The results of the total phenolic content are shown in Table 3. All sample extracts contain an amount of phenolic content ranging from 127.93-218.39 mg GAE/100 g of d.m. for red variety and 111.00-213.21 mg GAE/100 g of d.m. for yellow variety. The extraction of phenolic content using methanol was significantly less ($p<0.05$) effective than water, however, it was more effective than ethanol and acetone for RF40, RFFD, YF40, YF60 and YFFD. The suitable solvent for extraction target compounds should be selected carefully because the extracted compound will be based on the type of solvents used (Norra et al. 2016). The recovery of phenolic compounds is dependent on the solvent used in their extraction and its polarity (Alothman & Karim 2009). A polar solvent will isolate polar compound and non-polar solvent will extract non-polar compound, thus different solvents will yield different extracts and extract composition (Franco et al. 2008). Solvent polarity plays a key role in increasing phenolic solubility (Naczk et al. 2006). The present finding was in agreement with finding by Sofia et al. (2012), whereby water extracts give the highest value in total phenolic content compared to the methanol, ethanol and acetone extracts from apple pomace. This may be due to the better solvation of TPC present in plants as a result of interactions (hydrogen bonds) between the polar sites of the TPC molecule and the solvent. According to Boeing et al. (2014), the polarities of methanol and ethanol are similar. However, the obtained results showed that ethanol was less efficient in the extraction of TPC than methanol. This could be due to a low solvation of ethanol compared to methanol. The presence of longer ethyl radical in ethanol than the methyl radical in methanol results in a lower solvation of phenolic molecules (Boeing et al. 2014). In addition, the selectively characteristics of different polarities of the solvent to hydrophilic or hydrophobic phenolic compounds in extracts plays an

TABLE 1. The results of factorial experiments for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant properties, i.e. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and ferric reducing antioxidant potential (FRAP) assay, of red-fleshed watermelon rind powder

Source[1]	Sum of squares	df	Mean square	F	P-value
TPC					
X_1	9536.621	2	4768.311	12963.628	<0.0001
X_2	11837.973	3	3945.991	10727.984	<0.0001
X_1*X_2	1060.422	6	176.737	480.496	<0.0001
Error	8.828	24	0.368		
Total	1153529.269	36			
TFC					
X_1	2901.610	2	1450.805	4002.896	<0.0001
X_2	69266.865	3	23088.955	63704.408	<0.0001
X_1*X_2	17364.100	6	2894.017	7984.840	<0.0001
Error	8.699	24	0.362		
Total	243343.811	36			
DPPH					
X_1	6299.344	2	3149.672	17749.630	<0.0001
X_2	7003.598	3	2334.533	13156.004	<0.0001
X_1*X_2	892.937	6	148.823	838.675	<0.0001
Error	4.259	24	0.177		
Total	126540.185	36			
FRAP					
X_1	312568.157	2	156284.078	4736.539	<0.0001
X_2	237424.210	3	79141.403	2398.558	<0.0001
X_1*X_2	112575.955	6	18762.659	568.644	<0.0001
Error	791.890	24	32.995		
Total	8573807.089	36			

[1] X_1, drying method; X_2, type of solvent

important role in identifying the optimal concentration of phenolic compounds. From the obtained results, it could be concluded that the phenolic compounds of watermelon rinds were more hydrophilic.

Extraction with acetone showed the lowest value of TPC for red- and yellow-fleshed watermelon rinds powder extracts. This could be due to their lower efficiency of solvation, since acetone molecules are only proton acceptors while the other solvents such as methanol, ethanol, and water are proton donors (Boeing et al. 2014). Iloki-Assanga et al. (2015) also reported that acetone extracts (283.49 μg GAE/mg) has the overall lower TPC compared to ethanol extracts (304.34 μg GAE/mg) for stem of *Bucida buceras* (the non-edible black olive tree). From this, it can be concluded that extractability of a particular component appeared to depend on extraction medium polarity and the ratio of solute to solvent.

The TPC of the samples dried using hot-air oven drying methods was significantly higher (p<0.05) (162.33-218.39 mg GAE/100 g of d.m. for red variety; 147.48-213.21 mg GAE/100 g of d.m. for yellow variety) than those powders produced by the freeze drying (127.93-180.58 mg GAE/100 g of d.m. for red variety; 111.00-202.50 mg GAE/100 g of d.m. for yellow variety). The effect of drying method has been consistently demonstrated with respect to TPC. The freeze-drying technique, which is consider as the least aggressive drying method as compared to hot-air drying, has consistently produced extracts low in TPC. These results are in agreement with the findings reported by Hong et al. (2015), who prepare the senna leaves powder using hot-air oven drying and freeze drying technique. The authors reported that the prepared samples using hot-air oven drying contained the highest levels of phenolic compounds compared to freeze drying method. However, the hot-air drying temperature at 60°C resulted in decreasing of TPC in the sample extracted using water, methanol and acetone. According to Jahangiri et al. (2011), a high temperature and long drying time could destroy the phenols and decreasing water present in the plant cells, which makes the extraction with solvent more difficult.

TOTAL FLAVONOID CONTENT (TFC)

The results of the TFC are tabulated in Table 4. All extracts contain an amount of flavonoid ranging from 13.95 to 193.43 mg CEQ/100 g of d.m. for red variety. For yellow variety sample, the order of effectiveness in extraction of flavonoid was water (93.29-171.85 mg CEQ/100 g of d.m.) > methanol (42.46-64.65 mg CEQ/100 g of d.m.) > ethanol (23.27–53.65 mg CEQ/100 g of d.m.) > acetone (14.24-35.65 mg CEQ/100 g of d.m.). These results clearly

TABLE 2. The results of factorial experiments for total phenolic content (TPC), total flavonoid content (TFC), and antioxidant properties, i.e. 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and ferric reducing antioxidant potential (FRAP) assay, of yellow-fleshed watermelon rind powder

Source[1]	Sum of squares	df	Mean square	F	P-value
TPC					
X_1	3647.059	2	1823.530	6248.590	<0.0001
X_2	25987.025	3	8662.342	29682.778	<0.0001
X_1*X_2	1612.132	6	268.689	920.701	<0.0001
Error	7.004	24	0.292		
Total	1091017.091	36			
TFC					
X_1	1955.606	2	977.803	1648.245	<0.0001
X_2	51670.187	3	17223.396	29032.816	<0.0001
X_1*X_2	11683.307	6	1947.218	3282.350	<0.0001
Error	14.238	24	0.593		
Total	194732.608	36			
DPPH					
X_1	7811.920	2	3905.960	13086.754	<0.0001
X_2	5395.583	3	1798.528	6025.891	<0.0001
X_1*X_2	3547.563	6	591.260	1980.993	<0.0001
Error	7.163	24	0.298		
Total	133220.616	36			
FRAP					
X_1	142724.853	2	71362.427	2012.064	<0.0001
X_2	363840.462	3	121280.154	3419.495	<0.0001
X_1*X_2	128168.522	6	21361.420	602.285	<0.0001
Error	851.215	24	35.467		
Total	9211111.612	36			

[1] X_1, drying method; X_2, type of solvent

TABLE 3. Total phenolic content[1] (expressed as mg GAE/ 100 g dry matter) of red- and yellow-fleshed watermelon rind powders extracted using different solvents

Drying methods[2]	Types of solvent			
	Water	Methanol	Ethanol	Acetone
Red-fleshed watermelon rind powders				
RF40	218.39^{dC} ± 0.34	194.79^{cC} ± 0.90	177.45^{bB} ± 0.29	167.04^{aC} ± 0.85
RF60	210.27^{dB} ± 0.91	183.96^{bB} ± 0.25	195.81^{cC} ± 0.47	162.33^{aB} ± 0.17
RFFD	180.58^{dA} ± 0.57	163.42^{cA} ± 0.56	145.06^{bA} ± 0.39	127.93^{aA} ± 0.90
Yellow-fleshed watermelon rind powders				
YF40	211.69^{dB} ± 0.41	198.56^{cC} ± 0.78	159.44^{bB} ± 0.97	153.20^{aC} ± 0.44
YF60	213.21^{dA} ± 0.33	178.42^{cB} ± 0.42	166.68^{bC} ± 0.23	147.48^{aB} ± 0.08
YFFD	202.50^{dC} ± 0.44	169.15^{cA} ± 0.58	147.58^{bA} ± 0.40	111.00^{aA} ± 0.73

[1] Data are presented as mean ± standard deviation (n=3). Mean values in the same row with different superscript lower letters are significantly different at p<0.05. Mean values in the same column with different superscript capital letters are significantly different at p<0.05.

[2] RF40: red-fleshed watermelon rind dried using hot-air dryer at 40°C; RF60: red-fleshed watermelon rind dried using hot-air dryer at 60°C; RFFD: red-fleshed watermelon rind dried using freeze dryer; YF40: yellow-fleshed watermelon rind dried using hot-air dryer at 40°C; YF60: yellow-fleshed watermelon rind dried using hot-air dryer at 60°C; YFFD: yellow-fleshed watermelon rind dried using freeze dryer

indicate that the extraction was affected by the polarity of the solvents. The predominant positive effect of water and methanol may be due to the presence of polar substances such as phenolic glycosides. These findings were in agreement with a study conducted by Iloki-Assanga et al. (2015), who showed that the highest flavonoid content was observed in sample water extract from stem of *Bucida buceras*. Moreover, flavonoids are potent water-soluble super antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity (Johnson et al. 2012).

TFC for the sample prepared using hot-air oven drying at 40 and 60°C and freeze drying were ranged from 13.95-123.31 mg CEQ/100 g of d.m. for red variety; 27.23-93.29

TABLE 4. Total flavonoid content[1] (expressed as mg CEQ/ 100 g dry matter) of red- and yellow-fleshed watermelon rind powders extracted using different solvents

Drying methods	Types of solvent			
	Water	Methanol	Ethanol	Acetone
Red-fleshed watermelon rind powders				
RF40	$123.31^{dB} \pm 0.52$	$88.84^{cC} \pm 0.70$	$19.46^{bA} \pm 0.59$	$13.95^{aA} \pm 0.60$
RF60	$89.95^{dA} \pm 0.47$	$59.24^{cB} \pm 0.50$	$48.63^{bC} \pm 1.04$	$29.82^{aC} \pm 0.54$
RFFD	$193.43^{dC} \pm 0.24$	$57.70^{cA} \pm 0.54$	$34.71^{bB} \pm 0.62$	$25.33^{aB} \pm 0.56$
Yellow-fleshed watermelon rind powders				
YF40	$93.29^{dA} \pm 0.91$	$42.46^{cA} \pm 0.70$	$35.20^{bB} \pm 0.96$	$27.23^{aB} \pm 0.58$
YF60	$104.82^{dB} \pm 1.20$	$64.65^{cC} \pm 0.49$	$53.65^{bC} \pm 0.96$	$35.65^{aC} \pm 0.59$
YFFD	$171.85^{dC} \pm 0.78$	$53.15^{cB} \pm 0.55$	$23.27^{bA} \pm 0.59$	$14.24^{aA} \pm 0.59$

[1] Data are presented as mean ± standard deviation ($n = 3$). Mean values in the same row with different superscript lower letters are significantly different at $P < 0.05$. Mean values in the same column with different superscript capital letters are significantly different at $P < 0.05$.

[2] RF40: red-fleshed watermelon rind dried using hot-air dryer at 40°C; RF60: red-fleshed watermelon rind dried using hot-air dryer at 60°C; RFFD: red-fleshed watermelon rind dried using freeze dryer; YF40: yellow-fleshed watermelon rind dried using hot-air dryer at 40°C; YF60: yellow-fleshed watermelon rind dried using hot-air dryer at 60°C; YFFD: yellow-fleshed watermelon rind dried using freeze dryer

mg CEQ/100 g of d.m. for yellow variety, 29.82-89.95 mg CEQ/100 g of d.m. for red variety; 35.65-104.82 mg CEQ/100 g of d.m. for yellow variety, 25.33-193.43 mg CEQ/100 g of d.m. for red variety; 14.24-171.85 mg CEQ/100 g of d.m. for yellow variety, respectively (Table 4). Comparing the TFC of the watermelon rind samples prepared using different drying temperatures and extracted using water, freeze drying sample (193.43 mg CEQ/100 g of d.m. for red variety; 171.85 mg CEQ/100 g of d.m. for yellow variety) was found to be significantly higher ($p<0.05$) than sample dried using hot-air oven drying at 40°C (88.84 mg CEQ/100 g of d.m. for red variety; 93.29 mg CEQ/100 g of d.m. for yellow variety) as well as sample dried using hot-air oven drying at 60°C (89.95 mg CEQ/100 g of d.m. for red variety; 104.82 mg CEQ/100 g of d.m. for yellow variety). Freeze drying technique provided higher TFC due to its better ability in retaining bioactive compounds as compared to hot-air drying method. According to Chan et al. (2009), ice crystals will develop within the plant tissue matrix and the removal of moisture content leads to the tissue becoming more brittle during freeze drying process and consequently causes a greater rupturing cell structure for better solvent accessibility and compounds extraction. However, this was in contrast to the results obtained from TPC (Table 3). This could be explained by the different characteristics between phenolic and flavonoid compounds. Hence, further studies are needed to identify individual phenolic compound and flavonoid present in watermelon rind powders prepared using different drying conditions as well as extraction solvents, which might provide more details.

2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) FREE RADICAL-SCAVENGING PROPERTIES

From the results showed in Table 5, all sample extracts using different solvent extraction (water, methanol, ethanol and acetone) contain an amount of DPPH ranging from 23.49-84.88% and 12.90-85.28% for red- and yellow-fleshed watermelon rind powders, respectively. The extraction of DPPH using methanol was significantly more effective ($p<0.05$) than water, ethanol and acetone. Similarly, Emad and Sanaa (2013) found that methanol extract has higher DPPH value than water extract of algae (*Spirulina platensis*). Bauer (2002) reported that the compounds (lycopene, carotenoid and glucose oxydase enzyme) including phenol compounds and flavonoids found in watermelon rind to contain antioxidant properties. According to Boeing et al. (2014), phenolic compounds are usually mainly responsible for the antioxidant properties of plants and most of these compounds are categorised as hydrophilic antioxidants.

The antioxidant properties in free radical-scavenging ability of sample prepared using hot-air oven drying (40 and 60°C) was significantly higher ($p<0.05$) (23.49-84.88%) than sample produced using freeze drying (25.81-55.85%) method for red-fleshed variety. In addition, samples processed from hot-air oven drying at 60°C (54.70-84.88%) had higher value of DPPH than the sample processed from hot-air oven drying at 40°C (23.49-79.84%) and freeze drying technique (25.81-55.85%) for red-fleshed watermelon rind powder. Findings obtained from this study was found to be similar to the results as reported by Narmin et al. (2014), whereby scavenging activity of DPPH radical of pepper extract was shown to increase with the increase in the drying temperature. According to Narmin et al. (2014), DPPH of pepper dried at 60°C showed higher value than sample dried at 35°C due to the breaking down of free radical during high heating. Hossain et al. (2010) also reported that fresh sample which contains high level of moisture will lose its antioxidant compounds through the enzymatic degradation process due to the high level of active enzymes in fresh sample. Ho et al. (2016) reported that watermelon rinds dried using freeze drying technique has higher moisture content (18.86% and 17.55% for red-fleshed and yellow-fleshed watermelon rind powders,

TABLE 5. 2,2-diphenyl-1-picrylhydrazyl free radical-scavenging assay[1] (expressed as % ascorbic acid inhibition) of red- and yellow-fleshed watermelon rind powders extracted using different solvents

Drying methods[2]	Types of solvent			
	Water	Methanol	Ethanol	Acetone
Red-fleshed watermelon rind powders				
RF40	$23.49^{aA} \pm 0.10$	$79.84^{cB} \pm 0.20$	$58.67^{bB} \pm 0.20$	$59.54^{bB} \pm 0.30$
RF60	$54.70^{aC} \pm 0.47$	$84.88^{dC} \pm 0.40$	$73.19^{bC} \pm 0.53$	$76.41^{cC} \pm 0.40$
RFFD	$25.81^{aB} \pm 0.60$	$55.85^{dA} \pm 0.40$	$34.07^{bA} \pm 0.53$	$43.88^{cA} \pm 0.31$
Yellow-fleshed watermelon rind powders				
YF40	$39.92^{aB} \pm 0.20$	$50.94^{dA} \pm 0.58$	$42.61^{bB} \pm 0.23$	$49.13^{cA} \pm 0.20$
YF60	$68.28^{aC} \pm 0.31$	$84.68^{cB} \pm 0.53$	$73.99^{bC} \pm 0.53$	$83.80^{cC} \pm 0.31$
YFFD	$12.90^{aA} \pm 0.81$	$85.28^{dB} \pm 0.53$	$39.25^{bA} \pm 0.62$	$51.75^{cB} \pm 0.91$

[1] Data are presented as mean ± standard deviation ($n = 3$). Mean values in the same row with different superscript lower letters are significantly different at $p<0.05$. Mean values in the same column with different superscript capital letters are significantly different at $p<0.05$.

[2] RF40: red-fleshed watermelon rind dried using hot-air dryer at 40°C; RF60: red-fleshed watermelon rind dried using hot-air dryer at 60°C; RFFD: red-fleshed watermelon rind dried using freeze dryer; YF40: yellow-fleshed watermelon rind dried using hot-air dryer at 40°C; YF60: yellow-fleshed watermelon rind dried using hot-air dryer at 60°C; YFFD: yellow-fleshed watermelon rind dried using freeze dryer

respectively) than hot-air oven drying at 40°C (14.75% and 15.12% for red-fleshed and yellow-fleshed watermelon rind powders, respectively) and 60°C (14.98% and 14.80% for red-fleshed and yellow-fleshed watermelon rind powders, respectively). Therefore, the lower the moisture content, the higher the solvent extraction efficiency of antioxidant compounds (Hossain et al. 2010). In addition, according to Norra et al. (2016), the length of drying time is an important factor in influencing the concentration of antioxidant activity. It is crucial to shorten the drying time in order to maximise the antioxidant activity. In this study, the hot-air dried samples had been dried for overnight, while the freeze-dried samples for 2 days, showing that as the length of drying time increase, the antioxidant activity decrease. Therefore, the longer duration of oxygen exposure resulting in increased redox activity and degradation of phenolic compounds, hence decreasing the antioxidant activity (Pham et al. 2015).

FERRIC-REDUCING ANTIOXIDANT POTENTIAL (FRAP) ASSAY

From the results tabulated in Table 6, comparing the different solvent extraction, methanol had the highest value obtained for antioxidant properties (372.15-700.18 mg FeE/100 g d.m. for red variety; 440.78-768.66 mg FE/100 g d.m. for yellow variety) followed by acetone (344.60-616.54 mg FE/100 g d.m. for red variety; 412.33-608.08 mg FE/100 g d.m. for yellow variety), ethanol (338.30-602.94 mg FE/100 g d.m. for red variety; 399.36-563.08 mg FE/100 g d.m. for yellow variety) and water with the value of 319.43-367.28 mg FE/100 g d.m. for red variety; 304.32-377.06 mg FE/100 g d.m. for yellow variety. FRAP assay had a similar trend to DPPH radical assay. In addition, this finding was similar with the study reported by Iloki-Assanga et al. (2015), where the sample extract using methanol solvent exhibited comparatively high reducing power than ethanol and acetone due to the stability of

TABLE 6. Ferric reducing antioxidant potential assay[1] (expressed as mg FE/100 g dry matter) of red- and yellow-fleshed watermelon rind powders extracted using different solvents

Drying methods[2]	Types of solvent			
	Water	Methanol	Ethanol	Acetone
Red-fleshed watermelon rind powders				
RF40	$319.43^{aA} \pm 062$	$372.15^{cA} \pm 0.96$	$338.30^{bA} \pm 0.94$	$344.60^{bA} \pm 0.90$
RF60	$367.28^{aC} \pm 0.56$	$605.11^{cB} \pm 0.15$	$411.48^{bB} \pm 0.28$	$598.38^{cB} \pm 0.44$
RFFD	$348.70^{aB} \pm 0.20$	$700.18^{cC} \pm 0.74$	$602.94^{bC} \pm 0.03$	$616.54^{bC} \pm 0.61$
Yellow-fleshed watermelon rind powders				
YF40	$377.06^{aC} \pm 0.57$	$440.78^{cA} \pm 0.45$	$399.36^{bA} \pm 0.33$	$412.33^{bA} \pm 0.05$
YF60	$328.02^{aB} \pm 0.90$	$639.69^{dB} \pm 0.43$	$480.25^{bB} \pm 0.78$	$535.17^{cB} \pm 0.22$
YFFD	$304.32^{aA} \pm 0.96$	$768.66^{dC} \pm 0.49$	$563.08^{bC} \pm 0.07$	$608.08^{cC} \pm 0.32$

[1] Data are presented as mean ± standard deviation ($n = 3$). Mean values in the same row with different superscript lower letters are significantly different at $p<0.05$. Mean values in the same column with different superscript capital letters are significantly different at $p<0.05$

[2] RF40: red-fleshed watermelon rind dried using hot-air dryer at 40°C; RF60: red-fleshed watermelon rind dried using hot-air dryer at 60°C; RFFD: red-fleshed watermelon rind dried using freeze dryer; YF40: yellow-fleshed watermelon rind dried using hot-air dryer at 40°C; YF60: yellow-fleshed watermelon rind dried using hot-air dryer at 60°C; YFFD: yellow-fleshed watermelon rind dried using freeze dryer

methanol solvent and high polarity of the solvent system. Moreover, antioxidant compounds that exhibit antioxidant capacity in FRAP assay are usually proton donors. Thus, these results were in line with the results obtained from TPC analyses (Table 3). The reducing capacity of the extracts may serve as an indicator of potential antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom (Iloki-Assanga et al. 2015).

The antioxidant properties determined by FRAP assay for samples dried using hot-air oven drying at 40°C and extracted using methanol, ethanol and acetone showed significantly lower (p<0.05) (338.30-372.15 mg FE/100 g d.m. for red variety; 399.36-440.78 mg FE/100 g d.m. for yellow variety) than the sample produced using hot-air oven drying at 60ºC (411.48-605.11 and 480.25-639.69 mg FE/100 g d.m. for red variety and yellow variety, respectively) and freeze drying (602.94-700.18 mg FE/100 g d.m. and 563.08-786.66 mg FE/100 g d.m. for red variety and yellow variety, respectively). These results also indicated that increasing temperature of hot-air drying from 40 to 60°C resulted in the rise of antioxidant activity (DPPH and FRAP). According to Yi and Wetzstein (2011) the increase of antioxidant capacity was attributed to the result of the formation of novel compounds (i.e. myristicin, safrole and other secondary compounds) with increased the free radical scavenging activity.

Conclusion

Based on the findings from the above-mentioned section, in terms of suitability of solvent extraction, water was the best solvent in extracting total phenolic compounds and total flavonoid followed by methanol, ethanol and acetone. Results for DPPH and FRAP assays showed methanol extract had the strongest antioxidant properties followed by acetone, ethanol and water. In terms of drying conditions, samples prepared from hot-air oven drying at 40 and 60°C was found to contain significantly higher (p<0.05) value in total phenolic compounds than samples dried using freeze dryer. However, hot-air oven drying at 60°C was found to contain highest DPPH value. Furthermore, all freeze dried sample (both red- and yellow-fleshed watermelon rind powders) extracted using methanol, ethanol, and acetone as solvents showed significantly higher (p<0.05) FRAP value than sample obtained from hot-air oven drying (40 and 60°C). The results obtained from the present study can provide general information on the suitability drying technique and solvent extraction used for its potential application as functional ingredient in food and pharmaceutical application and the end product is expected to benefit consumers. Further studies are needed to identify and characterize individual phenolic and flavonoid compounds present in watermelon rinds.

Acknowledgements

The authors wish to thank Universiti Sultan Zainal Abidin for the financial support from the University Research Grant (UNISZA/2015/DPU/(2)).

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Received: 18 February 2017
Accepted: 5 June 2017