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Antioxidant and Mineral Content of Pitaya Peel Extract obtained using Microwave Assisted Extraction (MAE)

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ABSTRACT

Background: Microwave Assisted Extraction (MAE) was used to extract active compounds in pitaya peels by using water as a solvent. Pitaya peels can be seen as a potential form of fruit waste, especially in the food industry because its solution extraction can be applied as natural coloring and contained beneficial active compounds that have commercialize value. Besides, extraction can be a solution for minimizing wastes produced by food processing industry. These food wastes often contain several usable substances of high value including some that are beneficial for health**. Objective:** The aim of this study is to determine the total phenolic content (TPC), antioxidant activity and mineral content of pitaya peel extract solution. **Results:** The results indicate that antioxidant activity had a good correlation with phenolic content. Meanwhile, 14 out of 24 elements were identified, which are Ba, Ca, Cu, Cd, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, Ti, and Zn**.** All mineral data were validated using the CCLASS software**. Conclusion:** MAE is the best optional equipment for extraction since the integrity of the active compound is still maintained.

INTRODUCTION

The dragon fruit, also known as Pitaya, is of three types, namely (1) *Hylocereus undatus*, red skin with white flesh; (2) *Hylocereus polyrhizus*, red skin with red flesh, and (3) *Selinecereus megalanthus*, yellow skin with white flesh (Grimaldo-Juárez *et al*., 2007). *Hylocereus polyrhizus* was selected for this study as it contains high phenolic compound and antioxidant activity compared to other pitaya species. The utilization of waste fractions such as peels can provide environmental and economic benefits, particularly if green solvents, such as water, are used. Besides that, agro-industrial by-products are cheap, abundant, and sustainable resources, which contain compounds with antioxidant, cytotoxic, and antimicrobial activities that could be proposed as natural antimicrobial agent (Rodrigues *et al*., 2013; Vinardell *et al*., 2008). To maintain the integrity of the active compounds, Microwave Assisted Extraction (MAE) can be applied for pitaya peel extraction.

MAE has been applied in sample preparation techniques because it can extract bioactive compounds more rapidly and possibly has better recovery than the conventional extraction process (Azmir *et al*., 2013). For example Thirugnanasambandham and Sivakumar (2015) applied 8 min of MAE to extract betalain from *H.polyrhizus*, which resulted in 9mg/L. Besides that, MAE is accepted as a potential and powerful alternative for organic compounds extraction from plant materials (Li *et al*., 2012). It is also recognized as a green technology because it reduces the use of organic solvents (Alupului *et al*., 2012; Rombaut *et al*., 2014). A proof was provided by Pinela *et al*. (2016) who used 20ml of water in the extraction of phenolic acid from 0.9 gram tomato using MAE, which resulted in 8.99mg GAE/g extract.

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An important concern related to the extraction of natural ingredients is the desired limitation of toxic solvents (Chaudhari *et al*., 2011). In this study, water was used as a natural solvent in the sample preparation. Although water is an abundant solvent, it is not suitable for nonpolar compound extraction. The majority of antioxidant compounds in plants are flavonoids and polyphenols, which are polar and readily soluble in water (Amarnath, 2004). Therefore, in this experiment, solvent extraction for the active compounds was done by water. According to Michel *et al*. (2011), extraction methods using biodegradable and nontoxic solvents such as water and ethanol are well developed. Additionally, water is the preferred solvent as it increases the extraction yield because water may enhance swelling and increase the contact surface area between the sample and the solvent (Hayat *et al*., 2009). As a result, active compounds are easily released into the surrounding medium. In this research, 50 mL of water as solvent volume should be sufficient to ensure that the entire sample is immersed, especially when a sample swells during the extraction process (Dahmoune *et al*., 2013).

MATERIAL AND METHOD

2.1 Sample Collection:

The pitaya peels (*H. polyrhizus*) were obtained from a supermarket in Temerloh, Pahang. The peels were weighed and washed with distilled water. Then, the peels were cut into 2 cm portion. For the freeze-drying process, the samples were frozen overnight in the fridge at -80 ^{o}C and placed in the freeze dryer for 96 hr. Afterwards, the freeze-dried samples were ground and sifted through a 20 mesh sieve to obtain the powdered samples. The dried powder was stored in bags and kept in dry environment prior to conducting the experiments (Chaiwut *et al*., 2012).

2.2 Microwave Assisted Extraction (MAE):

Extraction was carried out using MAE. The selected optimum condition of power, temperature, and time setting for MAE were 400 watt, 45 ^{o}C and 20 min respectively and the weight of the sample was 1.2 g with slight modification method from Chaiwut *et al*. (2012). Water was used as an extraction solvent because it is nontoxic for active compounds. The liquid-solid extraction was done by adding of the freeze dried samples to 50 mL of extraction solvent in a 1000 mL extraction vessel. The extraction vessel was placed in MAE. Immediately after the extraction process, the homogenate was centrifuged at 9000 rpm for 40 min at 25 ^{0}C. The supernatant was collected after centrifugation and the same procedure was repeated twice to ensure maximum extraction of active compounds. All of the experiments were performed in triplicates (Thirugnanasambandham and Sivakumar, 2015).

2.3 Analysis of Solution Extraction:

2.3.1 Determination of Total Phenolic Content (TPC):

The antioxidant level for each sample was measured by the Total Phenolic Content (TPC) assay using the Folin-Ciocalteu method based on the modified method from Lim *et al*. (2007). Samples of 0.3 mL of each extract were measured into test tubes followed by 1.5 mL of the Folin-Ciocalteu reagent. Then, 1.2 mL of 7.5% w/v of sodium carbonate solution was added in the test tubes. The tubes were shook and incubated in the dark for 30 min at room temperature. The absorbance was measured at 765 nm using the Shimadzu Lambda. All samples and readings were measured in triplicates. A calibration curve was prepared using the regression equation of the calibration curve of gallic acid (y = 0.094x, r2 = 0.9985), and the contents were expressed as mg gallic acid equivalent (GAE)/g of the sample.

2.3.2 Measurement Uncertainty in TPC :

The guideline in calculating uncertainty followed the standard, ISO 21748-2010. The substance of TPC was represented by gallic acid with CAS NO 149-91-7. To calculate the uncertainty in TPC, few factors had to be considered, which are balance, volumetric flask, precision, and bias. These factors were described in the following fish bone diagram (diagram 1) and equation 1 as follows:

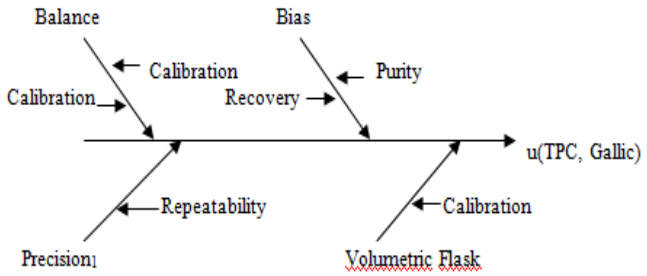

Diagram. 1: Fish Bone Diagram for Measuring Uncertainty of TPC

$$\frac{u(TPC)}{C_{TPC}} = \sqrt{\left(\frac{u(Precision_1)}{Precision_1}\right)^2 + \left(\frac{u(Balance)}{Wt}\right)^2 + \left(\frac{u(V.\ Flask\ )}{Vol_{50ml}}\right)^2 + \left(\frac{u(Bias)}{C_{std}}\right)^2} \quad (1)$$

2.3.3 Free Radical Scavenging Activity Assay:

In this experiment DPPH (2,2-diphenyl-2-picrylhydrazyl) was used as a synthetic free radical that can react with the antioxidant content in pitaya peel to form the DPPH complex compound. The reaction is visually noticeable as the colour changes from purple to yellow due to hydrogen donating ability (Ajila *et al*., 2007). The free radical scavenging activity was determined according to the method of Khamsah *et al*. (2006) with slight modifications. The reagent and solution for the analysis assay were prepared in 70% ethanol. Each sample was prepared in serial dilution (0.2 mg/ml, 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml, and 1.0 mg/ml) with a final volume of 10 mL. A pitaya peel extract of 1 mL was mixed with 2 mL of DPPH reagent (0.1mM) in a test tube. Then, the solution was mixed by a vortex mixer for 20 sec and incubated at room temperature in the dark for 30 min. The reduction of DPPH was measured at 517 nm against 70% ethanol as a blank assay. The percentage of the scavenging activity was measured after all absorbance were recorded. All tests were carried out in triplicates.

The antioxidant activity of the samples is defined as in Equation 2:

$$\text{DPPH scavenging activity (\%)} = \left[1 - \left(\frac{Atest}{Acontrol}\right)\right] X\ 100\% \quad (2)$$

Where Abs control is the absorbance of the DPPH solution without extract.

2.3.4 Determination of Mineral Content:

The equipment used in this analysis was the PerkinElmer Optima 8300 DV Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) system (German) equipped with an auto sampler and Mira mist nebuliser. Stock solutions of a single element standard solution (1000 ppm) consisting of 24 elements (Al, As, Ba, Be, Bi, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Mo, Ni, K, Na, Sr, Ti, V, and Zn) from the Perkin Elmer system (German) were employed to prepare the calibration standard. Ultrapure water was used in the standard preparation. Table 1 shows the operating conditions of the ICP-OES equipment.

All elements were detected in the radial mode, except for arsenic, lead and phosphorus which were detected in the axial mode. All samples were analyzed in triplicates to eliminate any specific error, to verify the homogeneity of the samples, and to evaluate the repeatability of the procedure. For data verification, the quality control (QC) standard with a concentration of 10 ppm containing 24 elements were analyzed together with the pitaya peel solution extraction.

Table 1: The operating conditions of the ICP-OES equipment

No	Parameter	Flow Rate
1	Argon plasma	12 L/min
2	Auxiliary gas	0.4 L/min
3	Nebuliser gas	0.5 L/min
4	Radio Frequency (RF)	1500 Watts
5	Pump	1.5 mL/min

2.3.5 Cclass Software:

This software was used to verify mineral content of pitaya peel that obtained from the instrument ICP-OES. The data was analyzed based on QC standard as reference material. In this experiment, QC standard with concentration 10 ppm was preferred to recover all elements in mineral content. Tolerance for each elements at QC standard has been set into the software. It enable for user to check the accuracy of data by observe the value of z-score. Based on laboratory quality standard international (LQSI), the qc data was accepted if the value fall within the range of the three sigma limit, which is -3≤ z≤ 3. The z-score value was calculated by the software as Equation 3.

$$\text{Z-score} = \left|\frac{Experiment\ value - Expected\ value}{standard\ deviation}\right| \quad (3)$$

RESULTS AND DISCUSSION

3.1 TPC and Antioxidant Activity:

Table 2 displays the calculation of uncertainty measurement in TPC following the method in ISO 21748-2010. In this experiment six readings were performed to read TPC. Gallic acid with a concentration of 50 ppm was used as quality control (QC) for each TPC reading. The final reading of uncertainty in TPC was 58.04 ± 1.98 mg/g with a confidence level of 95%.

Table 2: The calculation of uncertainty measurement in TPC following the method in ISO 21748-2010.

u(Precision)

No	Compound		TPC, mg/L/Recovery %								u(Precision$_1$) Precision$_1$
			1.2050	1.2067	1.2042	1.2033	1.2014	1.2	Average	SD	
			1	2	3	4	5	6			
1	Total Phenolic Content (Gallic Acid)	UV-VIS readout	58.22	58.12	57.90	58.05	58.21	57.75	58.04	0.185	
		Cal, %	55.20	55.10	55.34	55.20	55.40	55.18	55.24	0.111	0.0032
		Recovery, %	105.47	105.48	104.63	105.16	105.07	104.66	105.08	0.375	

U (Bias)

No	Compound	Observed Value, mg/L / Recovery, %						Average	SD	u(Precision$_2$) Precision$_2$	u(R. Std) Compound	u(Bias) C $_{std}$
		1	2	3	4	5	6					
1	Gallic Acid (50ppm)	55.20	55.10	55.34	55.20	55.40	55.28	55.24	0.111	0.0020	0.0144	0.0146
		100.3	100.2	100.6	100.4	100.7	99.2					

U (TPC)

No.	Compound	u(Precision$_1$) Precision$_1$	u(Balance) Wt	u(Vf) Vol $_{(50mL)}$	u(Bias) Cstd	u(TPC) C	Expanded uncertainty	Reported Uncertainty with 95% confidence
1	TPC (Gallic acid)	0.0032	0.0083	0.0007	0.0146	0.017	1.98	58.04 ± 1.98mg/g

Several studies showed total phenolic compound, TPC is strongly correlated with the antioxidant activity content, such as reported by Bertoncelj *et al*. (2007). In this study, the value of TPC contributed to antioxidant activity as presented in Table 3. There is a strong relationship between antioxidant activity and TPC in fruits, vegetables, and grain products as stated by Samarth *et al*. (2008). According to Pãixao *et al*. (2007), antioxidant properties are always associated with phenolic compounds, which are considered as one of the most important quality parameters in fruits.

Based on the study conducted by Ruzlan *et al*. (2010), the highest antioxidant activity as further supported by TPC was exhibited by *H. polyrhizus* peels, followed by *H. undatus* peels, *H. polyrhizus* pulps, and *H. undatus* pulps.

Table 3: Displays the result between TPC and antioxidant activity.

No.	Power (Watt)	Temperature (°C)	Time (min)	Weight (g)	TPC (mg GAE/g sample)	Antioxidant activity (%)
1	400	45	20	1.2	58.04+/- 1.98	88.21

3.2 Mineral Content of Samples:

Table 4: The Cclass Data for Mineral Content in Pitaya Peel.

Element	Wavelegth(nm)	Unit	Expected Value of QC (ppm)	Tolerance (%)	Standard Deviation	Experimental Value of QC (ppm)	Sample Concentration (ppm)	Z-Score
Al	308.215	PPM	10	35	3.50	10.50	N.D	0.14
As	188.979	PPM	10	12.5	1.25	11.14	N.D	0.91
Ba	233.527	PPM	10	11.25	1.13	10.71	0.14	0.63
Be	313.042	PPM	10	10.5	1.05	9.06	N.D	-0.90
Ca	317.933	PPM	10	22.5	2.25	11.16	9.75	0.52
Cd	214.440	PPM	10	10.2	1.02	9.85	0.03	-0.15
Co	230.786	PPM	10	11.25	1.13	8.66	N.D	-1.19
Cr	267.716	PPM	10	12.5	1.25	9.23	N.D	-0.62
Cu	327.393	PPM	10	11.25	1.13	10.12	0.22	0.11
Fe	273.955	PPM	10	35	3.50	12.14	0.31	0.61
K	766.490	PPM	10	100	10.00	10.05	1081	0.01
La	408.672	PPM	10	11.25	1.13	7.28	N.D	-2.42
Li	670.784	PPM	10	10.5	1.05	10.41	N.D	0.39
Mg	279.077	PPM	10	15	1.50	9.74	56.81	-0.17
Mn	403.075	PPM	10	12.5	1.25	10.36	1.73	0.29
Na	589.592	PPM	10	22.5	2.25	10.03	2.69	0.01
Ni	231.604	PPM	10	12.5	1.25	9.70	0.16	-0.24
Pb	220.353	PPM	10	15	1.50	10.46	0.05	0.31
Sc	361.383	PPM	10	10.2	1.02	9.93	N.D	-0.26
Sr	460.733	PPM	10	10.25	1.03	10.36	0.38	0.35
Ti	368.519	PPM	10	17.65	1.765	10.36	0.015	0.20
V	292.402	PPM	10	10.5	1.05	8.12	N.D	-1.79
Y	371.029	PPM	10	10.5	1.05	9.06	N.D	-0.90
Zn	206.200	PPM	10	11.25	1.125	9.60	0.95	-0.36

**N.D=Not Determined

**QC= Quality Control

In this research, 24 elements were analyzed using ICP-OES. However, only the concentration of 14 elements, namely Ba, Ca, Cu, Cd, Fe, K, Mg, Mn, Na, Ni, Pb, Sr, Ti, and Zn were identified in pitaya peel solution extract as presented in Table 4. Each element has a specific wavelength. During the experiment, any interference between elements was solved using Inter Element Correction (IEC) and Multi Spectral Fitting (MSF) that had already been set in the instrument software. Hence, ICP-OES is a powerful instrument to detect multi elements in a sample. Besides that, the Cclass software was applied in this research to check the QC result. In Cclass, tolerance value is readily provided for each element and the user has to provide the expected value of QC in the software. The standard deviation for each element is calculated using the tolerance information, whereby the tolerance and standard deviation are required to calculate the z-score. The z-score is used to observe the difference between the experimental and the expected values of QC. Pertaining to this, the Laboratory quality services international (LQSI) stated that an indication that a process is not in control or unacceptable is when a single point lies outside the three-sigma control limit, which is a z-score larger than +3 or -3. Referring to table 4, all elements in QC values fall within the z-score range of $-3 \leq z \leq 3$. This shows that the experimental value for the QC data were correct and acceptable. It is important to ensure that the z-score fall within the range of $-3 \leq z \leq 3$ because the reading of QC data will affect the mineral content result of pitaya peel concentration.

Based on Table 4, the element with the highest concentration is potassium with 1081 ppm. Potassiun is essential to maintain body water content and acid balance, thus pitaya peels may be a good source of potassium. In this study, the concentration of calcium was 9.75 ppm and magnesium was 56.8 ppm. These values were slightly higher than that reported by Chaiwut *et al*. (2012). Calcium allows the correct formation of skeleton during one's childhood, which is essential in preventing osteoporosis later in life. Magnesium is also essential for human because it helps to regulate blood sugar level and is involved in energy metabolism and protein synthesis (Mir-Marqués *et al*., 2015).

Besides that, sodium is also an essential element, but is only required in small amounts. In this study, it was found to be lower, which is 2.69 ppm. High intakes of this element are associated with increased blood pressure and risk of cardiovascular disease (Organization, 2012). Therefore, pitaya peel extract could be beneficial because its consumption is not associated with hypertension. The concentration of manganese, strontium, iron, and titanium in pitaya peel samples were less than 5 ppm. Manganese and iron are essential nutrients for human. Additionally, it is very important for females between 14 and 50 years old and babies in the first months of their life to have an adequate supply of iron. Excessive amount of strontium and titanium can cause health problems. Therefore, monitoring their levels in one's food is important as they are not essential nutrients for humans. In all cases, the content of lead and cadmium obtained by ICP-OES was lower than the LOD (limit of detection) values. Therefore, the lead and cadmium contents did not exceed the maximum permitted levels in fruits (0.10 ppm and 0.05 ppm, respectively) according to the European Commission (Van Egmond *et al*., 2007).

The mineral content and QC found in this study were interpreted by the cclass software. The data obtained in this study showed potential minerals in pitaya peels for pitaya grown in Malaysia. In conclusion, pitaya peel extract can be applied in various industries such as food, pharmaceutical, and cosmetic industries as it has a vital mineral composition for human health.

Conclusion:

The measurement of uncertainty in TPC was 58.04 ± 1.98 mg/g with a confidence level of 95%. This shows that the reported result was accurate and only had a potential error of ± 1.98 mg/g. Few factors were considered in the uncertainty calculation, namely balance, volumetric flask, precision, and bias. Next, the correlation between TPC and antioxidant activity was determined in pitaya peel extract, in which the TPC value displayed the value of antioxidant activity with 88.21%. In addition, 24 elements were analyzed in pitaya peels for the first time using ICP-OES in order to identify the essential elements that are important for human diet to maintain normal physiological function. The results showed that the data for the 14 identified elements were correct and acceptable as the z-score obtained for qc did not fall outside of the three sigma limit, which is $-3 \leq z \leq 3$. Based on mineral analysis, the extraction sample can be potentially used in food and cosmetic industries.

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