

EFFECT OF DIFFERENT OPERATING PARAMETERS ON EXTRACTION OF ACTIVE COMPOUNDS FROM PITAYA PEEL BY MICROWAVE ASSISTED EXTRACTION (MAE)

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Muhd Azlan Nazeri^a, Norashikin Mat Zain^{b*}

*Corresponding author
shikin@ump.edu.my

^aFaculty of Chemical Engineering and Natural Resources, University Malaysia Pahang, 26300, Gambang, Pahang, Malaysia

^bFaculty of Chemical Engineering and Natural Resources, University Malaysia Pahang, 26300, Gambang, Pahang, Malaysia

Graphical abstract



Abstract

Microwave-assisted extraction (MAE) has been recognised as a powerful potential alternative for the extraction of active compounds from plant materials compared to other advanced methods such as ultrasound-assisted extraction (UAE), pressurised liquid extraction (PLE), supercritical fluid extraction (SFE), and Soxhlet. The advantages of MAE processing technique are reduced processing time, higher yield, lower usage of solvent, and smaller energy demand. Nevertheless, most researchers used organic solvents which have toxic effect on the environment. Therefore, in this study, distilled water was used as a natural solvent in the sample preparation. Pitaya peel is a form of potential fruit waste, especially in the food industry. Notably, its liquid extract can be applied as natural colouring and it contains beneficial active compounds that have commercial value. Wastage during the processing of food is inevitable and disposal can be a major problem for the industry and the society. Negative impacts such as pollution to the environment, hazards to human health, and loss of income to the waste generator may occur. Thus, extraction can be an effective solution for minimising waste produced by the food processing industry. Food waste often contain several usable substances of high value including some of that are beneficial for health such as mineral contents and phenolic compounds. The aim of this research was to find the optimal operating parameters for extraction of total phenolic content (TPC) from pitaya peel using MAE method. These parameters were the (1) weight of the sample, (2) temperature, (3) power, and (4) extraction time. In this research, the results showed that the best condition for the parameters of MAE were at the power of 400 W, temperature of 45°C, and 20 min contact time when extracting 1.2 g pitaya peel in 50 mL distilled water. These figures were validated through statistical analysis using SPSS with Bonferroni post hoc tests. The TPC presented in the liquid extract was measured in GAE/g. In addition, the Inhibitory Concentration (IC₅₀) of the liquid extract was determined by applying the best condition for the parameters of MAE and DPPH reagent as the synthetic free radical. The IC₅₀ value obtained in this research was 0.52 mg/mL.

Keywords: Microwave, Pitaya Peel, Pitaya Extract, Parameter, Total Phenolic Content

Abstrak

Microwave-assisted extraction (MAE) telah dikenalpasti sebagai suatu alternatif berkuasa yang mempunyai potensi untuk pengekstrakan sebatian aktif daripada bahan tumbuhan berbanding dengan kaedah maju yang lain seperti *ultrasound-assisted extraction* (UAE), *pressurised liquid extraction* (PLE), *supercritical fluid extraction* (SFE), dan Soxhlet. Kelebihan MAE adalah pengurangan masa pemprosesan, hasil yang lebih tinggi, penggunaan pelarut yang minima, dan penggunaan tenaga yang lebih rendah. Walaubagaimanapun, kebanyakan pengkaji telah menggunakan pelarut organik yang mempunyai kesan toksik kepada persekitaran. Dalam kajian ini, air suling telah digunakan sebagai pelarut semulajadi untuk penyediaan sampel. Kulit pitaya merupakan sisa buah yang mempunyai potensi, terutamanya dalam industri makanan. Cecair ekstraknya boleh digunakan sebagai pewarna semulajadi dan mengandungi sebatian aktif yang berfaedah serta mempunyai nilai komersial. Pembaziran sewaktu pemprosesan makanan tidak dapat dielakkan dan pembuangan boleh menjadi masalah besar kepada industri tersebut dan masyarakat. Impak negatif seperti pencemaran kepada persekitaran, ancaman kepada kesihatan manusia, dan kehilangan pendapatan kepada penghasil sisa buangan boleh terjadi. Lantas, pengekstrakan boleh menjadi penyelesaian yang berkesan untuk mengurangkan sisa yang dihasilkan oleh industri pemprosesan makanan. Selalunya, sisa makanan mengandungi beberapa bahan yang berguna dan mempunyai nilai tinggi termasuklah yang berfaedah kepada kesihatan contohnya kandungan mineral dan sebatian fenolik. Tujuan kajian ini adalah untuk mendapatkan keadaan terbaik parameter operasi dalam MAE untuk pengekstrakan jumlah kandungan fenolik (TPC) kulit pitaya. Parameter tersebut adalah (1) berat sampel, (2) suhu, (3) kuasa, dan (4) masa pengekstrakan. Hasil kajian menunjukkan bahawa keadaan terbaik untuk parameter MAE berada pada kuasa 400 W, suhu 45°C, dan 20 minit masa sentuhan apabila pengekstrakan dilakukan pada 1.2 g kulit pitaya dalam 50 mL air suling. Nilai-nilai ini telah disahkan melalui analisis statistik SPSS dengan ujian Bonferroni *post hoc*. TPC dalam ekstrak cecair diukur dalam unit GAE/g. Di samping itu, kepekatan perencatan (IC₅₀) ekstrak cecair telah ditentukan dengan menggunakan keadaan terbaik bagi parameter MAE dan DPPH reagen sebagai sintetik radikal bebas. Nilai IC₅₀ yang diperoleh dalam kajian ini adalah 0.52 mg/mL.

Keywords: Gelombang ketuhar, kulit pitaya, ekstrak pitaya, parameter, jumlah kandungan fenolik

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1.0 INTRODUCTION

The dragon fruit, also known as pitaya, has three types, namely (1) *Hylocereus undatus* with red skin and white flesh; (2) *Hylocereus polyrhizus* with red skin and red flesh; and (3) *Selenicereus megalanthus* with yellow skin and white flesh [1]. *Hylocereus polyrhizus* (*H. polyrhizus*) was selected for this study as it contains high phenolic compound and antioxidant activity compared to other pitaya species.

The utilisation of waste fractions including peels can provide environmental and economic benefits, particularly if green solvents like water are used. Additionally, 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 [2, 3]. The integrity of these active compounds was preserved by applying

microwave-assisted extraction (MAE) on the pitaya peel.

MAE may be labelled as “green technology” according to environmental standards. Furthermore, a majority of the plant extracts obtained through MAE are dietary polyphenol such as flavonoids, anthocyanin, and carotenoid [1]. Notably, phenolic compounds from pitaya are highly soluble in water and alcohol due to their polar structure [2]. Therefore, various solvents such as methanol, ethanol, and acetone with different proportions of water were used to extract the polyphenol compound from different plant materials. Nonetheless, this practice resulted in harmful effects [3]. In previous studies, pitaya peel was extracted using organic solvents in the extraction process. For example, solvent with 50 % to 95 % ethanol was applied into MAE to extract active compounds [4]. Thirugnanasambandham *et al.* [5] also applied 95 % ethanol into MAE to extract pectin from pitaya peel.

The literature review revealed that very few total phenolic content (TPC) studies on *H. polyrhizus* were conducted using distilled water into MAE. In the current research, distilled water was used as an extraction solvent due to its nontoxic application [6].

MAE is equipped with a power sensor with power range of 0-1000 W, an infrared temperature sensor, and a temperature controller. The method is based on the direct effect of ionic conduction and dipole rotation on molecules [7]. Moreover, the operation of MAE implements microwave energy together with the polar solvent. The microwave energy stresses the sample thermally, resulting in increased temperature and pressure inside the sample. Consequently, the plant tissue ruptures and releases the targeted compounds into the surrounding solvent [8]. Chemat *et al.* [9] explained that the increased extraction yield under microwave radiation may be caused by the synergistic combination of two transfer phenomena – mass and heat – working in the same direction.

Nevertheless, to the best of the researchers' knowledge, MAE has not been employed for determining the best condition for the extraction of TPC from pitaya peel. Many factors can affect the performance and efficiency of MAE on phenolic compound extraction such as (1) power, (2) type of solvent, (3) particle size of the sample, (4) extraction time, (5) temperature, and (6) solid-liquid ratio [10]. Hence, the objective of this study was to estimate the influence of various MAE extraction conditions, namely the effects of power, time, temperature, and sample weight on the yield of TPC from pitaya peel.

The best condition for the extraction process of polyphenol is required to increase the amount of the compound in the solvent. Polyphenol is extracted from different plants and research demonstrated that power and irradiation time affect polyphenol yield positively. Meanwhile, degradation was also observed at high microwave power levels and long extraction time [11-14].

The liquid extract obtained from MAE was evaluated for inhibitory concentration (IC_{50}) which depicts its potential in reducing free radicals. Free radical is a toxic substance produced during the oxidation process in the human body [15]. DPPH or 2,2-diphenyl-2-picrylhydrazyl was applied as the synthetic free radical to represent the reaction with antioxidants in the pitaya peel extract. As stated by Ajila *et al.* [16], the lowest value of IC_{50} indicates the highest strength of the extracts to act as DPPH scavengers. Apart from that, lower IC_{50} value is associated with stronger DPPH scavenging capacity in the sample [17].

The pitaya peel contains various minerals and has a lot of antioxidant compounds compared to other subtropical fruits [18]. In this research, the pitaya peel of *H. polyrhizus* was used. Notably, *H. polyrhizus* contains higher phenolic content compared to other species of pitaya fruit [15]. The peel comprised outer and inner layers known as albedo. Both layers contained red pigments (betacyanin) and yellow pigments (betaxanthins).

The red colour from *H. polyrhizus* is highly demanded, especially in the market, because of its health benefits and potential to replace synthetic dyes [19]. The fruit has anti-inflammatory and antidiabetic properties, suppression effect on cardiovascular diseases, and potential for cancer prevention [21]. In addition, the colour of the pitaya fruit is appealing and attracts a lot of attention from the consumers [20].

2.0 METHODOLOGY

2.1 Sample Collection

The pitaya peels from *H. polyrhizus* were obtained from a supermarket in Temerloh, Pahang. The peels were weighed and washed with distilled water. Consecutively, the peels were cut into 2 cm portions. For the freeze-drying process, the samples were frozen overnight in the fridge at -80°C and then placed in the freeze dryer for 96 h.

Afterward, the freeze-dried samples were grounded and sifted through a 20 mesh (0.85 mm) sieve to produce the powdered samples. These samples were stored in bags and kept in a dry environment prior to conducting the experiment [4].

2.2 Microwave-assisted Extraction (MAE)

The extraction was carried out using the MAE approach with the one-factor-at-a-time method (OFAT). Liquid-solid extraction was conducted by adding the freeze-dried sample with 50 mL distilled water into a 1000 mL extraction vessel. Subsequently, the extraction vessel was placed into MAE. The conditions of MAE, particularly power, temperature, time, and weight of the sample were observed.

Immediately after the extraction process, the homogenate was centrifuged at 9000 rpm for 40 min at 25°C . After the centrifugation, the supernatant was collected and the same procedure was repeated twice to ensure maximum extraction of the active compounds. All experiments were performed in triplicates [5].

2.3 Effect of Parameters

The yield of active compounds from the extraction of pitaya peel depends on the parameters used in the experiment. In this research, only the effect of power, temperature, time of MAE, and weight of the pitaya peel were studied. These four parameters were anticipated to influence the yield of active compounds from the process significantly [6].

2.3.1 Effect of Power in MAE

Experiments were performed with different levels of extraction power while other parameters were kept constant [4, 5, 7]. The raw material was placed into

MAE at the power levels of 200 W, 400 W, 600 W, and 800 W. Simultaneously, the temperature was fixed at 45 °C, the time was constant at 20 min, and the weight of the pitaya peel was maintained to be 1.2 g. After the process, the liquid extract formed was treated using a centrifuge for 40 min to remove any residue. Subsequently, the TPC yield was determined using UV-VIS.

2.3.2 Effect of Temperature in MAE

Experiments were carried out with different extraction temperatures while other parameters were kept constant with slight modification [4, 5, 7]. The raw material was placed into MAE at the temperatures of 35 °C, 40 °C, and 45 °C. The power was fixed at 400 W, meanwhile time was maintained to be 20 min and the weight of the pitaya peel was kept constant at 1.2 g. After the process, the liquid extract formed was treated using a centrifuge for 40 min to remove any residue and the yield was analysed using UV-VIS to check for TPC.

2.3.3 Effect of Time in MAE

Experiments were executed with different extraction times while other parameters were fixed with slight modification [4, 5, 7]. The raw material was placed into MAE for 10 min, 15 min, 20 min, 25 min, and 30 min. Other parameters were kept constant: power at 400 W, temperature at 45 °C, and weight of the pitaya peel at 1.2 g. After the process, the liquid extract formed was treated for 40 min using a centrifuge to remove any residue and UV-VIS was applied to check for TPC in the yield.

2.3.4 Effect of Sample Weight in MAE

Various sample weights were used in the experiments while other parameters were maintained with only minor modification [4, 5, 7]. The raw materials weighing 1.2 g, 2.2 g, and 3.2 g were placed into MAE. The temperature, time, and power were fixed at 45 °C, 20 min, and 400 W, respectively. The liquid extract formed after the process was treated to remove any residue using a centrifuge for 40 min. Consecutively, the yield was examined using UV-VIS to check for TPC.

2.4 Analysis of Solution Extraction

2.4.1 Determination of Total Phenolic Content (TPC)

The antioxidant level for each sample was measured using the TPC assay with the Folin-Ciocalteu method which was modified by Lim *et al.* [8]. In the test tubes, the researchers measured 0.3 mL of each extract 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 to the test tubes. The tubes were shaken and incubated in the dark for 30 min at room temperature.

Subsequently, 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 for gallic acid ($y = 0.094x$, $r^2 = 0.9985$), and the contents were expressed as mg gallic acid equivalent (GAE)/g of the sample.

2.4.2 Free Radical Scavenging Activity Assay

In this experiment, DPPH was used as a synthetic free radical that can react with the antioxidant content in the pitaya peel to form the DPPH complex compound. The reaction was noticeable visually as the colour changed from purple to yellow due to the hydrogen-donating ability [9].

The free radical scavenging activity was determined using the method by Khamsah *et al.* [10] with minor adjustments. The reagent and solution for the assay analysis 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.

Furthermore, 1 mL pitaya peel extract was added to 2 mL DPPH reagent (0.1 mm) in a test tube. Then, the solution was mixed using 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 rates were recorded. Notably, all tests were carried out in triplicates.

The antioxidant activity of the samples was defined with Equation 1:

$$\text{DPPH scavenging activity (\%)} = \left[1 - \left(\frac{A_{\text{test}}}{A_{\text{control}}} \right) \right] \times 100\% \quad (1)$$

where A_{control} was the absorbance of the DPPH solution without the extract. Conversely, A_{test} was the absorbance of the DPPH solution in the presence of the extract comprising 2 mL DPPH and 1 mL test compound.

2.5 Statistical Analysis

Data from the triplicate experiments were presented in the forms of mean and standard error of the mean. The statistical analysis of the results was performed using one-way ANOVA with Bonferroni multiple comparison post hoc tests. All statistical analyses were performed using IBM SPSS Statistic 21.0 [11].

2.5.1 One-way ANOVA

In this research, one-way ANOVA was applied to compare the mean values of the TPC. The significant difference was determined by comparing p -value with the error that had been set in SPSS for this research, namely 5 % error. Two hypotheses were formulated for

the research. If $p < 0.05$, significant difference exist among the TPC values and hence, H_0 is rejected. On the contrary, if $p > 0.05$, no significant different is present among the TPC values and hence, H_0 is accepted.

3.0 RESULTS AND DISCUSSION

3.1 The Effect of MAE Parameters

MAE which is an innovative and green extraction technique offers high reproducibility in a shorter time, reduces solvent consumption, and has lower energy input without decreasing the extraction yield of the target species [12]. Due to these advantages, MAE was implemented as an alternative technique to release the active components from pitaya peel [5]. In this research, the effect of power, temperature, time of MAE, and weight of sample were studied by analysing the data using SPSS.

SPSS is a statistical tool that is mainly used for the modelling and investigation of a multivariable system in which a response of interest is influenced by several variables. The most common design under SPSS is indicated by one-way ANOVA with Bonferroni multiple comparison tests. This study may provide a crucial solution to environmental-related problems and extraction of valuable by-products from fruit waste.

3.1.1 The Effect of Power

The power applied in MAE ranged from 200 W to 800 W, and the optimum power was determined by the concentration of TPC. The effect of power on TPC value is shown in Figure 1.

Initially, the TPC value increased when the power varied from 200 W to 400 W. Prakash Maran *et al.* [6] explained that the higher microwave power means higher direct effect of microwave energy on the sample. Consequently, this accelerates the extraction of active compound. Moreover, the microwave radiation loosens the cell wall and skin tissues of the sample; this increases the interaction between solvent extraction and sample extraction [13].

Furthermore, during the extraction process, power spread inside the solvent and pitaya peel then generated molecular movement and heating. Vast electromagnetic energy was transferred to the samples through ionic conduction and dipole rotation to improve the efficiency and expedite the extraction system [14].

Nevertheless, the TPC value started to decrease when the power ranged from 600 W to 800 W. This may be attributed to the degradation of compounds with the higher microwave power during extraction [15].

From the statistical analysis, power of 200 W and 800 W had no significant difference with their TPC values of 3.411 mg GAE/g extract and 3.503 mg GAE/g extract, respectively. Moreover, application of 400 W (TPC = 5.808 mg GAE/g extract) produced a significant

difference with 600 W (TPC = 4.784 mg GAE/g extract). As a result, the best power to obtain the maximum TPC value was 400 W. This condition was further applied to study the effect of time, weight, and temperature.

This finding is similar to other studies on extraction of pitaya peel [7] and orange peel [6]. These researches showed that the active compound –pectin – increased with higher power and decreased when the power applied was over 400 W.

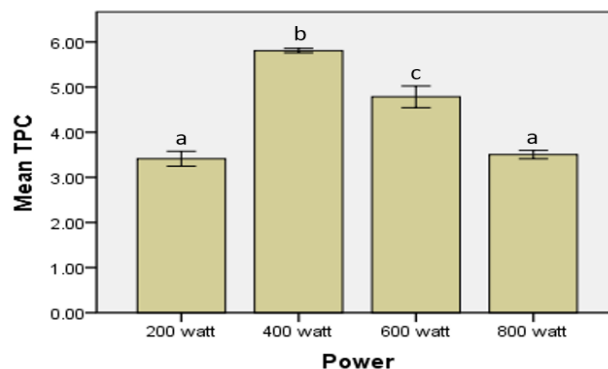


Figure 1 Effect of Various Power Applications on TPC yield

Notes:

(1) Error bars represent the standard deviation from the mean of triplicate experiments.

(2) Different letters indicate significant differences at $p \leq 0.05$.

3.1.2 The Effect of Temperature

The temperature for the MAE ranged from 35 °C to 50 °C and the optimum temperature was determined by the concentration of TPC. The effect of temperature on the TPC values is shown in Figure 2.

Initially, the TPC value increased when the temperature ranged from 35 °C to 45 °C due to the high formation of dipole rotation [5]. Conversely, the TPC value decreased at 50 °C. This corresponded with Lee *et al.* [16] who stated that further increase of temperature causes a negligible effect on the extraction of active compounds from pitaya peel using the MAE technique.

No significant difference was detected for temperatures of 35 °C and 40 °C with their TPC values of 5.126 mg GAE/g extract and 5.273 mg GAE/g extract, respectively. In contrast, 45 °C (TPC = 5.8 mg GAE/g extract) and 50 °C (TPC = 4.769 mg GAE/g extract) generated significant difference. Therefore, the best temperature to obtain the maximum TPC yield was 45 °C. Accordingly, this condition was further applied to study the effect of time and weight.

The result obtained agreed with previous work carried out by Thirugnanasambandham and Sivakumar [5] on pitaya peel extract. Their work proved that the active compound, namely betalain, increased with higher temperature and experienced negligible effect when extreme temperature was applied during MAE. Pinela *et al.* [17] also demonstrated that the extraction yield of phenolic acid from tomato increased with

higher temperature, followed by a gradual decrease with higher temperature of extraction.

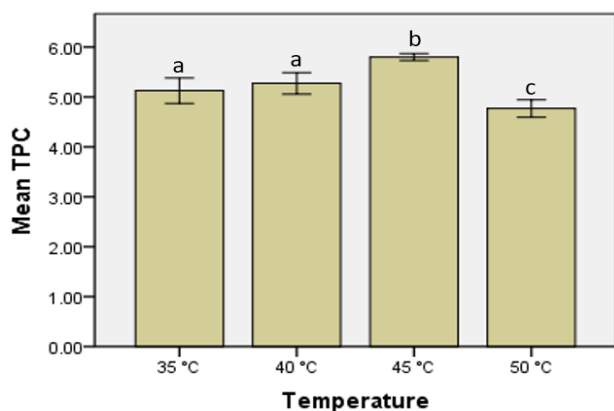


Figure 2 Effect of Various Temperatures on TPC Yield

Notes:

- (1) Error bars represent the standard deviation from the mean of triplicate experiments.
- (2) Different letters indicate significant differences at $p \leq 0.05$.

3.1.3 The Effect of Time

The time ranged from 10 min to 30 min and the optimum time was determined by the concentration of TPC. The effect of time on the TPC values is depicted in Figure 3.

Initially, the TPC value increased when the time increased from 10 min to 20 min as the microwave radiation accumulated heat for the reaction and produced effective extraction of phenolic compounds into the solvent [6, 7]. Nonetheless, degradation of phenolic compound occurred with excessive microwave time exposure [18, 19]. Thus, the TPC value decreased from 25 min to 30 min.

The statistical analysis indicated that 10 min and 30 min had no significant difference with TPC values of 4.806 mg GAE/g extract and 4.892 mg GAE/g extract, respectively. Additionally, times of 15 min (TPC = 51.36 mg GAE/g extract) and 25 min (TPC = 5.267 mg GAE/g extract) also produced no significant difference. The significant difference was attained at 20 min with TPC value of 5.723 mg GAE/g extract. Thus, the best time to obtain the maximum yield of TPC was 20 min. This condition was further used to examine the effect of sample weight.

This finding is similar to the result of a research by Cardoso *et al.* [20] which depicted that extraction of betalain from reed beet increased with longer extraction time but excessive time decreased the betalain content. Furthermore, Hayat *et al.* [21] proved that the extraction of phenolic acid content from citrus mandarin peel increased rapidly with longer extraction time. Nevertheless, the extraction yield gradually decreased with extreme extraction time.

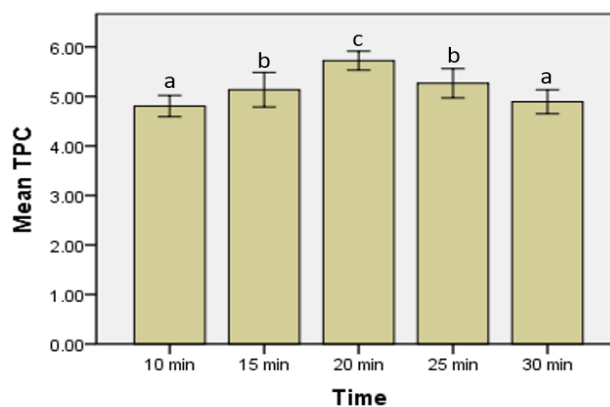


Figure 3 Effect of Various Times on TPC Yield

Notes:

- (1) Error bars represent the standard deviation from the mean of triplicate experiments.
- (2) Different letters indicate significant differences at $p \leq 0.05$.

3.1.4 The Effect of Weight

The optimum sample weight was determined by the concentration of TPC, and the sample weight varied in the range from 1.2 g to 3.2 g. All samples were extracted in the determined weight range and subsequently immersed in 50 mL of water as the extraction solvent. The effect of sample weight on the TPC values is presented in Figure 4.

The result depicted that sample weight of 1.2 g generated the highest TPC value. The cell walls of the sample ruptured when the extraction solvent caused excessive swelling on the pitaya peel and the compound was absorbed in the microwave directly. Therefore, phenolic compound may easily be released into the surrounding medium [22]. In this case, the solvent (water) volume of 50 mL was sufficient to ensure that the entire sample was immersed, especially as the sample would swell during the extraction process [23, 24].

Higher sample mass increases the surface area of the sample. The TPC value decreased sharply and reached its minimum at 2.2 g to 3.2 g as the solvent began to saturate with the sample and this resulted in inefficient extraction parameters. The mass transfer rate was affected negatively and the nonuniform distribution and exposure to microwave heating hindered the penetration of the phenolic compound into the solvent [6, 24].

The statistical analysis demonstrated significant difference for samples weighed 1.2 g, 2.2 g, and 3.2 g with TPC values of 5.708 mg GAE/g extract, 3.779 mg GAE/g extract, and 3.459 mg GAE/g extract, respectively. Hence, the best sample weight to obtain the maximum TPC value was 1.2 g.

This result is also parallel to other studies which depicted that the solution is saturated by the solute with increasing solid-liquid ratio. This decreased the extraction yield [6, 7, 17].

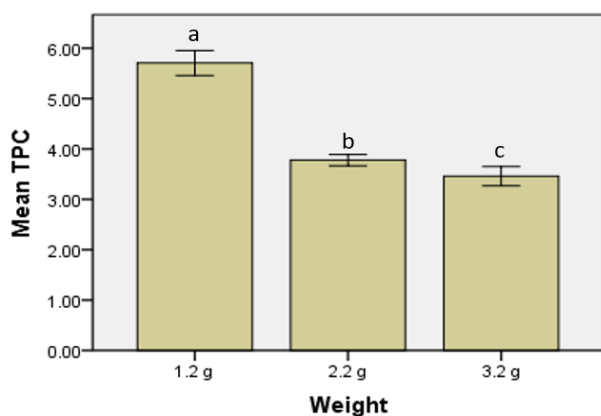


Figure 4 Effect of Various Sample Weights on TPC Yield

Notes:

- (1) Error bars represent the standard deviation from the mean of triplicate experiments.
- (2) Different letters indicate significant differences at $p \leq 0.05$.

3.2 Antioxidant Activity

Figure 5 illustrates the IC₅₀ value (in mg/mL) for the pitaya peel. IC₅₀ is the inhibitory concentration of DPPH at 50% activity to show the potential of the pitaya peel to reduce DPPH in a complex form. Small value of IC₅₀ means that the sample was more potent as an antioxidant. Siddhuraju *et al.* [25] supported this and explained that rapid decrease of absorbance indicates that the primary antioxidant activity is potent. In this study, the equation $y = 89.609x + 3.5776$ was applied to produce IC₅₀ concentration value of the pitaya peel extracted: 0.52 mg/mL.

The experiment conducted by Ruzlan *et al.* [26] revealed that the highest DPPH scavenging activity supported by the IC₅₀ data was exhibited by *H. polyrhizus* peel, followed *H. undatus* peel, *H. polyrhizus* pulp, and *H. undatus* pulp. In addition, many previous studies reported that high polyphenol content contributes towards high DPPH scavenging activity [27].

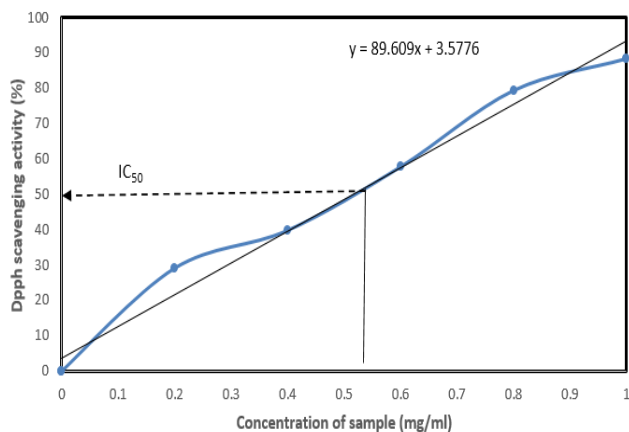


Figure 5 DPPH Scavenging Activity of Pitaya (*H. Polyrhizus*) Peel Extracts

4.0 CONCLUSION

In conclusion, the optimal MAE parameters were 400 W of power, 45°C of temperature, 20 min of time, and 1.2 g of sample weight. Evidently, these parameters were essential in the extraction of pitaya peel. Moreover, the pitaya peel extract produced the IC₅₀ value of 0.52 mg/mL.

Apart from that, SPSS software was a great tool to determine the significant analysis of experimental data. This software enabled the best conditions for the parameters of MAE to be identified successfully.

These results showed the potential of pitaya peel to be applied in the food industry as a natural colouring and the effectiveness of MAE process as a powerful extraction technique with minimal sample weight for the process. Furthermore, the process imposed low cost as distilled water was adopted as the extraction solvent and pitaya peel was used as the raw material.

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