Assessment of fatty acid composition and response surface optimization of ultrasonic-assisted extraction of phenolic compounds from *Pouteria campechiana* pulp

Nur Haziqah Che Marzuki a, Mariani Abdul Hamid b, Roswanira Abdul Wahab a.*

a Faculty of Science, Department of Chemistry, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia

b Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia

* Corresponding author: roswanira@kimia.uts.my

**INTRODUCTION**

Malaysia is one of few Southeast Asian countries that has several underutilized fruits and as such, they are rarely eaten (Ikram et al., 2009). Many of the nutritional or phytochemical components prevailing in these native fruit species are yet to be discovered. *Pouteria campechiana* (PC), belonging to the family Sapotaceae is among such fruit. PC is commonly called ‘Buah Kuning Telur’ in Malay while it bears a simpler English name ‘canistel’. Historically, PC is native to Central America but the fruit is widely distributed around the tropical and subtropical regions in South America and parts of Asia such as Sri Lanka, Indonesia and south Asian countries (de Lanerolle et al., 2009; Silva et al., 2009). The fruit of PC is ovoid-shaped measuring between 7.5−12.5 cm in length and 5−7.5 cm in breadth (Ma et al, 2004). When its skin is peeled away, it reveals a yellow pulp which centre may consist one to four hard seeds (Balerdi and Shaw, 1998). The richly textured PC pulp consists of a myriad of nutrients viz., carbohydrates, vitamin A and minerals (Coronel et al., 1986). The pulp can be eaten fresh and its rich texture makes it a popular ingredient in custards, ice creams, milkshakes, jam as well as marmalade. Claustro et al. (1997) reported that the seeds of PC can be made into a precooked drink that is comparable to coffee. Despite the various interesting uses, consumption of the PC fruit is unheard of among Malaysians.

The phytochemical composition of PC pulp mainly has triterpenes and flavonoids (Ma et al., 2004), in which the former may be found as long chains of acetate esters (Hernández et al., 2006; Lott and Jackes, 2001; Solís et al., 2004) Pertinently, bioactivity seen in extracts of PC pulp is associated with the plethora of polyphenolic antioxidants viz., gallic acid, myricitrin, (+)gallochatechin, (+)-catechin, dihydromyricetin, (+)-catechin-3-O-gallate and (−)-epicatechin (Ma et al., 2004). Natural fatty acids are also found in other *Pouteria* sp. i.e. the nuts of *P. lucuma* and are known for promoting skin regeneration (Rojo et al., 2010). Whereas the extracts of *P. ramiflora* have neuroprotective effects against oxidative damage. Other roles of these fatty acids also include restoring levels of myosin-Va protein in the brain and preventing hippocampal neuronal loss (Da Costa et al., 2013). In view of such interesting reports, the study believes the polyphenolic rich extract of PC pulp (Ma et al., 2004) may be suited as a bioactive ingredient in formulating an anti-ageing nanoemulsion for use on human skin.

To prevent the loss or destruction of potential bioactive components in the PC pulp during extraction, appropriate steps must, therefore, be taken. Selection of the extraction method for obtaining plant-based bioactive components usually depends on factors such as the nature of plant material and types of compounds present (Sasidharan et al., 2011). In this regard, the study resorted to using ultrasonic-assisted extraction (UAE) of the polyphenolic bioactive components in PC pulp powder were statistically optimized using the Central Composite Design (CCD). Conditions that maximized TPC in the crude extract of PC pulp powder were assessed for factors, ratio of ethanol:water, extraction temperature and extraction time. The established optimum conditions of the CCD model with a value of $R^2 = 0.8833$, were within the studied range and agreed well with the predicted values. Under an optimized condition [30 min, 35 °C and ratio of ethanol:water, 60:40 (% v/v)], the highest TPC was 1162.80 mg GAE/100 g in comparison to the predicted 1115.06 mg GAE/100 g. High Performance Liquid Chromatography confirmed that gallic acid and its derivatives were the major components, comprising a 0.03 % (w/w) of the PC pulp crude extract. Pertinently, a high recovery value in the HPLC validation data (109.84 %, $R^2 = 0.9995$) suggests that the method was accurate.

**Keywords:** *Pouteria campechiana*, fatty acid composition, response surface methodology, total polyphenolic content, ultrasound-assisted extraction.

© 2018 Penerbit UTM Press. All rights reserved
solvents i.e. applied in solid/fluid media (Esclapez et al., 2011). This extraction process is faster and more efficient as compared to conventional methods viz. maceration/stirring. This is due to the higher surface contact area between the solid and liquid phases induced by multiple particle disruption processes in UAE (Herrera and Luque de Castro, 2004). One main advantage of UAE is that additional modifications on the extracts are not required, except for filtration of the extracts before chromatographic analysis (Esclapez et al., 2011).

Conversely, UAE applications in solid/gas systems, however, are uncommon due to impedance mismatch and the poor transmission of ultrasound through air (Esclapez et al., 2011). Most importantly, the use of UAE is considerably greener for harvesting bioactive compounds in plant materials (Dal Pra et al., 2017). The operating process of UAE is simple, requiring the sample to be mixed with a suitable solvent(s) before placing into an ultrasonic bath/ultrasonic probe (Hromadkova and Ebringerová, 2003). One marked difference between an ultrasonic bath and probe is that the former only allows the optimization of extraction time and temperature. Conversely, the ultrasonic probe is more versatile where factors including extraction time and temperature, output of the ultrasonic source (amplitude) and energy pulsation possibility can be optimized (Adam et al., 2009).

Nonetheless, the different combination of UAE process parameters could have significant effects on the extraction yield of bioactive compounds from the PC pulp. To overcome this issue, the study employed a statistical approach called response surface methodology (RSM) to identify the optimum combination of extraction parameters that guarantees maximum extraction effectiveness. This second order polynomial prediction model has been commonly used to establish the best experimental process parameters. Unlike other statistical methods such as that of single factor experiment and orthogonal design method, RSM allows the observation of the interactive effects between process parameters while reducing the number of experiments (Ishah et al., 2017; Manan et al., 2018; Manan et al., 2016; Marzuki et al., 2015). Herein, the study focused on developing a highly efficient protocol for UAE of PC pulp bioactive compounds. To date, there are no reports detailing the optimization of extraction of polyphenolic compounds from PC pulp by UAE. By this virtue, there is a need to develop a suitable UAE method with favors maximum extraction of polyphenolic compounds with high antioxidant activity. In this study, significant extraction parameters were identified and the optimum conditions of UAE were predicted using the generated Central Composite Design (CCD) model.

**EXPERIMENTAL**

**Reagents**

Ciocalteu’s phenol reagent was purchased from Sigma-Aldrich (St. Louis, USA). The grapeseed oil was purchased from Borges (Catalonia, Spain). Absolute ethanol, methanol and anhydrous gallic acid standard were purchased from Merck (Darmstadt, Germany). Other chemicals such as potassium hydroxide, sodium carbonate, hydrochloric acid and boron trifluoride-methanol solution were also acquired from Sigma-Aldrich (St. Louis, USA). Nylon microfilter with a pore size of 0.45 µm was purchased from Borges (Catalonia, Spain). Absolute ethanol, methanol and anhydrous ammonium acetate were also acquired from Sigma-Aldrich (St. Louis, USA). Other chemicals such as potassium hydroxide, sodium carbonate, hydrochloric acid and boron trifluoride-methanol solution were also acquired from Sigma-Aldrich (St. Louis, USA). Nylon microfilter with a pore size of 0.45 µm was purchased from Sigma-Aldrich (St. Louis, USA). Nylon microfilter with a pore size of 0.45 µm was purchased from Sigma-Aldrich (St. Louis, USA). Nylon microfilter with a pore size of 0.45 µm was purchased from Sigma-Aldrich (St. Louis, USA).

**Sample preparation**

The mature ripened fruits of *P. campechiana* (PC) were collected from Nasuha Herbs and Spices (2° 2’ 21.38” N latitude, 102° 34’ 8.7’’ E longitude) in the Pagoh area of Johor, Malaysia. The fruits were cleaned from Nasuha Herbs and Spices (2° 2’ 21.38” N latitude, 102° 34’ 8.7’’ E longitude) in the Pagoh area of Johor, Malaysia. The fruits were cleaned from Nasuha Herbs and Spices (2° 2’ 21.38” N latitude, 102° 34’ 8.7’’ E longitude) in the Pagoh area of Johor, Malaysia. The fruits were cleaned from Nasuha Herbs and Spices (2° 2’ 21.38” N latitude, 102° 34’ 8.7’’ E longitude) in the Pagoh area of Johor, Malaysia. The fruits were cleaned

**Proximate analysis**

The moisture content of the PC pulp was analyzed according to the protocol set out by the Association of Analytical Communities based on the AOAC 993.01-Moisture (Loss on Drying) (AOAC 934.01, 1934). The PC pulp was dried in a vacuum oven at 70 °C to a constant weight before the measurement was carried out. Ash content of the PC pulp was determined according to the AOAC 923.03-Ash (Direct Method) whereas the Kjeldahl method was used to estimate the protein content, was done as described by the AOAC 988.05 and 981.10. Estimation of the protein content was calculated by multiplying the total nitrogen content (%) by a factor of 6.25. Lipids in the PC pulp were extracted using a Soxhlet apparatus maintained at temperatures between 40–60 °C using petroleum ether as the solvent (BS ISO 8262-3:2005–Fat Gravimetric Method). Total carbohydrate (TC) content including the fiber content in the PC pulp was estimated using the following equation: [TC = 100 – (Moisture + Total ash + protein + fat)]. Finally, calculation of total energy value, expressed in Kcal/100 g (wet basis), was based on Methods of Analysis for Nutrition Labelling (Nutrition Labelling and Education Act, 1991). All measurements were performed in triplicates.

**Fatty acid (FA) composition analysis**

Fatty acid methyl esters (FAME) were prepared as described by the following IUPAC methodology without heating. A 100 g sample of PC pulp extract was saponified with 1.2 mL of methanolic potassium hydroxide (0.5 M) at 60 °C for 10 min, neutralized with hydrochloric acid (0.7 M). The mixture was methylated in a 3.0 mL solution of boron trifluoride-methanol for approximately 10 min in a 60 °C water bath. The fatty acids were extracted with petroleum ether under varying temperatures that ranged between 40–60 °C. The obtained FAME was separated using a GCMS-QP2010 PLUS Shimadzu, Japan. Also, the FAME fractions were separated on a VARIAN Saturn 2100D GC-MS equipped with a CP7420 (100 m x 0.25 mm i.d.) column and helium (1 mL/min) was used as the carrier gas. The oven temperature was programmed as follows: initial oven temperature of 80 °C held for 1 min, increased to 160 °C at 20 °C/min, increased to 198 °C at 1 °C/min, and finally increased to 250 °C at 5 °C/min, held for 5 min. Injector and detector temperatures were 250 °C and 180 °C, respectively (Villa-Rodríguez et al., 2011). The retention times of the fatty acids were compared with those of standards for their identification and the composition of each fatty acid was reported as relative percent.

**Ultrasound-assisted extraction (UAE) process of PC**

The ultrasound-assisted extraction (UAE) of PC pulp powder was carried out using an ultrasonic processor (VCX 130, Sonics, UK). The power and frequency of the ultrasonic processor was set at 130 watts and 20 kHz, respectively, using a variable power output controller. The probe used in this process was a 6 mm in diameter titanium horn and the sample was irradiated directly from the horn. Sonication was carried out in a stainless steel beaker that consisted of a 10 g of fresh PC pulp mixed in variable ratio of ethanol:water (v/v) as the solvent (working volume 100 mL). The mixture was sonicated for durations ranging from...
5–30 min under continuous mode. The resulting suspension was centrifuged at 6 000 rpm for 20 min and filtered through a filter paper Whatman No. 1. Ethanol was removed by vacuum evaporation under 40 °C on a rotary evaporator. The samples were frozen, lyophilized and stored at 4 °C.

Experimental design and optimization of process parameter

Optimization experiment was carried out using response surface methodology (RSM) to extract polyphenolics in the PC pulp powder while maximizing the response i.e. antioxidant activity. A full-factorial three-level-three factor, Central Composite Design (CCD) required 20 experimental runs that comprised of six axial points, eight factorial points and six central points, were used in the experiment. The relevant extraction factors assessed were: extraction time (A: 10, 20 and 30 min), extraction temperature (B: 25, 30, 35 °C) and ratio of ethanol:water (C: 60, 70 and 80 %, v/v) to affect the response i.e. total phenolic content (TPC). Table 1 shows the independent variables and their levels, as well as the actual and coded values for the process optimization. Each factor was coded at three levels (−2, −1, 0, +1, +2).

Table 1 The actual and coded independent variables for the Central Composite design for the extraction of total phenolic content of Ficus racemosa L. fruit.

| Variables  | Coded Levels |
|------------|--------------|
| A: Extraction time (min) | −2 | 0 | +1 | +2 |
| B: Extraction temperature (°C) | 21.59 | 0 | 30 | 35 | 38.41 |
| C: Ratio of alcohol (% v/v) | 53.18 | 0 | 70 | 70 | 86.82 |

All experiments were performed in triplicates. For clarity, the range of values for the three independent factors were determined by an earlier screening study. Experimental data were fitted to a second-order polynomial model to obtain the regression coefficients (β). A mathematical regression model for the response of Y (the predicted response) was fitted in Equation 1.

\[ Y = \beta_0 + \sum_{i=1}^{a} \beta_i X_i + \sum_{i=1}^{a} \beta_i X_i^2 + \sum_{i=1}^{a} \sum_{j=1}^{a} \beta_{ij} X_i X_j \]

where Y is response (TPC, [mg GAE/100 g dw]), β0 is the coefficient constant, \( \beta_i \) is the linear coefficient, \( \beta_{ii} \) is the coefficient of squared effect, \( \beta_{ij} \) is the coefficient of interaction effect, and \( X_i \) and \( X_j \) are the coded values of variables i and j, respectively (extraction time \( X_1 \), extraction temperature \( X_2 \), and ethanol concentration \( X_3 \)).

Total phenolic content (TPC)

TPC was determined using Folini–Ciocluceau method described by (Ascevatham et al., 2014). Briefly, 100 μL of Folini–Ciocluceau reagent and 200 μL of Na2CO3 (2 %, w/v) were transferred into a test tube containing 100 μL of the PC extract (1 mg/mL). The mixture was incubated for 15 min at 45 °C with shaking at 200 rpm before the absorbance was read at 765 nm using a UV–visible spectrophotometer (SHIMADZU, 1800 UV-VIS, JAPAN). TPC was expressed as milligrams per gram of gallic acid equivalents (mg/g GAE). Different concentrations of gallic acid (GA) (0–10 μg/mL) were prepared by dissolving GA in methanol. A linear standard graph was obtained by plotting the various concentrations of GA along the x-axis and absorbance along the y-axis. All experiments were performed in triplicates.

High performance liquid chromatography (HPLC) analysis

PC crude extract was dissolved in HPLC grade methanol to make up a 20 mg/mL sample solution before filtering through a 0.45 μm nylon microfilter (Phenomenex). The extracts were analyzed using a HPLC (Agilent 1200 series, Agilent Technologies, USA) equipped with a UV detector (G1314B, Agilent) and an Eclipse XDB-C18 column (5 μm, 250 mm×4.6 mm, Agilent). The mobile phase consisted of 3 % aqueous acetic acid (A) and methanol (B) set at a flow rate of 1 mL/min. The mobile phase composition began with 100 % of A that was maintained for 1 min, followed by a linear increase to 63 % of B in 27 min, and then the composition returned to the initial condition within 5 min for the next run. Comparison of retention times and spectral data of the samples with those of the standards were used to identify and assignment the peak of the phenolic compounds. Quantification of phenolic compounds was estimated using the respective calibration curves for gallic acid.

RESULTS AND DISCUSSION

Proximate analysis

Table 2 presents the results for the proximate analysis of PC pulp (dry weight). Results revealed that the contents for moisture, ash and protein in PC pulp powder are very low, amounting to approximately 8.4, 1.7 and 4.0 %, respectively. The low moisture content (8.4 %) seen here indicates the PC pulp can be sufficiently dehydrated for storage over an extended period of time without the loss of quality. This is because a high moisture content can subsequently lead to a reduced keeping quality and shortened shelf life (Jaafar et al., 2009). The low ash content (1.7 %) infers a low quantity of total inorganic minerals whereas the high percentage of carbohydrates (84.9 %) agreed well with the high total energy value (364.6 kcal/100 g) of the PC pulp powder. These results are consistent with the richly textured fresh PC pulp, suggesting it is categorically a high calorie food, similar to an earlier description by Berto et al. (2015).

Table 2 Results of proximate analysis for the pulp of PC.

| No | Parameters | Test Method | Results  |
|----|------------|-------------|---------|
| 1  | Moisture   | In-house STP/FL313/002/07 (based on AOAC 934.01)Moisture (Loss on Drying) | 8.4 % |
| 2  | Ash        | In-house STP/FL313/001/07 (based on AOAC 923.03) – Ash (Direct Method) | 1.7 % |
| 3  | Protein    | In-house STP/FL313/005/07 (based on AOAC 988.05 & 981.10) – Protein (Kjeldahl Method) | 4.0 % |
| 4  | Fat        | In-house STP/FL313/003/07 (based on BS ISO 8262-3:2005) – Fat (Gravimetric Method) | 1.0 % |
| 5  | Carbohydrate | In-house STP/FL313/007/07 based on Methods of Analysis for Nutrition Labelling | 84.9 % |
| 6  | Total Energy Value | In-house STP/FL313/007/07 based on Methods of Analysis for Nutrition Labelling | 364.6 Kcal/100g |
Fatty acid (FA) composition analysis

It is worth mentioning here that reports on the fatty acid composition of PC pulp remains limited (Silva et al., 2009). Table 3 illustrates the fatty acid composition in PC pulp, expressed as the percentage of total fatty acid (TFA) content of the PC crude extract. A total of 14 different types of fatty acids was identified with palmitic acid (C16:0, 24.5 %), oleic acid (C18:1, 19.1 %), myristic acid (C14:0, 16.1 %) and linolenic acid (C18:2, 14.1 %) being the major types of fatty acids that cumulatively amounted to 73.8 % of the TFA content in the PC pulp. The present study confirmed that the PC pulp is comprised of a substantially high percentage of saturated fatty acid (SFA), suggesting that the fruit is a good source of SFA. SFA (55.9 %) formed a considerable proportion of the TFA composition in PC pulp, in which myristic acid (C14:0) and palmitic acid (16:0) were the dominant fatty acids, constituting 40.6 % and 24.5 % of the TFA content, respectively. An approximate 23.4 % of monounsaturated fatty acids (MUFA) was present whereas polyunsaturated fatty acids (PUFA) contributed 16.5 % of TFA. The order of abundance in terms of unsaturation of the fatty acids are as follows:

SFA > MUFA > PUFA

Table 3 Fatty acid content of PC pulp (dry weight).

| Fatty acid composition | Percentage (%) |
|------------------------|----------------|
| Hexanoic acid C6:0     | 0.1            |
| Caprylic acid C8:0     | 3.1            |
| Capric acid C10:0      | 1.2            |
| Lauric acid C12:0      | 3.9            |
| Myristic acid C14:0    | 16.1**         |
| Palmitic acid C16:0    | 24.5*          |
| Palmitoleic acid C16:1 | 4.4            |
| Stearic acid C18:0     | 3.3            |
| Oleic acid C18:1 cis   | 19.1*          |
| Oleic acid C18:1 trans | < 0.1          |
| Linoleic acid C18:2 cis| 2.4            |
| Linoleic acid C18:2 trans | < 0.1       |
| Linolenic acid C18:3 cis | 14.1*       |
| Linolenic acid C18:3 trans | < 0.1        |
| Arachidic acid C20:0   | 0.6            |
| Behenic acid C22:0     | < 0.1          |
| Lignoerotic acid C24:0 | 3.0            |
| Others                 | 4.2            |

Fatty acids found in natural oils have several beneficial properties suitable for application in cosmetic and personal care products. In cosmeceutical emulsions, plant oils can be constituents of the oily phase due to their low molecular weights and low viscosities. These qualities are the reasons for plant oils being preferred over mineral oils to prepare emulsions (Bwai et al., 2013). Interestingly, the fatty acid content in the PC pulp was found comparable to the pulp of the more widely known fruit, the Hass avocado (Persea americana) (Villa-Rodriguez et al., 2011). The findings are consistent with our observation on the pulps of ripened PC and Hass avocado being similarly textured, having a rich and yet unsweetened taste. The relatively high contents of oleic and palmitic acid imply their potential application for topical cosmetic uses. Various studies have shown that oleic acid exhibits the best permeation enhancing effect among unsaturated fatty acids (Kim et al., 2008; Rabasco Álvarez and González Rodríguez, 2000), whereas for the SFAs, palmitic acid is the most potent (Kim et al., 2008; Vermaak et al., 2011). Skin permeation enhancement effects were also reported for linoleic, lauric, myristic and stearic acids (Vermaak et al., 2011). Based on the findings, the PC pulp has a fatty acid profile that can act as an enhancer for drug permeation for transdermal and topical drug delivery. Moreover, the aforementioned fatty acids are natural and edible, which increases their applicability as an additive or component in cosmetics (Kanikkanthan and Singh, 2002; Tanojo et al., 1997).

Optimization of the ultrasound-assisted extraction condition for improving phenolic contents using response surface methodology (RSM)

RSM is a statistical method to analyse and estimate the optimum levels of selected factors within a design range (Sharmila et al., 2016). The use of RSM has been found practical for various optimization experiments to extract polyphenolic compounds from several types of fruits viz. Citrus sinensis L. peel (Khan et al., 2010), Mangifera pajang peels (MPP) (Prasad et al., 2011) and Malus domestica (Alberti et al., 2014). In this study, the effects of process parameters viz. solvent ratio (ethanol:water, % v/v), extraction temperature and extraction time on the yield and TPC in the PC pulp extract were investigated using a full factorial three-factor-three-level Central Composite Design (CCD).

RSM experiments and fitting the models

The present study attempted to identify the best factors for extracting phenolic compounds. Subsequently, the optimized conditions were validated for the highest TPC in the UAE of PC pulp. The interaction was represented as a response surface plot, plotted as a function of two targeted variables, while the other variable was held constant. The CCD model used various statistical analysis parameters viz. P-value, F-value, adjusted determination of coefficient (Adj. R²) and coefficient of determination (R²) to represent the statistical significance of the developed quadratic model. The value of adequate precision was used as a measure of the signal to noise ratio. Adequacy of the generated model was assessed using analysis of variance (ANOVA) to describe the data and to express the quality of the fitted model. A model that is significant can be represented by a P-value < 0.05 while a P-value < 0.001 suggests the term is highly significant. P-value describes the significance and interaction capability of each variable, in which variables showing lower P-values exhibit greater significance. In this study, regression analysis was used to determine the best fitting model whereas the best combination of factors was established using the optimization function based on the ridge maximum and canonical analyses. Table 4A represents the generated equation in terms of coded factors. Fitting of the data to various models (linear, two factorial, quadratic and cubic), the corresponding ANOVA revealed that the TPC was well described by a quadratic polynomial model. The values of R² and Adj. R² were 0.8833 and 0.7536, respectively, indicating acceptable accuracy and general availability of the polynomial model. A R² > 0.80 usually implies a satisfactory model (Poojary and Mugeraya, 2012; Mohamad et al., 2015) while a value of adequate precision, 8.773 (measures the ratio signal to noise) higher than 4, suggests sufficient signals were attained (Table 4A).

The positive sign in front of terms imply synergistic effects, whereas the negative sign infers an antagonistic effect influencing the independent variables affecting the TPC in the UAE of PC pulp. The antagonistic interaction between extraction time versus extraction temperature (~5.07AB) (Table 4A) and extraction time versus ratio of EtOH:water (~137.14AC) (Table 4A), as well as their corresponding linear terms were also significant (P-value 0.0297) (Table 5). The data also indicate that simultaneously increasing both factors will not improve the value of TPC. Conversely, a positive term for the effect of temperature versus ratio of ethanol:water, (+ 0.97BC) (Table 4A) strongly implies their synergistic interaction would improve the TPC.

Likewise, a positive term for the effect of temperature versus ratio of ethanol:water, (+ 0.97BC) (Table 4A) strongly implies their synergistic interaction would improve the TPC. The relatively high computed F-value of 6.81 (> 4.0), a small P-value (0.0040) and the insignificant lack-of-fit (P-value 0.1073) obtained for the model further affirmed its suitability to predict the experiment (Table 4B). This was supported by the computed F-value of the model (6.81) being higher than the tabular F0.05 (4,5) = 3.02, implying that the degree of freedom relative to the residual was significant at the 5% confidence level. The lack of fit (F-value 3.37) that was lower than the tabular F0.05 (4,5) = 5.19,
density and viscosity are also reduced, resulting in higher rates of mass transfer. The number of cavitation bubbles within the sonication mixture are also increased, producing a cohesive force that decreased the tensile strength following a reduction in solvent viscosity (Kong et al., 2015). This behaviour is also consistent with reports describing higher extraction temperatures that incite the breaking of phenolic matrix bonds that ultimately affect the membrane structure of plant cells and increase coagulation of lipoproteins (Prasad et al., 2011). The data also demonstrate that the use of a 60:40 (v/v) of ethanol:water can help solubilize into the surrounding medium, hence the TPC is increased. This might be due to the intensified swelling which increased disruption of plant cells due to presence of higher amounts water. Also, the contact surface area between the solvent and plant matrix are inadvertently increased when the extraction temperature (B) was increased up to −0.500 before a decline was seen. As the temperature increased, solvent surface area between the solvent and plant matrix are inadvertently increased when the extraction temperature (B) was increased up to −0.500 before a decline was seen. As the temperature increased, solvent viscosity is also reduced, resulting in higher rates of mass transfer.

Table 4 (A) Quadratic polynomial equations for the estimated coded and processed factors for TPC in the PC pulp crude extract and (B) ANOVA for the second-order polynomial model of the CCD.

| (A) Response | Quadratic polynomial model equations | R² | Adj. R² | Adequate precision |
|--------------|-------------------------------------|----|---------|-------------------|
| Total Phenolic Content (mg GAE/100 g dw) | 601.97 + 190.85A - 154.74B + 231.94C - 5.07AB - 137.14AC + 0.97BC - 19.89A² - 16.56B² - 26.42C² | 0.8833 | 0.7536 | 8.7733 |

Note: A : Extraction time (min), B: Extraction temperature (°C), C: Ratio of ethanol: water (% v/v)

(B) ANOVA

| Source of Variation | Sum of Squares | Degree of freedom | Mean Square | F-value | P-value |
|---------------------|----------------|------------------|-------------|---------|---------|
| Model               | 1538784.95     | 10               | 153878.49   | 6.81    | 0.0040* |
| Residual            | 203292.28      | 9                | 22588.03    |         |         |
| Lack of Fit         | 148328.67      | 4                | 34020.05    | 3.37    | 0.1073**|
| Pure Error          | 54963.61       | 5                | 12702.01    |         |         |
| Corr Total          | 1742077.22     | 19               |             |         |         |

*s: Significant at P < 0.05. **ns: Not significant at P > 0.05.

suggests that the lack of fit was insignificant relative to the pure error (Table 4B). The ANOVA for the linear terms for extraction time, (A) (P-value < 0.0011) and ratio of ethanol:water, (C) (P-value < 0.0003) were found highly significant, while the extraction temperature (B) was significant.

Table 5 ANOVA for the second order polynomial model and coefficient values for TPC in PC pulp extract.

| Source       | Degrees of Freedom | F-value | P-value |
|--------------|--------------------|---------|---------|
| Linear       |                    |         |         |
| A            | 1                  | 22.02   | 0.0011**|
| B            | 1                  | 6.00    | 0.0368**|
| C            | 1                  | 32.52   | 0.0003**|
| Interactions |                    |         |         |
| AB           | 1                  | 0.01    | 0.9261**|
| AC           | 1                  | 7.01    | 0.0297**|
| BC           | 1                  | 0.00    | 0.9858**|
| Quadratic    |                    |         |         |
| A²           | 1                  | 0.46    | 0.6274**|
| B²           | 1                  | 1.94    | 0.6855**|
| C²           | 1                  | 0.71    | 0.5214**|

Note: A : Extraction time (min), B: Extraction temperature (°C), C: Ratio of ethanol: water (% v/v)

** Significant at P < 0.01. *** Significant at P < 0.05.

Pertinently, the data evidently showed that the ratio of ethanol:water, (C) had the largest impact on the TPC in the UAE process (Table 5).

Correspondingly, the experimentally obtained values (Table 6) for the response variables agreed well with the predicted values (R² = 0.8933), indicative of a satisfactory model. The relationship between the predicted and experimental percentage conversions is shown in Figure 2a. For this study, TPC values from the UAE of PC pulp extract was within 136.07–1047.54 mg GAE/100 g dry weight.

Table 6 Experimental design and results of the CCD.

| A: | B: | C: |
|----|----|----|
| Run No. | Extraction time (min) | Extraction temperature (°C) | Ratio of ethanol: water (% v/v) | Actual TPC (mg GAE/100 g dw) | Predicted TPC (mg GAE/100 g dw) |
| 1   | 30 | 25  | 80  | 240.98 | 342.89 |
| 2   | 10 | 25  | 60  | 406.15 | 416.86 |
| 3   | 36.82 | 30  | 70  | 1045.08 | 866.67 |
| 4   | 20 | 30  | 70  | 524.59 | 601.97 |
| 5   | 30 | 25  | 60  | 971.31 | 1082.99 |
| 6   | 10 | 25  | 80  | 136.07 | 225.32 |
| 7   | 10 | 35  | 60  | 414.34 | 469.22 |
| 8   | 30 | 35  | 60  | 1047.54 | 1115.06 |
| 9   | 20 | 30  | 70  | 469.26 | 601.97 |
| 10  | 3.18 | 30  | 70  | 268.03 | 224.73 |
| 11  | 20 | 30  | 70  | 589.34 | 601.97 |
| 12  | 20 | 21.59 | 70  | 926.23 | 815.37 |
| 13  | 10 | 35  | 80  | 236.48 | 281.57 |
| 14  | 20 | 38.41 | 70  | 405.74 | 294.88 |
| 15  | 30 | 35  | 80  | 232.79 | 378.85 |
| 16  | 20 | 30  | 70  | 615.57 | 601.97 |
| 17  | 20 | 30  | 86.82 | 288.93 | 137.19 |
| 18  | 20 | 30  | 53.18 | 987.30 | 917.33 |
| 19  | 20 | 30  | 70  | 778.69 | 601.97 |
| 20  | 20 | 30  | 70  | 596.31 | 601.97 |
Mutual effect of experimental factors on the extraction of PC fruits

Effect of extraction time and ratio of ethanol:water

When compared to the conventional Soxhlet extraction method, UAE works more rapidly and is more convenient. The generated ultrasound transfers energy while concurrently accelerating movement in a material to produce the cavitation effect that disrupts plant cell walls and liberates the compounds into the surrounding fluid. The sonication process also creates heat which further speeds up the extraction process (Teng et al., 2016), and promotes mass transfer. Figure 3 illustrates the (a) response surface and (b) contour plots for the effect of extraction time (A) and ratio of ethanol:water (C), and their mutual interaction on the TPC in the UAE of PC pulp powder. In this assessment, the temperature was held at 30 °C. The F-value indicated that the effect of extraction time (22.02) was more significant than extraction temperature (6.00), implying that the former has a greater influence in improving extracted TPC in the UAE process. The outcome seen here generally agrees with the use of a prolonged extraction time that tended to improve extraction efficiency. It promotes the complete cracking the PC pulp cell through acoustic cavitation, permitting higher diffusion of solvent molecules to dissolve the phenolic compounds (Sharmila et al., 2016).

Effect of extraction time and extraction temperature

The (a) response surface curve and (b) contour plot for the effect of extraction time (A) and extraction temperature (B), and their mutual interaction on the TPC in the UAE of PC pulp powder is represented in Figure 4. In this evaluation, the ratio of ethanol:water was maintained at 70 % (v/v). The F-value indicated that the effect of extraction time (22.02) was more significant than extraction temperature (6.00), implying that the former has a greater influence in improving extracted TPC in the UAE process. The outcome seen here generally agrees with the use of a prolonged extraction time that tended to improve extraction efficiency. It promotes the complete cracking the PC pulp cell through acoustic cavitation, permitting higher diffusion of solvent molecules to dissolve the phenolic compounds (Sharmila et al., 2016).
The data indicate the extraction process carried out between 27−30 min and extraction temperatures between 27.5−30 °C gave the highest TPC at 760.55 mg GAE/100 g. TPC was increased with increasing extraction time due to the higher accessibility of solvent molecules and more phenolic compounds that could permeate into the ethanol:water mixture, given enough time. Also, the result may have something to do with the longer extraction time that tends to increase the temperature of the ethanol:water mixture. This decreases the dielectric constant of water along with increasing solubility of the phenolic compounds, enhancing extraction rate, diffusion rate and reducing both solvent viscosity and surface tension (Prasad et al., 2011). These changes synergistically support the higher TPC in the UAE process of PC.

However, the study was mindful that the use of higher extraction temperatures can also adversely affect stability of the extracted phenolic compounds. This is clearly demonstrated when further increment in the reaction temperature was counterproductive to the response of this study and yielded lower TPC likely due to degradation of phenolic compounds. Under elevated temperatures, higher interferences on compound stability can occur, invoked by enzymatic and chemical degradation or reaction with other plant components. These circumstances would normally reduce efficiency of the UAE process (Durling et al., 2007).

**Effect of extraction temperature and ratio of ethanol:water**

Figure 5 depicts the (a) response surface curve and (b) contour plot for the effect of extraction temperature (B) and ratio ethanol:water (C), and their mutual interaction on the TPC in the UAE of PC pulp powder. Based on the contour plot, it can be seen that a maximum TPC of 929.23 mg GAE/100 g was attained using a solvent ratio of ~ 60% (v/v) and any values of extraction temperatures between 25−27 °C. As indicated by the F-value, the effect solvent ratio (32.52) was more significant in relevance to the extraction temperature (6.00). Such outcome can be correlated to the similar polarity between ethanol:water used in the extraction fluid with that of phenolic compounds during extraction, as reported by (Sharmila et al., 2016). Moreover, Naczk and Shahidi (2004) reported that several phenolic compounds can occur naturally as glycosides.

It is possible that the presence sugar in PC pulp contributed to improved solubility of the phenolic compounds in the extraction fluid. Alberti et al. (2014) also described a similar observation. The study noted that the lowest TPC was obtained when using 80 % (v/v) of ethanol:water. Conversely, high concentrations of ethanol to water have been known to promote protein denaturation, and consequently prevent the dissolution of phenolic compounds into the fluid medium (Odabaş and Koca, 2016), hence consistent with the obtained lower TPC values. Moreover, an elevated extraction temperature naturally increases decomposition of phenolics (Naczk and Shahidi, 2004) and intensifies solvent loss through vaporization. Combination of such changes would generally reduce mass transfer during extraction (Tan et al., 2013). So, lower concentrations of phenolic compounds are transferred out of the PC cells into the outer fluid, to yield lower TPCs.

**Model verification**

Verification of the model equation to predict the optimum response values was carried out using the optimization function in the Design Expert 7.1.6 software. The optimized condition was established as follows: extraction time (A) of 30 min, extraction temperature (35 °C) and solvent ratio (ethanol:water, 60:40, % v/v) to obtain a predicted
yield of 1132.74 mg GAE/100 g. The experimental values for the highest TPC (1162.80 mg GAE/100 g) accorded quite well with the predicted TPC (1115.06 mg GAE/100 g). Since the corresponding percentage deviation was an acceptable 4.28 % (deviation < 5 %), optimization by the CCD model can be deemed accurate and therefore, applicable. The highest TPC in the crude extract of PC in this study was similar to that result obtained by Aservatham et al. (2014), with the former obtaining a slightly lower TPC of 1150 ± 2.3 mg GAE/100 g.

Analysis of PC crude extract using High Performance Liquid Chromatography (HPLC)

Figure 6 illustrates the HPLC chromatogram of PC pulp crude extract, spiked sample and standard GA. Linearity of the method was assessed by diluting the GA standard solution into a series of concentrations. All calibration curves were constructed by plotting the peak areas of the standard solutions versus their corresponding concentrations.

The PC crude extract was spiked with standard GA, and the HPLC validation data. The value of the regression coefficient ($R^2$) was 0.98, indicating a good linearity of the HPLC method.

CONCLUSION

The results of the present study indicate that each factor, for the exception of extraction temperature, in the UAE of PC pulp powder has a significant effect on the TPC. The study successfully optimized the extraction of the phenolic compounds from the pulp of PC using the CCD. Under an optimized condition, the experimental values obtained for a maximum TPC was 1162.80 mg GAE/100 g (dw), fitted well with the predicted result (1115.06 mg GAE/100 g) with only a 4.28 % deviation. Hence, the protocol for an UAE of PC pulp pulp proposed in this study permits a rapid and maximum extraction of phenolic compounds.

ACKNOWLEDGEMENT

This work was supported by Universiti Teknologi Malaysia, Johor, through a Research University Grant, grant number Q.J130000.2526.17H48. We are grateful to the staff of Nasuha Herbs Farm, Pagoh for providing the fruit samples used in this study.

REFERENCES

Adam, M., Dobias, P., Eisner, A., Ventura, K., 2009. Extraction of antioxidants from plants using ultrasonic methods and their antioxidant capacity. *Journal of Separation Science* 32, 288-294.

Addai, Z.R., Abdullah, A., Mutalib, S.A., 2013. Effect of extraction solvents on the phenolic content and antioxidant properties of two papaya cultivars. *Journal of Medicinal Plants Research* 7, 3354-3359.

Alberti, A., Zielinski, A.A.F., Zardo, D.M., Demiate, I.M., Nogueira, A., Mafra, L.I., 2014. Optimisation of the extraction of phenolic compounds from apples using response surface methodology. *Food Chemistry* 149, 151-158.

Aservatham, G.S.B., Sivasudha, T., Sasikumar, J., Christabel, P.H., Jeyadevi, R., Ananth, A.A., 2014. Antioxidant and hepatoprotective potential of *Pouteria campechiana* on acetaminophen-induced hepatic toxicity in rats. *Journal of Physiology and Biochemistry* 70, 1-14.

Balerdi, C., Shaw, P., 1998. *Sapodilla, sapote and related fruit: Tropical and subtropical fruits*. Auburndale: AgSci. pp. 78-136.

Berto, A., da Silva, A.F., Visentainer, J.V., Matsuhashi, M., de Souza, N.E., 2015. Proximate compositions, mineral contents and fatty acid compositions of native Amazonian foods. *Food Research International*. 77, 441-449.

Bwai, M., Adedirin, O., Akanji, F., Muhammad, K., Idoko, O., Useh, M., 2013. Physicochemical properties, fatty acids profiles and antioxidant properties of seed oil of breadfruit (*Treculia africana*). *International Journal of Research in Pharmacy & Science* 3(3), 44-54.

Cintas, P., Luche, J.-L., 1999. Green chemistry. The sonochemical approach. *Green Chemistry* 1, 115-125.

Claustror, A.L., Mahulid, R.S., Mayo, S.G., 1997. *A compendium of the trees and shrubs of the UST campus*. Department of Biological Science, College of Science, University of Santo Tomas.

Coronel, R.E., Zuño, J.C., Sotto, R.C., 1986. *Promising fruits of the Philippines*. College of Agriculture, University of the Philippines at Los Baños. Da Costa, A.V., Calabria, L.K., Furtado, F.B., de Gouveia, N.M., da Silva Oliveira, R.J., de Oliveira, V.N., Beletti, M.E., Espindola, F.S., 2013. Neuroprotective effects of *Pouteria ramiflora* (Mart.) Radl. (Sapotaceae) extract on the brains of rats with streptozotocin-induced diabetes. *Metabolic and Brain Disorder* 28, 411-419.

Dal Pra, V., Lunelli, F.C., Vendruscolo, R.G., Martins, R., Wagner, R., Lazzaretti, A.P., Jr., Freire, D.M., Alexandri, M., Kourtinas, A., Mazutti, M.A., da Rosa, M.B., 2017. Ultrasound-assisted extraction of bioactive compounds from palm pressed fiber with high antioxidant and photoprotective activities. *Ultrasonic Sonochemistry* 36, 352-366.

de Lannerolle, M., Priyadarshani, A., Sumithraachchi, D., Jansz, E., 2009. The carotenoids of *Pouteria campechiana* (Sinhala: ratalawulu). *Journal of National. Science Foundation of Sri Lanka* 36.

Durling, N.E., Catchpole, O.J., Grey, J.B., Wobby, R.F., Mitchell, K.A., Foo, L.Y., Perry, N.B., 2007. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol–water mixtures. *Food Chemistry* 101, 1417-1424.

Esclapez, M.D., García-Pérez, J.V., Mulet, A., Cárcel, J.A., 2011. Ultrasound-Assisted Extraction of Natural Products. *Food Engineering Reviews* 3, 108-129.

Handa, S.S., 2008. Extraction technologies for medicinal and aromatic plants. *Place Published: International Centre for Science and High Technology.*
Ma, J., Yang, H., Basile, M.J., Kennelly, E.J., 2004. Analysis of polyphenolic Hromadkova, Z., Ebringerová, A., 2003. Ultrasonic extraction of plant Lu, C.-L., Li, Y.-M., Fu, G.-Q., Yang, L., Jiang, J.-G., Zhu, L., Lin, F.-L., Chen, Ikram, E.H.K., Eng, K.H., Jalil, A.M.M., Ismail, A., Nazri, Lu, C.-L., Li, Y.-M., Fu, G.-Q., Yang, L., Jiang, J.-G., Zhu, L., Lin, F.-L., Chen, HERNÁNDEZ, L., LUNA, H., SOLIS, A., VÁZQUEZ, A., 2006. Application of crude preparations of leaves from food plants for the formation of cyanohydrins with high enantiomeric excesses. Tetrahedron: Asymmetry 17, 2813-2816. Herrera, M.C., Luque de Castro, M.D., 2004. Ultrasound-assisted extraction for the analysis of phenolic compounds in strawberries. Analytical and Bioanalytical Chemistry 379, 1106-1112. Hromadkova, Z., Ebringerová, A., 2003. Ultrasonic extraction of plant materials—investigation of hemicellulose release from buckwheat hulls. Ultrasonics sonochemistry 10, 127-133. Ikram, E.H.K., Eng, K.H., Jalil, A.M.M., Ismail, A., Idri, S., Azlan, A., Nazri, H.S.M., Diton, N.A.M., Mokhtar, R.A.M., 2009. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. Journal of Food Composition and Analysis 22, 388-393. Isas, A.A., Mahat, N.A., Jamalis, J., Attan, N., Zakaria, I.L., Huyop, F., Wahab, R.A., 2017. Synthesis of geranyl propionate in a solvent-free medium using Rhizomucor miehei lipase covalently immobilized on chitosan–graphene oxide beads. Preparative Biochemistry and Biotechnology 47, 199-210. Jaafar, R.A., Ahmad Ridhwan, A., Zaini, N., Vazulevan, R., 2009. Proximate analysis of dragon fruit (Hylocereus polyrhizus). American Journal of Applied Sciences 6, 1341-1346. Kamikkanna, N., Singh, M., 2002. Skin permeation enhancement effect and skin irritation of saturated fatty alcohols. International Journal of Pharmaceutics 245, 219-228. Khan, M.K., Abert-Vian, M., Fabiano-Tixier, A.-S., Dangles, O., Chemat, F., 2010. Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (Citrus sinensis L.) peel. Food Chemistry 119, 851-858. Kim, M.-J., Doh, H.-J., Choi, M.-K., Chung, S.-J., Shim, C.-K., Kim, D.-D., Kim, J.S., Yong, C.-S., Choi, H.-G., 2008. Skin permeation enhancement of diclofenac by fatty acids. Drug Delivery 15, 373-379. Kong, F., Yu, S., Feng, Z., Wu, X., 2015. Optimization of ultrasound-assisted extraction of antioxidant compounds from Guava (Psidium guajava L.) leaves using response surface methodology. Pharmacognosy Magazine 11, 463. Lott, R.H., Jackes, B.R., 2001. Isozyme analysis of rain forest plants using immature seeds. Biotropica 33, 197-204. Lu, C.-L., Li, Y.-M., Fu, G.-Q., Yang, L., Jiang, J.-G., Zhu, L., Lin, F.-L., Chen, J., Lin, Q.-S., 2011. Extraction optimisation of daphnoretin from root bark of Wikstroemia indica (L.) CA and its anti-tumour activity tests. Food Chemistry 124, 1500-1506. Ma, J., Yang, H., Basile, M.J., Kennelly, E.J., 2004. Analysis of polyphenolic antioxidants from the fruits of three Pouteria species by selected ion monitoring liquid chromatography– mass spectrometry. Journal of Agricultural and Food Chemistry 52, 5873-5878. Manan, F.M.A., Attan, N., Zakaria, Z., Keyon, A.S.A., Wahab, R.A., 2018. Enzymatic esterification of engenol and benzoic acid by a novel chitosan-chitin nanowhiskers supported Rhizomucor miehei lipase: Process optimization and kinetic assessments. Enzyme and Microbial Technology 108, 42-52. Manan, F.M.A., Rahman, I.N.A., Marzuki, N.H.C., Mahat, N.A., Huyop, F., Wahab, R.A., 2016. Statistical modelling of engenol benzoate synthesis using Rhizomucor miehei lipase reinforced nanobioconjugates. Process Biochemistry 51, 249-262. Marzuki, N.H.C., Huyop, F., Aboul-Enein, H.Y., Mahat, N.A., Wahab, R.A., 2015. Modelling and optimisation of Candida rugosa nanobioconjugates catalysed synthesis of methyl oleate by response surface methodology. Biotechnology and Biotechnological Equipment 29, 1113-1127. Mason, T., Peters, D., 1999. An introduction to the uses of power ultrasound in chemistry. Sonochemistry, Oxford University Press: New York. Masturah, M., Masstah, M., Wan Ramli, W., Harcharan, S., Jamaliah, M., 2006. Extraction of hydrosoluble tannins from Phyllanthus niruri Linn: Effects of solvents and extraction method, Separation and Purification Technology, 487-498. Mohamad, N.R., Huyop, F., Aboul-Enein, H.Y., Mahat, N.A., Wahab, R.A. 2015. Response surface methodological approach for optimizing production of geranyl propionate catalysed by carbon nanotubes nanobioconjugates. Biotechnology and Biotechnological Equipment 29(4), 732-739. Naczk, M., Shahidi, F., 2004. Extraction and analysis of phenolics in food. Journal of Chromatography A 1054, 95-111. Odaiba, H.L., Koca, I., 2016. Application of response surface methodology for optimizing the recovery of phenolic compounds from hazelnut skin using different extraction methods. Industrial Crops and Products 91, 114-124. Oniszczuk, A., Olech, M., 2016. Optimization of ultrasound-assisted extraction and LC-ESI-MS/MS analysis of phenolic acids from Brassica oleracea L. var. sabellica. Industrial Crops and Products 83, 359-363. Poopjoy, H., Mugeraya, G., 2012. Laccase production by Phellinus noxius hpF17: Optimization of submerged culture conditions by response surface methodology. Research in Biotechnology 3. Prasad, K.N., Hassan, F.A., Yang, B., Kong, K.W., Ramanan, R.N., Azlan, A., Ismail, A., 2011. Response surface optimisation for the extraction of phenolic compounds and antioxidant capacities of underutilised Mangifera pajong Kosterm. peels. Food Chemistry 128, 1121-1127. Rascarbo Álvarez, Á.M., González Rodríguez, M.L., 2000. Lipids in pharmaceutical and cosmetic preparations. Grasas y aceites, 51, 74-96. Rojo, I.E., Villano, C.M., Joseph, G., Schmidt, B., Shalaev, V., Shumlan, J.L., Lila, M.A., Raskin, I., 2010. Original contribution: Wound-healing properties of nut oil from Pouteria lucuma. Journal of Cosmetic Dermatology 9, 185-195. Sasidharan, S., Chen, Y., Saravanaan, D., Sundram, K., Latha, L.Y., 2011. Extraction, isolation and characterization of bioactive compounds from plants’ extracts. African Journal of Traditional, Complementary and Alternative Medicines 8. Sharmila, G., nikitha, V.S., Ilayarasi, S., Dhivyra, K., Rajasekar, V., Kumar, N.M., Muthukumar, K., Muthukumaran, C., 2016. Ultrasound assisted extraction of total phenolics from Cassia auriculata leaves and evaluation of its antioxidant activities. Industrial Crops and Products 84, 13-21. Silva, C.A., Simeoni, I.A., Silveira, D., 2009. Genus Pouteria: Chemistry and biological activity. Revista Brasileira de Farmacognosia 19, 501-509. Solis, A., Luna, H., Manjarrez, N., Perez, H.I., 2004. Study on the (R-) oxyvinilacte acid of Pouteria sapota. Tetrahedron 60, 10427-10431. Tan, M., Tan, C., Ho, C., 2013. Effects of extraction solvent system, time and temperature on total phenolic content of henna ( Lawsonia inermis) stems. International Food Research Journal 20, 3117-3123. Tanojo, H., Bouwstra, J.A., Junginger, H.E., Bodde, H.E., 1997. In vitro human skin barrier modulation by fatty acids: Skin permeation and thermal analysis studies. Pharmaceutical Research 14, 42-49. Teng, H., Chen, L., Huang, Q., Wang, J., Lin, Q., Liu, M., Lee, W.Y., Song, H., 2016. Ultrasound-assisted extraction of raspberry seed oil and evaluation of its physicochemical properties, fatty acid compositions and antioxidative activities. PLoSOne 11, e0153457. Vermaak, I., Kamatou, G.P.P., Komane-Mofokeng, B., Viljoen, A.M., Beckett, K., 2011. African seed oils of commercial importance — Cosmetic applications. South African Journal of Botany 77, 920-933. Villa-Rodriguez, J.A., Molina-Corral, F.J., Ayala-Zavala, J.F., Olivas, G.I., Gonzalez-Aguilar, G.A., 2011. Effect of maturity stage on the content of fatty acids and antioxidative activity of ‘Hass’ avocado. Food Research International 44, 1231-1237. Zhang, Q.A., Shen, H., Fan, X.H., Shen, Y., Wang, X., Song, Y., 2015. Changes of gallic acid mediated by ultrasound in a model extraction solution. Ultrasónico sonochemistry 22, 149-154.