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Optimisation of ultrasound assisted extraction of pomposia (*Syzygium cumini* L.) anthocyanins and phenolic compounds based on response surface methodology

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Abstract

Ultrasound-assisted extraction in combination with stirring was used to extract bioactive compounds from peel and flesh of pomposia (*Syzygium cumini* L.) fruits. Two independent factors including sonication power (90 - 150 W) and extraction time (10 - 30 min) were studied using 3^2 full factorial design to achieve optimal yields of both total polyphenols content (TPC) and total monomeric anthocyanins content (TMAC). The experimental data were fitted using reduced-order cubic models. The optimum conditions for simultaneous extraction of TPC and TMAC from flesh and peel were 135.73 W at 30 min and 100.30 W at 30 min, respectively. High performance liquid chromatography (HPLC) analysis was used to identify and quantify the polyphenol components. Comparing IC_{50} of butylated hydroxytoluene (BHT) (23.19 mg/L) with that of peel and flesh extracts (2.82 and 10.24 mg GAE/L, respectively) indicates the superior scavenging activity of these extracts against DPPH radicals.

Introduction

Pomposia (*Syzygium cumini* L.) is an edible Egyptian tropical fruit with a purple peel. It is a member of the Myrtaceae family, and is called Indian black plum, java plum, and jambolan in other countries (Tavares *et al.,* 2016). Its taste is described as a blend of sour, sweet, and astringent (Lestario *et al.,* 2017). Recently, studies have shown that pomposia fruits contain high level of antioxidants such as phenolic acids and anthocyanins (Coelho *et al.,* 2016). These natural antioxidants have the ability to replace harmful synthetic antioxidants (Singh *et al.,* 2015), and are helpful in preventing cardiovascular diseases and cancers (Ayya *et al.,* 2015; Varadharajan *et al.,* 2017).

Ultrasound-assisted extraction (UAE) is more effective than traditional techniques for extracting bioactive compounds (He *et al.,* 2016), since it facilitates solvent penetration into cellular materials and disruption of cell walls (Tao *et al.,* 2014; Sun *et al.,* 2015; Maran *et al.,* 2017). Solvent type, ultrasound intensity, temperature, and extraction time affect the yield of extractable materials (Mason and Lorimer, 2002).

Response surface methodology (RSM) is a statistical method that describes the interactions between extraction parameters and product properties, and is used for the optimisation of extraction methods (Frontuto *et al.,* 2019). Numerous studies have been performed to assess the efficiency of different methods of extracting polyphenols and/or anthocyanins from pomposia fruit and its peel (Chaudhary and Mukhopadhyay, 2013; Maran *et al.,* 2014; 2015).

However, the literature is very scarce on the extraction of these components from pomposia fruits using ultrasound method. The present work was therefore carried out to investigate the optimal ultrasound-assisted extraction conditions for maximising the recovery of polyphenols and anthocyanins from peel and flesh of pomposia fruits using full factorial experimental design. Additionally, HPLC profile of phenolic compounds and DPPH radical scavenging activity of the extract containing highest yield of TPC were also evaluated.

Materials and methods

Materials

Plant material

Three kilograms of pomposia fruits at optimum maturity were collected from the farm of the Faculty of Agriculture, Cairo University, Egypt in August 2019. The fruits were manually sorted, washed, peeled, and stored at 4°C prior to extraction and analyses.

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Chemicals and reagents

Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), and authentic standards of the phenolic and flavonoid compounds were purchased from Sigma Chemical Co., Ltd (St. Louis, MO, USA).

Methods

Extraction procedure

Separately, samples of peel and flesh were homogenised using a high-speed blender. The homogenised samples were extracted at room temperature (25°C) using a combination of ultrasound waves and magnetic stirring (Medline Scientific, Model MS 300, UK) at maximum speed. The temperature during ultrasound-assisted extraction was carefully monitored by a thermocouple. The recorded increment of temperature during the 30 min of sonic stirring was 2.5, 3.0, and 3.4°C, when the ultrasonic intensity was set at 90, 120, and 150 W, respectively. The temperature did not exceed 28.4°C. This temperature increase was attributable to the ultrasonic intensity and the extraction time. The high speed of the magnetic stirring, besides controlled temperature (air-conditioned laboratory), reduced the increase in temperature to a great extent as resulted from the relatively low ultrasonic intensity used. The present work was focused on the optimisation of ultrasonic intensity and extraction time as independent factors for the extraction of bioactive compounds from the investigated materials. Briefly, 5 g of either flesh or peel samples were extracted with 100 mL of 80% aqueous ethanol (sample to solvent ratio of 1:20, w/v). This solvent was used for solid-liquid extraction at the suggestion of other investigators (Bucic-Kojic *et al.,* 2011). The domain of the experimental independent factors (ultrasonic power, 90 - 150 W; ultrasonic time, 10 - 30 min) was selected according to previous research (Belwal *et al.,* 2019) and preliminary experiments.

An ultrasound generator (Fisher Sonic Dismembrator, Model 300, 50 Hz, USA) fitted with an ultrasound probe (19 mm diameter tip) was used to sonicate the mixture. The probe was submerged under the surface of the mixture to a depth of 0.5 cm. The obtained extracts were directly filtered through Whatman No. 1, and analysed.

Physicochemical analyses

The moisture content of fruit flesh and peel was determined under vacuum (100 mm Hg pressure) at 60 ± 2°C for 24 h (AOAC, 2005). Total soluble solids and titratable acidity were determined using a hand refractometer (ATAGO, Tokyo, Japan) and pH meter, and expressed as °Brix and g malic acid/100 g sample, respectively.

Total polyphenolic content (TPC)

The TPC was spectrophotometrically determined by Folin-Ciocalteau method (Singleton and Rossi, 1965) using gallic acid (GA) as a standard. Finally, the absorbance was measured at 750 nm with a UV-Vis Spectrophotometer (UV-2000, Unico, USA). A calibration curve (R^2 = 0.9990) of GA with concentrations that varied between 50 and 800 mg/L was used to calculate TPC, which was expressed as mg gallic acid equivalents (GAE)/g fresh sample.

Total monomeric anthocyanin content (TMAC)

The TMAC was determined according to the pH differential method (AOAC, 2005). The sample was properly diluted using KCl buffer solution (pH 1) and CH_3COONa buffer solution (pH 4.5). Absorbance (A) of the diluted samples was determined at 520 and 700 nm, using UV-Vis Spectrophotometer (UV-2000, Unico, USA). The concentration of monomeric anthocyanin pigments was expressed as cyanidin-3-glucoside equivalent (cyn-3-gly E) with a molecular weight (MW) and molar extinction coefficient (ε) of 449.2 g/mol and 26,900 L/mol.cm, respectively (Branco *et al.,* 2016). Absorbance (A) was calculated using Eq. 1, and consequently the TMAC was calculated using Eq. 2:

$$A = (A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5} \quad \text{(Eq. 1)}$$

$$TMAC\ (mg\ \text{Cyn}-3-\text{gly}/g\ fresh\ sample) = \frac{(A \times MW \times DF \times 1000 \times V)}{(\varepsilon \times l \times M)} \quad \text{(Eq. 2)}$$

where, DF = dilution factor, V = diluted volume (L), l = path length in cm, and M = weight of the sample.

DPPH radical scavenging activity

Scavenging activity of peel and flesh extracts against DPPH radicals was evaluated. The absorbance of the samples (As) and control (Ac) was read at 517 nm using a spectrophotometer after an incubation period of 30 min in the dark at 25°C (Brand-Williams *et al.,* 1995). BHT as a standard antioxidant with concentrations varying from 6 to 36 mg/L was used. DPPH radical inhibition percentage was calculated using Eq. 3.

$$Inhibition\ \% = \left[\frac{(Ac-As)}{Ac}\right] \text{x}100 \quad \text{(Eq. 3)}$$

Results were expressed as IC_{50} value (the sample concentration that caused 50% inhibition of DPPH

radicals, µg/mL).

HPLC profiles of polyphenols and flavonoids

The phenolic and flavonoid compounds of the fruit flesh and peel extracts were analysed by HPLC (Hewlett Packard, series 1050 M, CA). Fractionation was conducted on a BDS-Hypersil C_{18} column (5 µm particle size, 250 × 4.6 mm, i.d.). Mobile phase solvent systems were: (i) phosphoric acid 0.01 M (A); acetonitrile (B). The elution gradient of B started with 2% B and increased to 15% at 10 min and 35% at 25 min. The solvent flow rate was 2 mL/min, and (ii) phosphoric acid 0.01 M (A); methanol (B). The elution gradient of B started with 5% and increased to 50% at 10 min and 100% at 25 min (Escarpa and González, 1999). Flow rate was kept at 1 mL/min. The detection of phenolic and flavonoid components was carried out at 280 and 330 nm, respectively (Mattila *et al.,* 2000). Relative retention time and chromatographic peak area of each elute were compared with that of the standard to identify and calculate its concentration.

Experimental design and data analysis

RSM with 3^2 FFD was applied to determine the extraction conditions that exhibit maximum extraction yield of TPC and TMAC from pomposia fruit flesh and peel (Bezerra *et al.,* 2020). The studied independent variables were extraction time and sonication power (Table 1).

The one-factor-at-a-time technique was used for the preliminary screening of the factors (sonication power and extraction time) before proceeding to the optimisation study. The variables were coded using Eq. 4 (Myers *et al.,* 2009):

$$X_i = \frac{\left(x_i - \frac{[\max(x_i)+\min(x_i)]}{2}\right)}{\left(\frac{[\max(x_i)-\min(x_i)]}{2}\right)} \quad \text{(Eq. 4)}$$

where, X_i, x_i, max(x_i), and min (x_i) = coded value, corresponding actual value, maximum value, and lowest value of the variable x_i, respectively.

The effects of each independent variable on the yield of TPC and TMAC were tested in triplicate. The Brown-Forsythe test was implemented to examine the homogeneity of variable variances. The significance of the experiments was analysed using one-way ANOVA test, followed by Tukey's test that was performed to determine the significant difference between treatments at $p < 0.05$. The reduced cubic model (Eq. 5) was used to fit the experimental results of nine runs using Design-Expert© software version 11 (Stat-Ease Inc., Minneapolis, MN, USA).

$$Y = \beta_0 + \sum_{j=1}^{k}\beta_j X_j + \sum_{j=1}^{k}\beta_{jj} X_j^2 + \sum_i \sum_{<j=2}^{k}\beta_{ij} X_i X_j + \sum_i \sum_{<j=2}^{k}\beta_{ij} X_i X_j^2 + e_i \quad \text{(Eq. 5)}$$

where, Y = estimated response; Xi and Xj = coded independent variables; β_0 = intercept; β_j, β_{jj}, and β_{ij} = coefficients of linear, quadratic, and interaction effect, respectively; and e_i = error. Analysis of variance (ANOVA) was implemented to determine the statistical significance of the model and their various terms.

Results and discussion

Physicochemical characteristics

Results indicated that physicochemical characteristics

Table 1. Factors of 3^2 full factorial design and the mean values of the obtained responses.

Run	Time (min), X_1 (coded)	Sonication power (W), X_2 (coded)	Total monomeric anthocyanin (mg cyn-3-gly E/g)		Total polyphenols (mg GAE/g)	
			Flesh	Peel	Flesh	Peel
1	10 (-1)	90 (-1)	0.32 ± 0.016^{e}	8.33 ± 0.104^{c}	7.40 ± 0.200^{e}	8.36 ± 0.380^{cd}
2	20 (0)	90 (-1)	0.36 ± 0.010^{e}	8.79 ± 0.049^{b}	11.80 ± 0.800^{d}	9.56 ± 0.396^{bc}
3	30 (1)	90 (-1)	0.48 ± 0.017^{e}	9.57 ± 0.050^{a}	19.33 ± 0.306^{b}	10.95 ± 0.478^{a}
4	10 (-1)	120 (0)	1.23 ± 0.004^{d}	8.42 ± 0.062^{c}	16.47 ± 0.231^{c}	7.33 ± 0.306^{d}
5	20 (0)	120 (0)	1.29 ± 0.017^{d}	8.99 ± 0.076^{b}	16.73 ± 0.115^{c}	10.33 ± 0.503^{ab}
6	30 (1)	120 (0)	1.38 ± 0.010^{d}	9.34 ± 0.135^{a}	24.20 ± 0.529^{a}	10.67 ± 0.306^{ab}
7	10 (-1)	150 (1)	2.06 ± 0.130^{c}	7.06 ± 0.218^{d}	6.10 ± 0.300^{e}	5.77 ± 0.603^{e}
8	20 (0)	150 (1)	2.48 ± 0.103^{b}	8.35 ± 0.043^{c}	11.30 ± 0.520^{d}	7.70 ± 0.70^{d}
9	30 (1)	150 (1)	2.69 ± 0.084^{a}	8.89 ± 0.165^{b}	12.47 ± 0.808^{d}	9.73 ± 0.231^{ab}
	p (ANOVA)		< 0.001	< 0.001	< 0.001	< 0.001
	p (Brown-Forsythe)		0.217	0.800	0.657	0.901

Values are expressed as means ± standard deviations of three replicates (n = 3). Different letters in the same column indicate significant differences at $p < 0.05$.

of the fully ripened pomposia fruits were as follows: whole fruit weight, 4.08 ± 0.21 g; peel weight, 0.45 ± 0.027 g/fruit; seed weight, 1.19 ± 0.140 g/fruit; total soluble solids, 18.17 ± 2.72 Brix; total acidity, 1.008 ± 0.003 g malic acid/100 g; pH, 3.71 ± 0.12; and flesh moisture content, 80.58 ± 1.10%. These results are in agreement with those obtained by de Carvalho *et al.* (2017). The moisture content of fruit peel was 70.71 ± 1.14%.

Fitting the model

A preliminary study was conducted to determine the effect of sonication time (10, 20, 30, and 40 min) and ultrasonic power (90, 120, and 150 W) on the recovery of phytochemical compounds from pomposia fruits. These experiments indicated that extending extraction time to 30 min at 90 W was accompanied by a significant increase in the yield of extractable constituents, after which, no significant increase in the yield was recorded. Meanwhile, yield of polyphenols in the fruit flesh significantly decreased after 30 min of extraction.

In addition, increasing sonication power to levels higher than 120 W at extraction time of 30 min significantly decreased the yield of polyphenols and peel anthocyanin, while the highest yield of flesh anthocyanin was obtained at 150 W.

TMAC and TPC yields of pomposia fruit flesh and peel extracts obtained by UAE are shown in Table 1.

All response variables were found to be significantly different using one-way ANOVA ($p < 0.05$). Probability values of Brown-Forsythe's tests were higher than 0.05 ($p > 0.05$), which indicated that all responses were homoscedastic (Table 1). These tests are crucial for applying RSM (Granato *et al.,* 2014; Pedro *et al.,* 2016).

Data in Table 1 show that the experimental values of TMAC significantly varied from 0.32 to 2.69 mg cyn-3-gly E/g flesh, and from 7.06 to 9.57 mg cyn-3-gly E/g peel according to the combinations of independent variables. Meanwhile, TPC significantly varied from 6.10 to 24.20 mg GAE/g flesh, and from 5.77 to 10.95 mg GAE/g peel. The maximum TMAC and TPC yields from peels were observed in run 3 with the extraction conditions of 30 min and sonication power of 90 W. Meanwhile, the highest TPC yield from flesh was obtained in run 6 (30 min and 120 W sonication power). The TMAC and TPC of the fresh fruit pulp were 2.13 mg cyn-3-gly E/g and 2.06 mg GAE/g, respectively (Branco *et al.,* 2016). The TMAC and TPC of the frozen pulp were 0.066 mg cyn-3-gly E/g and 3.219 mg GAE/g, respectively (Coelho *et al.,* 2016). These variations in TMAC and TPC of the flesh of pomposia fruits could be due to cultivation regions and extraction conditions (Singh *et al.,* 2015).

Fitting experimental data using linear, interactive, quadratic, cubic, and reduced cubic models revealed that the best model to represent responses variability was the reduced cubic model. The highest values of R^2, adjusted R^2, and predicted R^2 were recorded for the reduced cubic models. The lack of fit test values of reduced cubic models for extracting anthocyanins from different fruit parts in contrast to other models was insignificant and the model was not aliased.

The results of fitting experimental data using reduced cubic-order models are outlined in Table 2. The empirical mathematical models derived for coded variables are given below (Eqs. 6 - 9):

$$TMAC\ (flesh) = 1.313 + 0.076X_1 + 1.059X_2 + 0.117X_1X_2 + 0.099X^2_2 - 0.070X_1^2X_2 + 0.118X_1X_2^2 \quad \text{(Eq. 6)}$$

$$TMAC\ (peel) = 8.987 + 0.461X_1 - 0.2228X_2 + 0.147X_1X_2 - 0.107X_1^2 - 0.416X^2_2 - 0.266X_1^2X_2 + 0.307X_1X_2^2 \quad \text{(Eq. 7)}$$

$$TPC\ (flesh) = 18.433 + 3.876X_1 - 1.392X_1X_2 + 1.050X^1_2 - 7.733X^2_2 - 1.792X_1^2X_2 \quad \text{(Eq. 8)}$$

$$TPC\ (peel) = 9.709 + 1.667X_1 - 0.932X_2 - 0.764X^2_2 \quad \text{(Eq. 9)}$$

The goodness and fitness of the obtained models and their significance were tested by multiple regression analysis and analysis of variance (ANOVA). The obtained models were highly significant ($p < 0.0001$), which indicates their suitability for representing the relationship between variables. All regression coefficients were significant except coefficients of X_1^2 for anthocyanin extraction from fruit flesh, X_2 and $X_1X_2^2$ for polyphenol extraction from fruit flesh, and X_1X_2,X_1^2,$X_1^2X_2$,and $X_1X_2^2$ for polyphenol extraction from fruit peel. R^2 values for flesh TMAC, peel TMAC, flesh TPC, and peel TPC were 0.996, 0.982, 0.970, and 0.917, respectively; while Adj. R^2 values were 0.995, 0.975, 0.960, and 0.887 for flesh TMAC, peel TMAC, flesh TPC, and peel TPC, respectively. Increasing the values of R^2 and Adj. R^2 to close to one indicated the adequacy of the model to predict the experimental data (Jiang *et al.,* 2017). The predicted R^2 value was 0.992, 0.961, 0.945, and 0.840 for flesh TMAC, peel TMAC, flesh TPC, and peel TPC, respectively. The predicted R^2

Table 2. ANOVA analysis and statistical parameters of the reduced cubic-order models.

Source	Flesh TMAC			Peel TMAC			Flesh TPC			Peel TPC		
	RC	**SS**	***p*-value**	**RC**	**SS**	***p*-value**	**RC**	**SS**	***p*-value**	**RC**	**SS**	***p*-value**
Model	1.313	19.168	< 0.0001	8.987	12.869	< 0.0001	18.433	779.953	< 0.0001	9.709	70.963	< 0.0001
X_1	0.076	0.034	0.0083	0.461	1.275	< 0.0001	3.867	89.707	< 0.0001	1.667	16.667	< 0.0001
X_2	1.059	6.725	< 0.0001	-0.222	0.296	0.0001	-0.250	0.375	0.5904	-0.932	5.208	0.0009
X_1X_2	0.117	0.163	< 0.0001	0.147	0.259	0.0002	-1.392	23.241	0.0004	0.343	1.408	0.0548
X_1^2	-0.016	0.001	0.5469	-0.107	0.069	0.0317	1.050	6.615	0.0330	-0.396	0.941	0.1106
X_2^2	0.099	0.059	0.0011	-0.416	1.037	< 0.0001	-7.733	358.827	< 0.0001	-0.764	3.506	0.0044
$X_1^2X_2$	-0.070	0.020	0.0389	-0.266	0.282	0.0002	-1.792	12.840	0.0047	-0.023	0.002	0.9389
$X_1X_2^2$	0.118	0.056	0.0013	0.307	0.378	< 0.0001	0.708	2.007	0.2207	-0.026	0.003	0.9299
Residual		0.075			0.242			23.774			6.387	
Lack of Fit		0.004	0.3382		0.000	0.8827		19.508	< 0.0001		2.635	0.0023
Pure Error		0.072			0.242			4.267			3.752	
Cor. Total		19.244			13.111			803.727			77.351	
Std. Dev.	0.063			0.113			1.119			0.580		
Mean	1.368			8.639			13.978			8.935		
C.V.%	4.603			1.307			8.003			6.489		
PRESS	0.159			0.513			44.210			12.355		
R^2	0.996			0.982			0.970			0.917		
Adj. R^2	0.995			0.975			0.960			0.887		
Pred. R^2	0.992			0.961			0.945			0.840		
Adeq. Precision	69.001			40.869			27.632			17.010		

should not be more than 0.2 less than the adjusted R^2 (Aziz and Arof, 2016). The coefficients of variation were low and ranged from 1.307 to 8.003. All adequate precision values were higher than 17.00, thus indicating the adequacy of the model.

Effect of process variables

The individual and interactive influences of process factors on the studied responses were illustrated using perturbation plots (Figure 1) and three-dimensional (3D) response surface plots (Figure 2).

Extraction time

To compare the effects of extraction factors (extraction time and sonication power) on the different responses at the midpoint [coded 0.0 (20 min and 120 W)] for all the studied factors, perturbation plots (Figure 1) were drawn. In these plots, response values corresponded to changes in a single factor, while all the other factors were kept constant. The sensitivity of response to a particular factor is increased as that factor shows curvature or a steep slope. Contrarily, flat lines indicate insensitivity of response to changes occurring in that factor. Therefore, data in Figure 1 suggest that the sensitivity of TPC to changes in extraction time was higher than that of TMAC from different fruit parts. Extraction time (factor A) lines for TPC were more curved than those of TMAC from different fruit parts, especially from fruit flesh, which showed relatively flat line (Figure 1a).

During the first 10 min of the extraction process, the yield of TMAC was higher than that of TPC, as shown in (Table 1). Thus, the slope of TMAC surface plots at different sonication powers was lower than that of TPC surface plots (Figure 2). This reveals that the effect of extraction time on the yield of TMAC was lower than that of TPC yield. Furthermore, the regression coefficients for extraction time (X_1) (Table 2) were 0.076 and 0.461 for TMAC yield, and 3.867 and 1.667 for TPC yield, from flesh and peel, respectively. This represents the high effect (slope) of extraction time on TPC yield. This might be due to the binding affinity between polyphenols and proteins, which induce protein and polyphenol to form complexes with various solubility characteristics

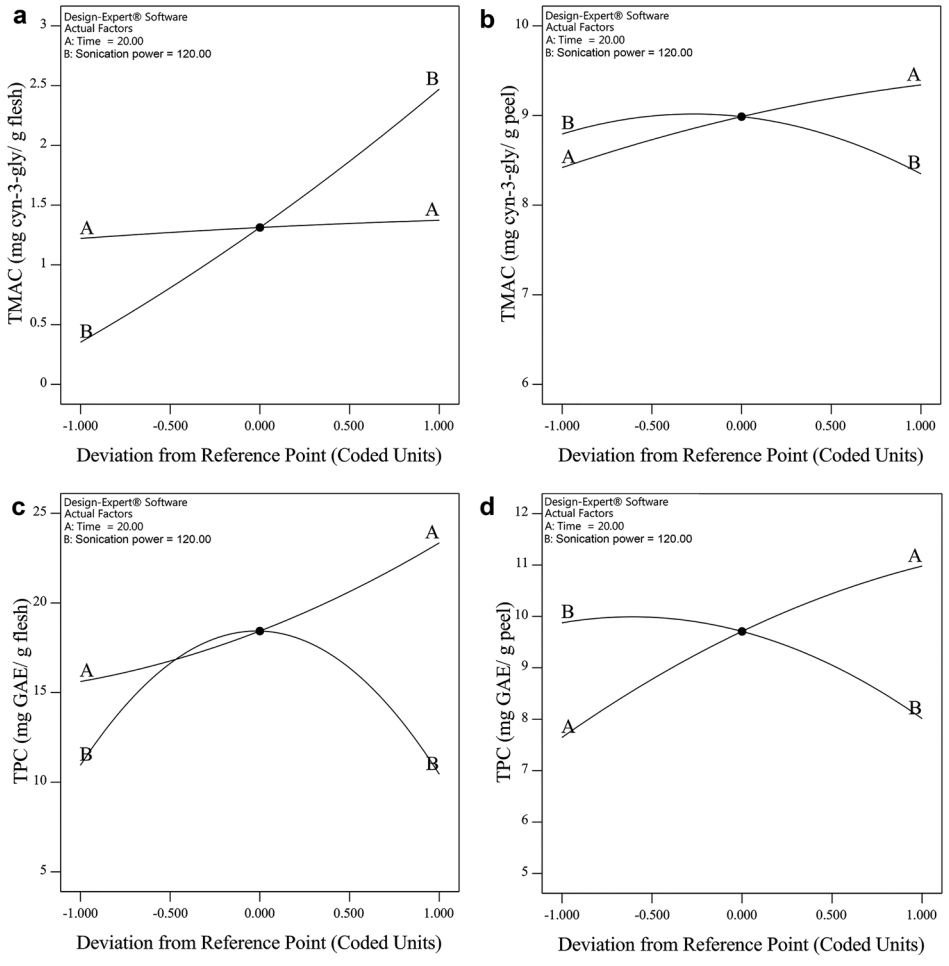

Figure 1. Perturbation plots of (a) flesh TMAC, (b) peel TMAC, (c) flesh TPC, and (d) peel TPC as a function of extraction time (factor a) and sonication power (factor b).

(Papadopoulou and Frazier, 2004).

Extending extraction time from 10 to 30 min significantly increased ($p < 0.0001$) the extraction yield of TPC regardless of sonication power and investigated material used (Table 1, Figure 2c and 2d). The same trend could be noticed for the TMAC yield of fruit flesh and peel (Figure 1a and 1b).

Sonication power

Perturbation curves of the sonication power variable (Figure 1) reveal that all the studied responses were more sensitive to change with sonication power than with extraction time. Ultrasonic extraction efficiency relies highly on the sonication power (Chemat *et al.,* 2017). Table 1 and Figure 2a show that increasing sonication power caused significant ($p < 0.0001$) increase of TMAC yield from fruit flesh. This effect could be due to cavitation phenomena that stimulates the mass transfer process (Tiwari *et al.,* 2008). Table 1 and Figure 1b and 1d show that increasing sonication power from 90 to 120 W at each extraction time did not significantly affect the yield of TMAC and TPC from the peels. A further increase in the sonication power to 150 W resulted in a significant decrease of TMAC and TPC yields from peels except TPC yield obtained after 30 min. These reductions of TMAC and TPC yields at high sonication power could be explained by the degradation of these compounds (Agcam *et al.,* 2017).

Optimisation of extraction process and model verification

The desired function methodology was used to perform simultaneous optimisation of studied responses from the same fruit part. The optimum conditions for extracting bioactive compounds from the fruit flesh were 30 min at 135.73 W, and from the fruit peel were 30 min at 100.30 W, with desirability of 0.712 and 0.951, respectively. The optimal sonication power was rounded to the nearest integer, and the validation experiments were performed at the adjusted optimal conditions. The predicted and experimental values for TPC and TMAC from fruit peel were 11 and 11.65 ± 0.250 mg GAE/g peel, and 9.5 and 8.70 ± 0.125 mg cyn-3-gly E/g peel, respectively; whereas their values from fruit flesh were 19.6 and 19.25 ± 0.902 mg GAE/g flesh, and 2 and 1.95 ± 0.016 mg cyn-3-gly E/g flesh, respectively. The predicted and actual values for all responses were similar with an absolute error percentage within the acceptable limit (< 10%), which indicates the

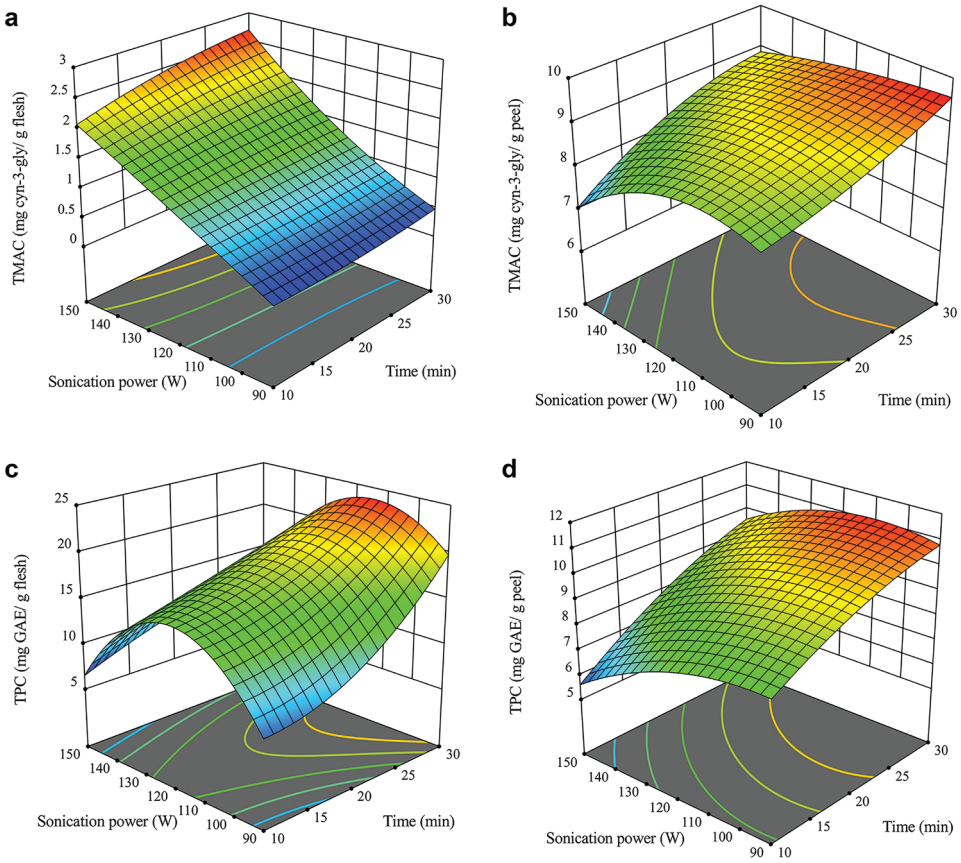

Figure 2. Response surface plot showing the interactive effect of ultrasonic intensity and extraction time on the extraction yield of (a) flesh TMAC, (b) peel TMAC, (c) flesh TPC, and (d) peel TPC.

adequacy of the obtained model (Nag and Sit, 2018).

Identified polyphenol compounds

HPLC analysis was used to identify and quantify the phenolic compounds of fruit flesh and peel extracts containing the highest yield of polyphenols. The concentrations of the identified phenolic compounds are shown in Table 3.

Table 3. Identified phenolic compounds in pomposia fruit flesh and peel extracts, and their antioxidant activity.

Compound	Fruit flesh (mg/100 g)	Fruit peel (mg/100 g)
Phenolic		
Gallic acid	1.56	2.86
Pyrogallol	230.60	337.34
4-Aminobenzoic acid	1.17	1.86
Tyrosol	3.19	3.74
Protocatechuic acid	7.07	11.69
Catechin	27.82	112.98
Chlorogenic acid	5.93	9.04
Catechol	26.87	7.82
Epicatechin	4.94	4.69
Caffeine	1.40	4.53
4-Hydroxybenzoic acid	8.36	6.39
Caffeic acid	11.54	11.86
Vanillic acid	17.54	3.25
ρ-Coumaric acid	1.83	0.62
Ferulic acid	6.29	4.13
Isoferulic acid	5.17	3.00
Resveratrol	0.17	0.98
E-Vanillic acid	138.01	18.11
Ellagic acid	4.04	8.68
Coumaric acid	1.92	0.73
Benzoic acid	31.13	3.67
3,4,5-Trimethoxycinnamic acid	2.80	2.49
Coumarin	1.58	0.50
Salicylic acid	3.91	1.98
Cinnamic acid	0.32	0.08
Flavonoid		
Luteolin	1.00	5.33
Naringin	2.35	1.47
Rutin	0.83	1.12
Hesperidin	2.70	1.17
Rosmarinic acid	0.29	0.33
Quercetrin	0.39	0.25
Quercetin	0.12	0.25
Hispertin	0.33	0.87
Kaempferol	0.12	0.00
Apigenin	0.03	0.03
7-Hydroxyflavone	0.03	0.02
***DPPH radical scavenging activity (IC_{50}) (mg GAE/L)**	10.24	2.82

*DPPH radical scavenging activity of BHT (IC_{50}) = 23.19 (mg/L).

Twenty-five phenolic compounds and 11 flavonoid compounds were identified in the extracts of flesh and peel of pomposia fruits. Pyrogallol and E-vanillic acid were the predominant polyphenols in the flesh (230.60 and 138.01 mg/100 g, respectively), while pyrogallol and catechin were the major polyphenols in the peel (337.34 and 112.98 mg/100 g, respectively). Benzoic acid, catechol, and vanillic acid were also found in appreciable amounts in the investigated parts of the fruits. Their concentrations in the flesh were greater than five times their levels in the fruit peel.

Chlorogenic acid (20.5 mg/100 g) and gallic acid (2.8 mg/100 g) were the major phenolic compounds present in the fresh pomposia pulp (Branco *et al.,* 2016). In addition, the major phenolic compounds of frozen fruit pulp were gallic acid (2.29 mg/100 g) and chlorogenic acid (1.37 mg/100 g) (Coelho *et al.,* 2016). Phenolic acids concentration decreased at the over-ripe stage of fruit maturity (Gruz *et al.,* 2011). This decrement could be due to deficiency of essential substances that are required for the formation of phenolic compounds at this stage (Seraglio *et al.,* 2018).

HPLC analysis indicated that hesperidin (2.70 mg/100 g) and naringin (2.35 mg/100 g) were the major flavonoid compounds in the fruit flesh extract, while luteolin (5.33 mg/100 g) was the main flavonoid in the fruit peel. Myricetin (3.54 mg/100 g) was the major flavonoid compound in fruit pulp (Branco *et al.,* 2016).

DPPH radical scavenging activity

The antioxidant activity of the flesh and peel extracts containing the highest level of phenolic compounds against DPPH radicals was measured and compared with BHT.

This test is recommended for screening the antioxidant activity of plant extracts (Hamlaoui *et al.,* 2018). Peel and flesh extracts exhibited DPPH radical inhibition percentage of 88.11 and 82.56% at 5.07 and 17.55 mg GAE/L, respectively. The inhibition percentage of DPPH reached only 73.96% when BHT concentration was 36 mg/L. The IC_{50} for peel extract, flesh extract, and BHT were 2.82 mg GAE/L, 10.24 mg GAE/L, and 23.19 mg/L, respectively (Table 3). This indicates high antioxidant activity of peel and flesh extracts as compared to that of BHT. The low IC_{50} value of peel extract could be explained by its high anthocyanin content. Our observation is in accordance with other study (Veigas *et al.,* 2007). A significant correlation was found between polyphenolic content and antioxidant activity of the grape seed extracts obtained by UAE (Vural *et al.,* 2018).

Conclusion

In the present work, RSM successfully optimised the UAE of anthocyanins and polyphenols from the flesh and peel of the pomposia fruit. The reduced cubic-order model fit experimental data well. The optimum extraction conditions of anthocyanins and polyphenols from the fruit flesh and peel using UAE were 30 min at 135.73 W, and 30 min at 100.30 W, respectively. The major phenolic compounds in flesh and peel of pomposia fruit were pyrogallol, catechin, and E-vanillic. The flesh and peel extracts showed superior scavenging activity against DPPH.

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