Arabian Journal of Chemistry (2017) **10**, S1145–S1157

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Ultrasound assisted extraction of bioactive compounds from *Nephelium lappaceum* L. fruit peel using central composite face centered response surface design

J. Prakash Maran [a,*], S. Manikandan [b], C. Vigna Nivetha [a], R. Dinesh [a]

[a] *Department of Food Technology, Kongu Engineering College, Perundurai, Erode 638 052, TN, India*
[b] *Department of Food and Process Engineering, SRM University, SRM Nagar, Kattankulathur, Chennai 603 203, TN, India*

Received 18 September 2012; accepted 4 February 2013
Available online 20 February 2013

KEYWORDS

Extraction;
Ultrasound;
Optimization;
Anthocyanin;
Phenolics;
Flavonoid

Abstract In this study, four factors at three level central composite face centered design was employed to study and optimize the process variables on extraction of bioactive compounds (total anthocyanin, phenolic and flavonoid content) from *Nephelium lappaceum* L. fruit peel. The effect of process variables such as extraction temperature (30–50 °C), power of ultrasound (20–40 W), extraction time (10–30 min) and solid–liquid ratio (1:10–1:20 g/ml) is studied. Multiple regression analysis was done on the experimental data to develop second-order polynomial models with high coefficient of determination value ($R^2 > 0.99$). The optimal conditions based on both individual and combinations of all process variables (extraction temperature of 50 °C, ultrasound power of 20 W, extraction time of 20 min and solid–liquid ratio of 1:18.6 g/ml) were determined by Derringer's desired function methodology. Under these conditions, total anthocyanin (10.26 ± 0.39 (mg/100 g)), phenolics (552.64 ± 1.57 (mg GAE/100 g)) and flavonoid (104 ± 1.13 (mg RE/100 g)) content values were determined and it is closely related with the predicted values (10.17 mg/100 g of total anthocyanin, 546.98 mg GAE/100 g of total phenolics and 100.93 mg RE/100 g of total flavonoid content) and indicted the suitability of the developed models.

1. Introduction

The globalization of the food industry has shown a highly increasing demand for functional foods, value addition and reutilization of agricultural waste. Recently, there has been an increasing interest in bioactive compounds from fruits, which function as free radical scavengers. Pigments and polyphenols are ubiquitous bioactive compounds which belong to a diverse group of secondary metabolites and are universally present in higher plants. In this prospect, phytochemicals have been shown to possess significant antioxidant capacities that can

* Corresponding author. Tel.: +91 4294 226606; fax: +91 4294 220087.
E-mail addresses: prakashmaran@gmail.com (J. Prakash Maran), manift@gmail.com (S. Manikandan), cvn.chand@gmail.com (C. Vigna Nivetha), dineshft008@gmail.com (R. Dinesh).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2013.02.007

protect human body from the aforementioned health problems. Interestingly, recent research has revealed that fruit peels and seeds, such as grape seeds and peel (Jayaprakasha et al., 2003; Jayaprakasha et al., 2001; Negro et al., 2003), pomegranate peel (Singh et al., 2002), sweet orange peel (Anagnostopoulou et al., 2006) and mango seed kernel (Kabuki et al., 2000) may potentially possess antioxidant and/or antimicrobial properties.

The extraction of bioactive compounds from permeable solid plant materials using solvents constitutes an important step in the manufacture of phytochemical-rich products. The biologically functional phenolics from various plants were isolated by extraction methods such as maceration and soxhlet extraction. However, these conventional extraction methods were generally time-consuming and had low efficiency (Ma et al., 2008). Recent years have seen an increasing demand for new extraction techniques enabling automation, shortening extraction times, and reducing consumption of organic solvent. In contrast, ultrasound in combination with conventional extraction is a potential technique in enhancing the rates and extent of mass transfer to and from the interfaces. The beneficial effects of ultrasound are derived from its mechanical effects on the process by increasing the penetration of solvent into the product due to disruption of the cell walls produced by acoustical cavitation (Toma et al., 2001). Moreover, it is achieved at lower temperatures and hence more suitable for enhancing the extraction of thermally unstable compounds (Wu et al., 2001) as compared to conventional methods. Recently, ultrasound-assisted extraction (UAE) has been widely used in the extraction of phenolics from different vegetable materials (Wang et al., 2008; Tiwari et al., 2010; Rostagno et al., 2003, 2007; Toma et al., 2001; Rodrigues and Pinto, 2007).

Nephelium lappaceum L. (rambutan) belongs to the family Sapindaceae, which is common in Southeast Asia. Due to its refreshing flavor and exotic appearance, this fruit is consumed as fresh, canned or processed. After being processed, the residues such as seeds and peels were discarded. From the previous findings, it was found that, the discarded peel of rambutan fruit comprises powerful phenolic antioxidants and scavenging activities (Thitilertdecha et al., 2010; Okonogi et al., 2007; Khonkarn et al., 2010; Palanisamy et al., 2008;) and possess various biological properties such as antibacterial and antiherpes (Nawawi et al., 1999; Thitilertdecha et al., 2010).

Ultrasound-assisted extraction (UAE) is an ideal extraction method capable of producing high quantities of bioactive compounds with a shorter extraction time. Development of an economical and efficient UAE technique for the separation of biologically active compounds, such as pigments and polyphenols from rambutan fruit peel is an emerging interest in the biomedical area and also creates novel opportunities to exploit its valuable properties. Hence, in this present work, four factors with three level central composite face-centered design was used to optimize and study the effects of extraction temperature, power of ultrasound, extraction time and solid–liquid ratio in UAE on the maximum yield of total anthocyanin, phenolic and flavonoid content from rambutan fruit peel.

2. Materials and methods

2.1. Plant materials

Fresh fruits of rambutan (*N. lappaceum* L.) at the commercial mature stage were procured from a commercial fruit market near Chennai, India on May 2012. Fruits were selected based on their uniformity in shape and color. The fruits were washed thoroughly in potable water and then air-dried. Thick layer of skin and thorns were peeled off from the fruits manually. Fruit peels were washed thoroughly in running tap water to get rid of adhered impurities on the surface. The fruit peels were dried in a vacuum oven at 40 °C until to obtain the constant weight. The dried peels were pulverized and sieved through a 40-mesh sieve to obtain the powdered samples. Powder samples (moisture content 12–14%) were stored in dark bags to prevent oxidation of active compounds due to sun light and kept in dry environment prior to the experiments.

2.2. Chemicals and reagents

Cyanidin-3-glucoside (C-3-G), gallic acid (GA) and rutin were purchased from Sigma chemicals, Mumbai. Folin–Ciocalteu reagent, sodium carbonate, Aluminum chloride, sodium nitrite and sodium hydroxide were obtained from Loba chemicals, Mumbai. All the chemicals used in this study are of analytical and HPLC grade.

2.3. UAE of pigment and polyphenols

UAE was performed according to the method described by Ying et al. (2011) in an ultrasonic bath (Power sonic, Korea) equipped with digital sonication power, time and temperature controller with a useful volume of 10 L (internal dimensions: $30 \times 24 \times 15$ cm) to carry out the extraction. About 10 g of ground powder was mixed with an appropriate quantity of distilled water. Triplicate experiments were carried out according to Table 1. After extraction, the extracts were centrifuged at 2600*g* for 15 min (Remi R-24 Centrifuge, India) and filtered through a filter paper (Whatman No. 1, England). The obtained supernatants (extracts) were collected in a screw-capped dark glass container and stored in the refrigerator until further analysis.

2.4. Total anthocyanin content

The total anthocyanin content (TAC) was estimated by a pH differential method which relies on the structural transformation of the anthocyanin chromophore as a function of pH value (Giusti and Wrolstad, 2000), which can be measured using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). Aliquots of extracts were brought to pH 1.0 using potassium chloride (0.025 M) and pH 4.5 using sodium acetate (0.4 M) and allowed to equilibrate for 1 h. The absorbance was recorded at 530 and 700 nm using a spectrophotometer calibrated with distilled water as the blank. The difference in absorbance between pH values and wavelengths was calculated using the formula:

$$A = (A_{530\text{nm}} - A_{700\text{nm}})_{\text{pH 1.0}} - (A_{530\text{nm}} - A_{700\text{nm}})_{\text{pH 4.5}} \quad (1)$$

The total monomeric anthocyanin content was obtained by

$$\text{TMA} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad (2)$$

where A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}), DF is the dilution factor, ε is the molar absorptivity of cyanidin-3-glucoside

Table 1 Uncoded and coded values of the independent variables and observed responses.

Run order	Extraction temperature (X_1, C)	Power of ultrasound (X_2, W)	Extraction time (X_3, min)	Solid–liquid ratio (X_4, g/ml)	TAC (mg/100 g)	TPC (mg GAE/100 g)	TFC (mg RE/100 g)
1	30 (−1)	40 (1)	10 (−1)	1:20 (1)	3.47	192.53	37.70
2	30 (−1)	20 (−1)	30 (1)	1:20 (1)	4.49	241.78	45.63
3	40 (0)	30 (0)	20 (0)	1:15 (0)	7.03	373.86	70.48
4	50 (1)	20 (−1)	30 (1)	1:20 (1)	7.76	421.68	77.72
5	40 (0)	40 (1)	20 (0)	1:15 (0)	6.85	362.28	68.60
6	40 (0)	30 (0)	30 (1)	1:15 (0)	5.07	264.62	50.01
7	30 (−1)	30 (0)	20 (0)	1:15 (0)	6.06	325.27	60.73
8	50 (1)	20 (−1)	10 (−1)	1:20 (1)	10.16	540.31	99.31
9	30 (−1)	20 (−1)	10 (−1)	1:10 (−1)	3.18	165.11	32.10
10	30 (−1)	40 (1)	30 (1)	1:10 (−1)	5.52	291.55	57.55
11	30 (−1)	20 (−1)	30 (1)	1:10 (−1)	3.92	207.47	39.66
12	50 (1)	20 (−1)	30 (1)	1:10 (−1)	7.53	405.45	76.36
13	50 (1)	40 (1)	30 (1)	1:20 (1)	6.92	369.01	67.96
14	50 (1)	30 (0)	20 (0)	1:15 (0)	9.89	527.95	98.85
15	30 (−1)	20 (−1)	10 (−1)	1:20 (1)	6.57	346.39	66.17
16	40 (0)	30 (0)	20 (0)	1:15 (0)	7.09	377.05	70.89
17	50 (1)	40 (1)	10 (−1)	1:20 (1)	7.58	405.10	78.25
18	50 (1)	40 (1)	10 (−1)	1:10 (−1)	7.55	407.51	77.84
19	40 (0)	30 (0)	20 (0)	1:20 (1)	7.37	391.94	73.69
20	50 (1)	20 (−1)	10 (−1)	1:10 (−1)	7.27	384.62	72.85
21	40 (0)	30 (0)	20 (0)	1:15 (0)	7.05	374.92	70.06
22	40 (0)	30 (0)	20 (0)	1:15 (0)	7.08	376.51	71.57
23	40 (0)	30 (0)	20 (0)	1:15 (0)	7.04	374.39	71.35
24	40 (0)	20 (−1)	20 (0)	1:15 (0)	7.27	389.62	72.69
25	30 (−1)	40 (1)	10 (−1)	1:10 (−1)	3.69	198.23	36.69
26	40 (0)	30 (0)	20 (0)	1:10 (−1)	7.14	379.71	71.50
27	40 (0)	30 (0)	10 (−1)	1:15 (0)	4.96	260.77	50.38
28	50 (1)	40 (1)	30 (1)	1:10 (−1)	9.72	512.91	98.65
29	40 (0)	30 (0)	20 (0)	1:15 (0)	7.07	375.98	71.29
30	30 (−1)	40 (1)	30 (1)	1:20 (1)	3.17	167.58	33.46

(26,900 L cm^{-1} mol^{-1}), and 1 is for a standard 1 cm path length. Total anthocyanins were reported in milligrams anthocyanins per 100 g of fruit peel (mg cyanidin-3-glucoside/100 g fruit peel).

2.5. Total phenolic content

The total phenolic content (TPC) was measured by Folin–Ciocalteu method as described by Vatai et al. (2009) with minor modifications. A known amount of (0.5 ml) diluted sample was mixed with 2.5 ml of Folin–Ciocalteu reagent (0.2 mol/L), and after 5 min, 2.0 ml of sodium carbonate (7.5 g/100 ml) was added. The mixture was kept at room temperature for 2 h after shaken and absorbance of the mixture was measured at 754 nm against a reagent blank (0.5 ml distilled water instead of the sample) with a UV–visible spectrophotometer (Elico, SL 244, India). Results were calculated on the basis of the calibration curve of gallic acid and expressed as gallic acid equivalents (mg GAE/100 g). All samples were analyzed in triplicates and the average values were calculated.

2.6. Total flavonoid content

Aluminum chloride assay method (Zhishen et al., 1999) was used to determine the total flavonoid content (TFC) of the samples. A known volume of extract (0.5 ml) was mixed with distilled water to make 5 ml solution, and 0.3 ml 5% $NaNO_2$ was added. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6th min, 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured at 510 nm. Total flavonoid content was calculated on the basis of the calibration curve of rutin and expressed as rutin equivalents (mg RE/100 g). All samples were analyzed in triplicates and the average values were calculated.

2.7. Experimental design

Box-Wilson design, also called central composite design (CCD), is an experimental design used to achieve maximal information about a process from a minimal number of experiments (Yang et al., 2009). In CCD, the central composite face-centered (CCFC) experimental design was used in this study to determine the optimal conditions and study the effect of four variables (extraction temperature (°C), power of ultrasound (W), extraction time (min) and solid–liquid ratio (g/ml)) on three responses (total anthocyanin content (TAC), total phenolic content (TPC) and total flavonoid content (TFC)) of ultrasound-assisted *N. lappaceum* L. peel extracts. From the preliminary experimental results, process variables and their ranges (extraction temperature (30–50 °C), power of ultrasound (20–40 W), extraction time (10–30 min) and solid–liquid ratio (1:10–1:20 g/ml)) were determined. After selection of independent variables and their ranges, experiments were established based on a CCFC design with four factors at three levels and each independent variable was coded at three levels between −1, 0 and +1. The coding of the variables was done by the following equation (Prakash Maran et al., 2013):

$$x_i = \frac{X_i - X_z}{\Delta X_i} \qquad i = 1, 2, 3 \ldots k \tag{3}$$

where x_i, is the dimensionless value of an independent variable; X_i, the real value of an independent variable; X_z, the real value of an independent variable at the center point; and ΔX_i, step change of the real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i. In this design, the star points are at the center of each face of the factorial space, thus $\pm\alpha = \pm 1$. The coded and uncoded independent variables used in this study are listed in Table 1.

In this study, total number of 30 experiments (consisting of 16 factorial points, eight star points and six replicates at the center points (in order to allow the estimation of pure error)) were carried out and the total number experiments were calculated from the following equation (Azargohar and Dalai, 2005):

$$N = 2^n + 2n + n_c \tag{4}$$

where N is the total number of experiments required; n is the number of factors; and c is the number of center points.

The experimental sequence was randomized in order to minimize the effects of unexpected variability in the responses due to extraneous factors. A second-order polynomial equation was used in order to develop an empirical model which correlated the responses to the independent variables. The general form second order polynomial equation is

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{i} \sum_{<j=2}^{k} \beta_{ij} X_i X_j + e_i \tag{5}$$

where Y is the response; X_i and X_j are variables (i and j range from 1 to k); β_0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are interaction coefficients of linear, quadratic and the second-order terms, respectively; k is the number of independent parameters ($k = 4$ in this study); and e_i is the error (Prakash Maran et al., 2013).

2.8. Statistical analysis

The statistical analysis was performed using Design Expert Statistical Software package 8.0.7.1 (Stat Ease Inc., Minneapolis, USA). The experimental data were analyzed by multiple regression analysis through the least square method. Two different tests namely sequential sum of squares and model summary statistics were carried out on the experimental data in order to find out adequacy of various models. The regression coefficients of all the terms (linear, quadratic, and interaction) involved in the model and their effect were analyzed by Pareto analysis of variance (ANOVA) and ANOVA tables were generated. All the terms of the model were tested and verified statistically by F-test at probability levels ($p \leqslant 0.05$). Adequacy of the developed models was tested by performing coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}) and predicted coefficient of determination (R^2_{pre}). After fitting the models, surfaces and contour plots were constructed to predict the relationship between the independent variables and responses.

2.9. Optimization

A numerical optimization technique (Derringer's desired function method) was employed for optimizing the various responses simultaneously involved in the UAE process. The desirability function method was applied for generating

optimum conditions having some specific desirability value. This optimization technique depends on whether a particular response (Y_i) is to be maximized or minimized or targeted based on the requirement of the process, while the independent variables are kept within the range. The general approach is to first convert each response (Y_i) into a dimensionless individual desirability function (d_i) and it was done with the following equation

$$d_i = h_n(Y_i) \tag{6}$$

Different desirability functions $d_i(Y_i)$ can be used to obtain the individual desirabilities (d_i) for each responses i.e., minimize, maximize, in range or target of the response (Derringer and Suich, 1980). Let L_i, U_i and T_i be the lower, upper, and target values, respectively, that are desired for response Y_i, with L_i, T_i, U_i. The individual desirabilities (d_i) for each response are obtained by specifying the goals, i.e., minimize, maximize, in range or target of the response. Depending on whether a particular response Y_i is to be maximized or minimized, different desirability functions $d_i(Y_i)$ can be used (Derringer and Suich, 1980). Let L_i, U_i and T_i be the lower, upper, and target values, respectively, that are desired for the response Y_i, with L_i, T_i, and U_i. The maximization of the response depends on its individual desirability function, which in turn relies on the target value hit by the exponent s. For $s = 1$, the desirability function increases linearly toward T_i which denotes a large enough value for the response; for $s < 1$, the function is convex, and for $s > 1$, the function is concave:

$$d_i(Y_i) = \begin{cases} 0 & Y_i(x) < L_i \\ \left(\frac{Y_i(x)-L_i}{T_i-L_i}\right)^s & L_i \le Y_i(x) \le T_i \\ 1 & Y_i(x) > T_i \end{cases} \tag{7}$$

If a response is to be minimized, then its individual desirability function is with the T_i denoting a small enough value for the response:

$$d_i(Y_i) = \begin{cases} 1 & Y_i(x) < U_i \\ \left(\frac{Y_i(x)-U_i}{T_i-U_i}\right)^s & T_i \le Y_i(x) \le U_i \\ 0 & Y_i(x) > U_i \end{cases} \tag{8}$$

After desirability values are computed for each response variable, they are combined to obtain a single global desirability index (D), which varies from 0 (completely undesirable response) to 1 (fully desired response) (Prakash Maran and Manikandan, 2012) and that are equal to their geometric mean of the individual desired functions. For all the desired functions, a total desired function D ($0 \le D \le 1$) is defined by Eren and Ertekin (2007)

$$D = (d_1^{v_i} \times d_2^{v_i} \times \cdots \times d_n^{v_i}) \tag{9}$$

$$D = \left(\prod_{i=1}^{n} d_i^{v_i}\right)^{1/\sum v_i} \tag{10}$$

where v_i is a number indicating the relative importance of the ith response.

In present study, desirability functions were developed for the following responses: maximum total anthocyanin, phenolic and flavonoid content. A weight factor, which defines the shape of the desirability function for each response, is then assigned. Weights must be between 1 and 10, with larger weights corresponding to more important responses. A weight factor of 1 was chosen for all individual desirability's in this work. The "importance" of a goal can be changed in relation to the other goals. It can range from 1 (least important) to 5 (most important). The default is three representing all goals to be equally important.

2.10. Validation of optimized conditions and predictive models

The suitability of the developed model equations for predicting the optimum response values was verified using the optimal condition. Triplicate experiments were carried out in the optimal condition and the mean experimental values were compared with the predicted values in order to determine the validity of the models.

3. Results and discussions

In this study, four factors with three level- CCFC design was employed to optimize the combination of UAE process variables (extraction temperature, power of ultrasound, extraction time, and solid–liquid ratio) and to study the linear, interactive and quadratic effect of process variables on the extraction of pigment (total anthocyanin content) and polyphenolic content (total phenolic and total flavonoid content) from rambutan fruit peels. A total number of 30 experiments including six center points (used to determine the experimental error) were carried out in order to find out the optimal extraction conditions. The various combinations of experimental conditions (coded and uncoded) with their respective experimental responses (mean response data) are presented in Table 1.

3.1. CCFC design analysis

The experimental data were analyzed and fitted to the four high degree polynomial models viz., linear, interactive (2FI), quadratic and cubic models. Two different tests namely the sequential model sum of squares and model summary statistics were carried out in this study to decide about the adequacy of models among various models to represent the total anthocyanin content, phenolic content and flavonoid content of the extracts and the results are listed in Table 2.

The adequacy of models tested output indicates that, cubic model was found to be aliased. The linear and quadratic models are found to have lower p-value (< 0.0001). But the R^2, adjusted R^2 and predicted R^2 values were found to be low in linear model (Table 2). Thus the linear and cubic models cannot be chosen for further modeling of experimental data. Quadratic model was not only found to have maximum R^2, adjusted R^2, predicted R^2, but also exhibited low p-values. Hence the quadratic model was chosen for further analysis.

3.2. Development of second order polynomial models

Second-order polynomial equation with interaction terms was fitted to the experimental results to develop mathematical model, which will help to predict the extraction efficiency of different sets of combinations of four process variables on the responses. Three empirical models were developed from this study to predict the UAE efficiency of TAC, TPC and TFC from rambutan fruit peels. The final model obtained in terms of coded factors is given below

Table 2 Adequacy of the models.

Source	Sum of squares	Degree of freedom	Mean square	*F* value	Prob > *F*	Remarks
Sequential model sum of squares for total anthocyanin content						
Mean	1273.62	1	1273.62			
Linear	66.37	4	16.59	12.28	< 0.0001	
2FI	19.34	6	3.22	4.24	0.0071	
Quadratic	14.29	4	3.57	334.03	< 0.0001	Suggested
Cubic	0.11	8	0.01	1.97	0.1929	Aliased
Residual	0.05	7	0.01			
Total	1373.78	30	45.79			
Sequential model sum of squares for total phenolic content						
Mean	3613723.80	1	3613723.80			
Linear	190801.80	4	47700.45	12.64	< 0.0001	
2FI	52180.22	6	8696.70	3.92	0.0102	
Quadratic	41862.81	4	10465.70	523.62	< 0.0001	Suggested
Cubic	228.69	8	28.59	2.81	0.0953	Aliased
Residual	71.12	7	10.16			
Total	3898868.44	30	129962.28			
Sequential model sum of squares for total flavonoid content						
Mean	129363.26	1	129363.26			
Linear	6403.90	4	1600.97	12.14	< 0.0001	
2FI	1922.50	6	320.42	4.43	0.0058	
Quadratic	1363.17	4	340.79	438.57	< 0.0001	Suggested
Cubic	3.83	8	0.48	0.43	0.8708	Aliased
Residual	7.82	7	1.12			
Total	139064.48	30	4635.48			
Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	Remarks
Model summary statistics for total anthocyanin content						
Linear	1.16	0.6627	0.6087	0.4670	53.39	
2FI	0.87	0.8557	0.7797	0.6933	30.72	
Quadratic	0.10	0.9984	0.9969	0.9870	1.30	Suggested
Cubic	0.08	0.9995	0.9980	0.8855	11.47	Aliased
Model summary statistics for total phenolic content						
Linear	61.43	0.6691	0.6162	0.4819	147727.40	
2FI	47.11	0.8521	0.7743	0.7013	85181.82	
Quadratic	4.47	0.9989	0.9980	0.9919	2319.49	Suggested
Cubic	3.19	0.9998	0.9990	0.9470	15101.42	Aliased
Model summary statistics for total flavonoid content						
Linear	11.48	0.6601	0.6057	0.4628	5211.57	
2FI	8.51	0.8583	0.7837	0.7168	2746.96	
Quadratic	0.88	0.9988	0.9977	0.9921	76.78	Suggested
Cubic	1.06	0.9992	0.9967	0.8575	1382.59	Aliased

$$\mathrm{TAC} = 7.06 + 1.91X_1 - 0.2X_2 - 0.018X_3 + 0.11X_4 + 0.085X_1X_2 - 0.051X_1X_3 - 0.065X_1X_4 + 0.41X_2X_3 - 0.78X_2X_4 - 0.65X_3X_4 + 0.92X_1^2 + 8.772E-003X_2^2 - 2.04X_3^2 + 0.2X_4^2 \quad (11)$$

$$\mathrm{TPC} = 374.91 + 102.41X_1 - 10.87X_2 - 1.03X_3 + 6.88X_4 + 3.33X_1X_2 - 2.16X_1X_3 - 3.77X_1X_4 + 18.61X_2X_3 - 41.47X_2X_4 - 34.14X_3X_4 + 52.24X_1^2 + 1.58X_2^2 - 111.67X_3^2 + 11.45X_4^2 \quad (12)$$

$$\mathrm{TFC} = 70.66 + 18.78X_1 - 1.43X_2 - 0.24X_3 + 0.93X_4 + 0.91X_1X_2 - 0.7X_1X_3 - 1.21X_1X_4 + 3.64X_2X_3 - 7.57X_2X_4 - 6.84X_3X_4 + 9.41X_1^2 + 0.26X_2^2 - 2.0.19X_3^2 + 2.21X_4^2 \quad (13)$$

3.3. Statistical analysis

Multiple regression analysis and Pareto analysis of variance (ANOVA) were used to check the adequacy and fitness of the developed models and results of ANOVA are given in Table 3. Table 3 summarized the effects of the model terms and associated *p* values for all three responses. At a 95% confidence level, a model was considered significant, if the *p* value < 0.05. The sign and value of the quantitative effect represent tendency and magnitude of the term's influence on the response, respectively. A positive value in the regression equation exhibits an effect that favors the optimization due to synergistic effect, while a negative value indicates an inverse relationship or antagonistic effect between the factor and the

response (Verma et al., 2009). From the ANOVA table, it was found that the developed models for all the responses were highly significant at probability level $p < 0.0001$ and also showed higher Fisher *F*-value (667.71 for TAC, 1017.95 for TPC and 890.68 for TFC) with significant *p*-value ($p < 0.0001$), which indicates that most of the variation in the response could be explained by the developed models.

Determination coefficient (R^2), adjusted determination coefficient (R^2_{adj}), predicted determination coefficient (R^2_{pre}) and coefficient of variation (CV%) were calculated to check the goodness and behavior of the developed models with the experimental data. A high R^2 coefficient ensures a satisfactory adjustment of the quadratic model to the experimental data. The calculated R^2 (0.998 for TAC, 0.999 for TPC and 0.998 for TFC), R^2_{adj} (0.996 for TAC, 0.998 for TPC and 0.997 for TFC) and R^2_{pre} (0.987 for TAC, 0.991 for TPC and 0.992 for TFC) values were high and advocate a high correlation between the observed and the predicted values. The analysis shows that the form of the model chosen to explain the relationship between the factors and the response is well-correlated and proved that the developed models defined exactly the true behavior of the process. The low CV values (1.59, 1.29 and 1.34) clearly indicated that, the deviations between experimental and predicted values are low and also showed a high degree of precision and a good deal of reliability in conducted experiments. Adequate precision is greater than four is desirable (Prakash Maran et al., 2013b) and the ratio was found to be >95, which indicates an adequate signal and confirms that this model can be used to navigate the design space.

3.4. Adequacy of the models

In general, the exploration of fitted response surface models may produce poor or misleading results, unless the model exhibits a good fit, which makes checking of the model adequacy essential (Prakash Maran et al., 2013a). Diagnostic plot such as predicted versus actual values (Fig. 1) is used to evaluate the relationship and model satisfactoriness between experimental data and predicted data obtained from the developed models. From Fig. 1, it was observed that, the data points lie very closely to the straight line. It exhibited high correlation between the experimental data and predicted data obtained from the models.

Data were also analyzed to check the residuals. The residual gives the difference between the observed value of a response measurement and the value that is fitted under the theorized model. Small residual value indicates that model prediction is accurate (Herbach et al., 2004). By constructing internally studentized residuals plot, a check was made to analyze the experimental data and to find out the satisfactory fit of the developed models and the plots are shown in Fig. 2.

Normal probability plot represents the normal distribution of the residuals and constructing a normal probability plot of the residuals allows the assessment of the normal distribution of the data. The normal probability plots of the residuals are shown in Fig. 3 and the data points on this plot lie reasonably close to a straight line indicating an acceptable fit.

3.5. Effect of process variables

In the present study, four factors at three level CCFC design used to study the influence of process variables such as

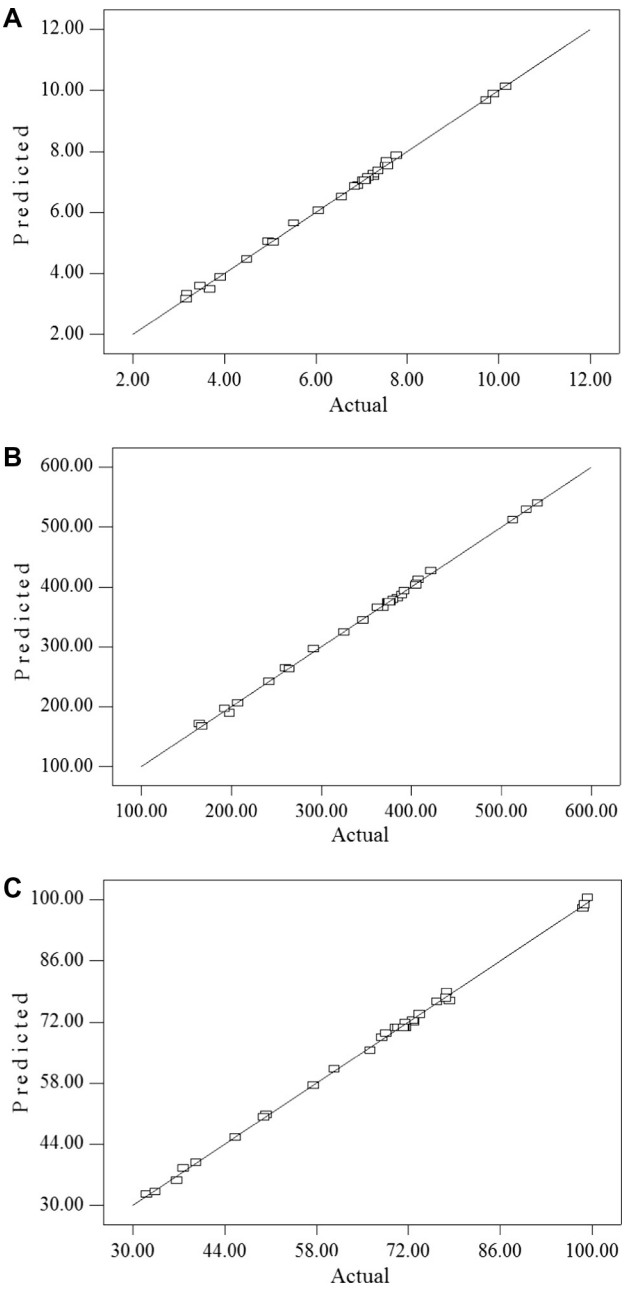

Figure 1 Diagnostic between experimental and predicted values for TAC (A), TPC (B), and TFC (C).

extraction temperature, power of ultrasound, extraction time, and solid–liquid ratio were investigated on the UAE extraction of pigment and polyphenols from rambutan fruit peels. The three dimensional response surface and contour plots were constructed from the developed models. Response surface and contour plots are graphical representations of a regression equation that illustrate the main and interactive effects of independent variables on a response variable. These graphs are drawn by maintaining two factors constant (in turn at its central level) and varying the other two factors in order to understand their main and interactive effects on the dependent variables (Prakash Maran et al., 2013). These graphs are easy to understand and represent the interactions between pairs of

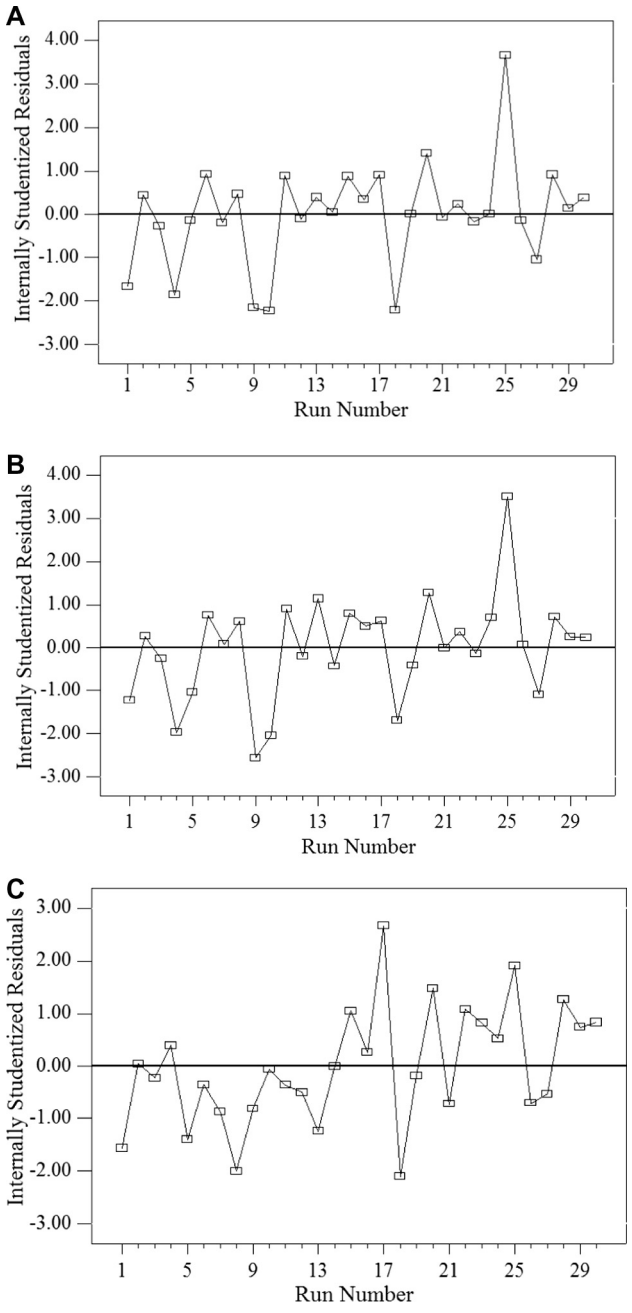

Figure 2 Internally studentized residuals plot for TAC (A), TPC (B), and TFC (C).

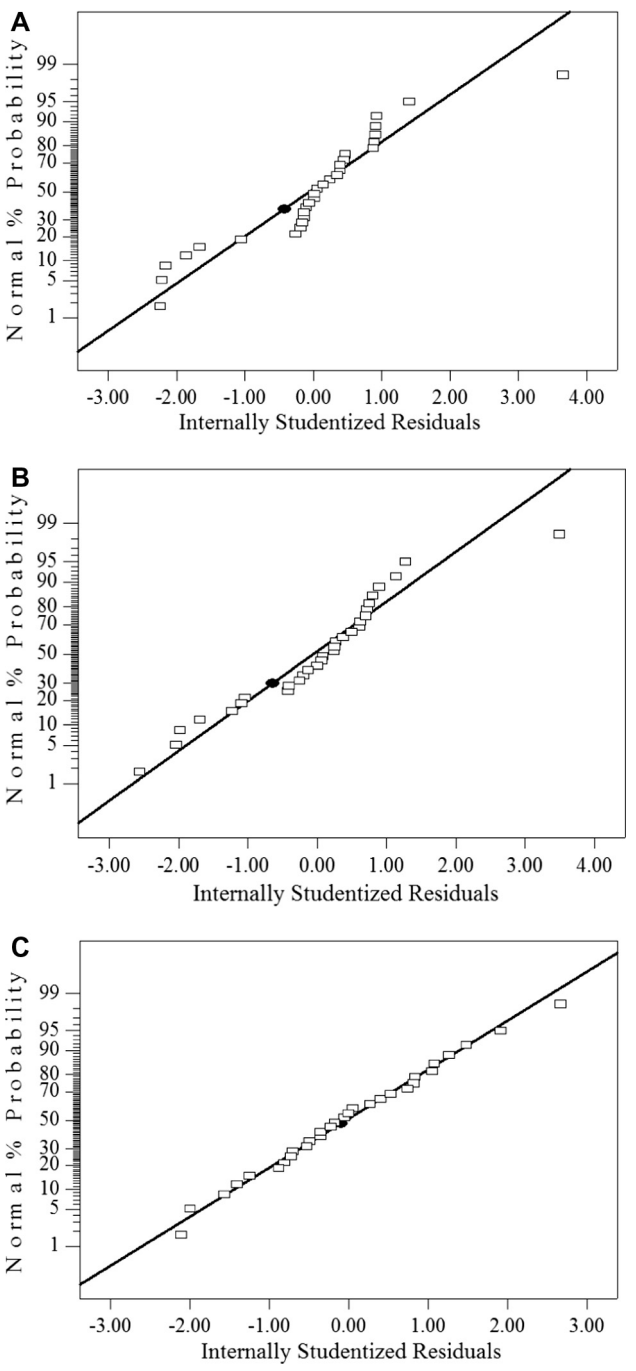

Figure 3 Normal probability plot for TAC (A), TPC (B), and TFC (C).

independent variables on the responses and also used to locate their optimal levels.

3.5.1. Effect of extraction temperature

The effect of extraction temperature on the yield of anthocyanin and polyphenols was studied. The results showed that, the extraction temperature exhibited a positive linear and quadratic effect (Table 3) on the yield. When the temperature was increased from 30 to 50 °C, the extraction yield of pigment and polyphenols was increased (Fig. 4), which is due to the high number of cavitational nucleus formed during higher extraction temperatures as a result of high cavitation threshold which is responsible for acoustic cavitation. The relative greater force ruptured the cavitational nucleus and disrupted the cell tissues during extraction, which will enhance the mass transfer (Toma et al., 2001). However, higher temperature could enhance the solubility of pigment and phenolic compounds in rambutan fruit peel and decrease the viscosity and density of the extracts (Chen et al., 2012). Reduced viscosity and density facilitate the solvent penetration deeper into the sample matrix which in turn enhances the extraction efficiency by exposing more surface area of the sample to the solvent used.

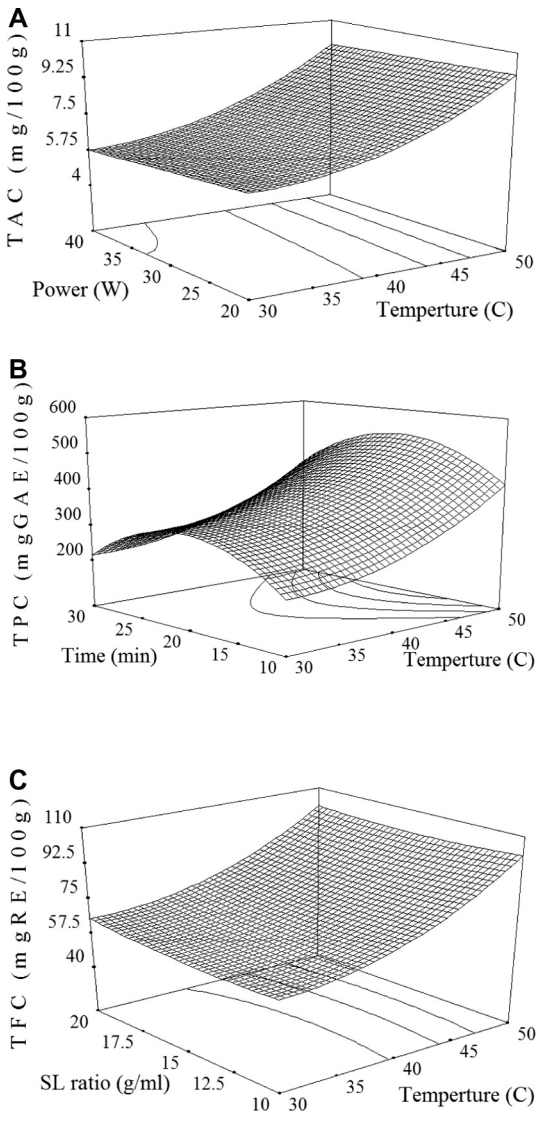

Figure 4 Response surface plots representing the effect of extraction temperature on the responses.

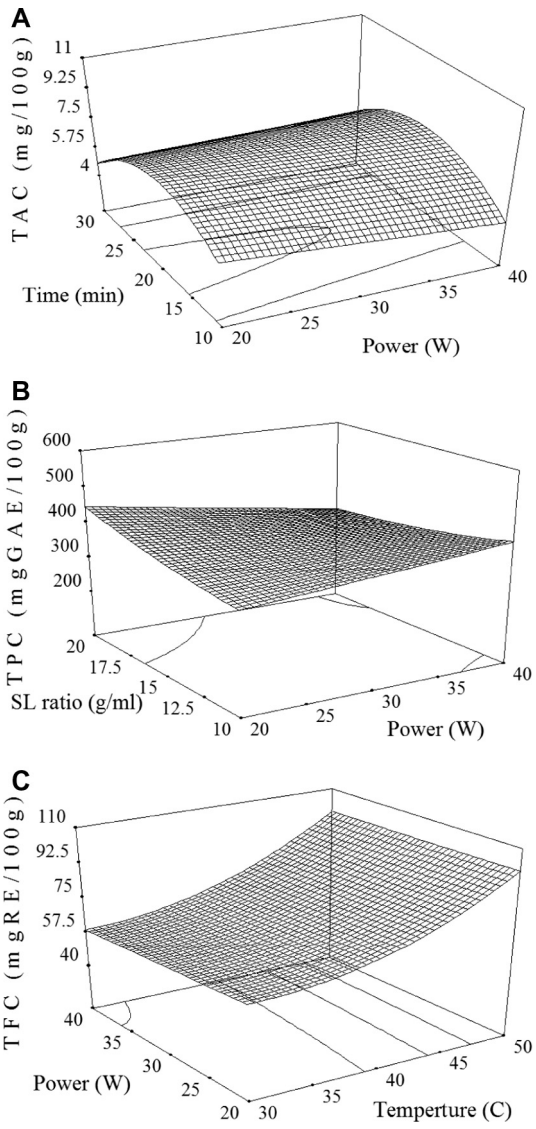

Figure 5 Response surface plots representing the effect of power of ultrasound on the responses.

3.5.2. Effect of extraction power of ultrasound

The efficiency of extraction power of ultrasound on the anthocyanin and polyphenols was evaluated and the results showed that, the yield increased with the increasing power of ultrasound (Fig. 5). The increase in extraction power facilitates the disruption of the cell walls of the peels, enhances the solubility of the compounds present in the peels and increases the extraction yield (Ying et al., 2011). This enhancement in extraction with ultrasound could be attributed to the ultrasonic effects such as micro jet formation and acoustic streaming (Sivakumar et al., 2009). Moreover, the intensity of ultrasound transmitted to the medium is directly related to the vibration amplitude of sonication, producing greater number of cavitation bubbles and therefore increased extraction efficiency (Dash et al., 2005).

3.5.3. Effect of extraction time

The pigment and polyphenols' yield was increased when the duration was maintained from 10 to 20 min but slowly decreased when the duration continued to be extended (Fig. 6). Most of the anthocyanin and polyphenols in broken cells are released at the early period of extraction, because ultrasound enhanced the release of those compounds into the exterior solvent and increased the yield in the first 20 min. However, longer extraction time with ultrasound treatment might induce the degradation of pigment and polyphenols (Tiwari et al., 2009).The number of cavitation micro-bubbles created by ultrasound increased with the duration extended. The asymmetric collapse of micro-bubbles near surfaces was also associated with micro-jets that could scour surfaces and damage substance in solution (Vilkhu et al., 2008). The structure of pigment and polyphenols was destroyed and its stability decreased because of continual collapse of micro-bubbles. The results are in agreement with Rostagno et al. (2007), who clearly indicated that 20 min of sonication time was sufficient enough to extract phenolics from soy beverages.

3.5.4. Effect of solid–liquid ratio

As shown in Fig. 7, the yield of anthocyanin and polyphenols was increased gradually when the solid–liquid ratio ranged

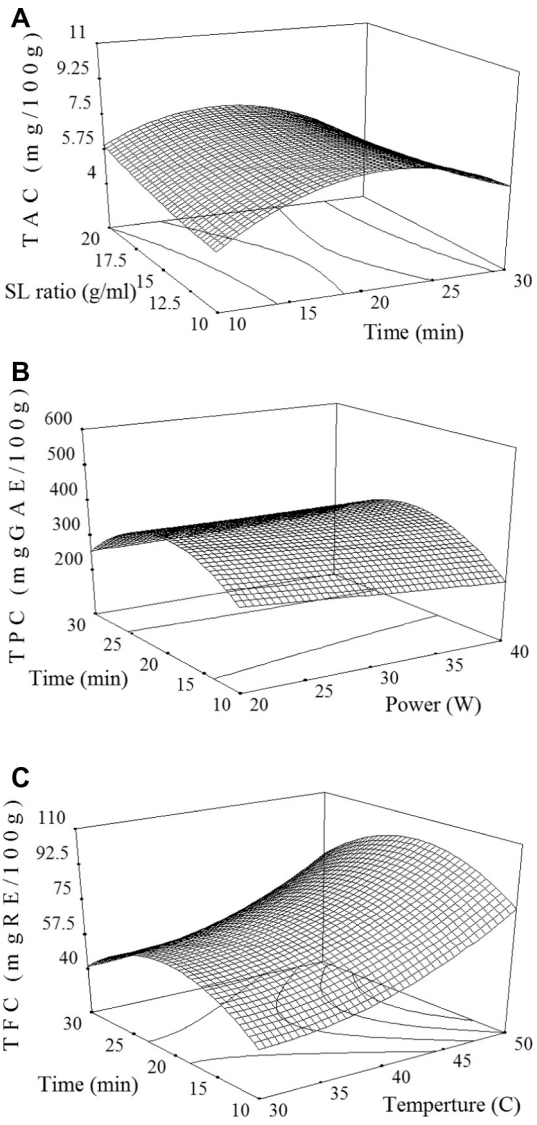

Figure 6 Response surface plots representing the effect of extraction time on the responses.

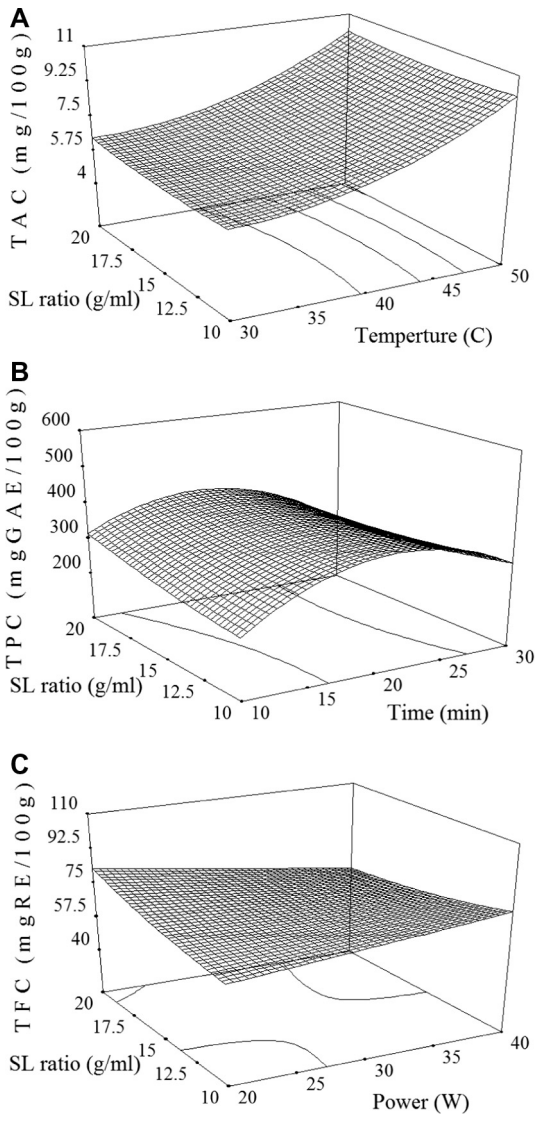

Figure 7 Response surface plots representing the effect of solid–liquid ratio on the responses.

from 1:10 to 1:20 (g/ml). In general, a higher solvent volume can dissolve target compounds more effectively and result in a better extraction yield. The concentration difference between the interior plant cell and the exterior solvent was caused by the higher solid–liquid ratio, which could dissolve the constituents (anthocyanin and polyphenols) more effectively leading to an enhanced mass transfer rate (Ying et al., 2011) and increase the extraction yield. The efficiency of ultrasonication can be explained by the fact that sonication simultaneously enhances the hydration and fragmentation processes while facilitating mass transfer of solutes to the extraction solvent, without causing significant decomposition of the solvent (Toma et al., 2001).

3.6. Determination and validation of optimum conditions

An optimum condition for the UAE of total anthocyanin and total polyphenols from rambutan fruit peel was determined to obtain maximum anthocyanin, phenolic and flavonoid content. Second order polynomial models obtained in this study were utilized for each response in order to obtain specified optimum conditions. Simultaneous optimizations of the multiple responses were carried out using Derringer's desirability function method. This function searches for a combination of factor levels that simultaneously satisfies the requirements for each response in the design. This numerical optimization evaluates a point that maximizes the desirability function. All the factors and responses with the respective high-limit and low-limit experimental region have to satisfy the criteria defined for the optimum operations as stated in Table 4. Applying the methodology of desired function, the optimum level of various parameters was obtained and it indicates that an extraction temperature of 50 °C, ultrasound power of 20 W, extraction time of 20 min and solid–liquid ratio of 1:18.6 g/ml give 10.17 mg/100 g of total anthocyanin content, 546.98 mg GAE/100 g of total phenolic content and 100.93 mg RE/100 g of total flavonoid content respectively with an overall desirability value of 0.927. These optimize

Table 3 Analysis of variance for the fitted second order polynomial models.

Source	Coefficient estimate	Sum of squares	*DF*	Standard error	Mean square	*F*-value	*p*-Value
Total anthocyanin content							
Model	7.06	100.00	14	0.03	7.14	667.71	< 0.0001
X_1	1.91	65.40	1	0.02	65.40	6113.33	< 0.0001
X_2	−0.20	0.75	1	0.02	0.75	70.33	< 0.0001
X_3	−0.02	0.01	1	0.02	0.01	0.57	0.4637
X_4	0.11	0.22	1	0.02	0.22	20.15	0.0004
X_{12}	0.09	0.12	1	0.03	0.12	10.81	0.0050
X_{13}	−0.05	0.04	1	0.03	0.04	3.93	0.0661
X_{14}	−0.07	0.07	1	0.03	0.07	6.32	0.0238
X_{23}	0.41	2.66	1	0.03	2.66	248.36	< 0.0001
X_{24}	−0.78	9.64	1	0.03	9.64	901.22	< 0.0001
X_{34}	−0.65	6.81	1	0.03	6.81	636.78	< 0.0001
X_1^2	0.92	2.21	1	0.06	2.21	206.68	< 0.0001
X_2^2	0.01	0.00	1	0.06	0.00	0.02	0.8932
X_3^2	−2.04	10.74	1	0.06	10.74	1004.19	< 0.0001
X_4^2	0.20	0.11	1	0.06	0.11	10.06	0.0063
Residual error	0.16						
Lack of fit	0.16						
Mean	6.52						
C.V.%	1.59						
Adeq. precision	95.51						
Total phenolic content							
Model	374.91	284844.83	14	1.39	20346.06	1017.95	< 0.0001
X_1	102.14	187803.96	1	1.05	187803.96	9396.19	< 0.0001
X_2	−10.87	2127.75	1	1.05	2127.75	106.46	< 0.0001
X_3	−1.03	19.12	1	1.05	19.12	0.96	0.3436
X_4	6.88	850.98	1	1.05	850.98	42.58	< 0.0001
X_{12}	3.33	177.72	1	1.12	177.72	8.89	0.0093
X_{13}	−2.16	74.86	1	1.12	74.86	3.75	0.0720
X_{14}	−3.77	227.31	1	1.12	227.31	11.37	0.0042
X_{23}	18.61	5540.33	1	1.12	5540.33	277.19	< 0.0001
X_{24}	−41.47	27514.15	1	1.12	27514.15	1376.58	< 0.0001
X_{34}	−34.14	18645.85	1	1.12	18645.85	932.89	< 0.0001
X_1^2	52.24	7070.70	1	2.78	7070.70	353.76	< 0.0001
X_2^2	1.58	6.47	1	2.78	6.47	0.32	0.5777
X_3^2	−111.67	32310.62	1	2.78	32310.62	1616.56	< 0.0001
X_4^2	11.45	339.71	1	2.78	339.71	17.00	0.0009
Residual error	299.81						
Lack of fit	291.89						
Mean	347.07						
C.V.%	1.29						
Adeq.precision	117.60						
Total flavonoid content							
Model	70.66	9689.56	14	0.27	692.11	890.68	< 0.0001
X_1	18.78	6350.41	1	0.21	6350.41	8172.36	< 0.0001
X_2	−1.43	36.98	1	0.21	36.98	47.59	< 0.0001
X_3	−0.24	1.02	1	0.21	1.02	1.31	0.2708
X_4	0.93	15.49	1	0.21	15.49	19.93	0.0005
X_{12}	0.91	13.32	1	0.22	13.32	17.14	0.0009
X_{13}	−0.70	7.84	1	0.22	7.84	10.09	0.0063
X_{14}	−1.21	23.52	1	0.22	23.52	30.27	< 0.0001
X_{23}	3.64	211.70	1	0.22	211.70	272.44	< 0.0001
X_{24}	−7.58	918.09	1	0.22	918.09	1181.49	< 0.0001
X_{34}	−6.84	748.02	1	0.22	748.02	962.63	< 0.0001
X_1^2	9.41	229.18	1	0.55	229.18	294.94	< 0.0001
X_2^2	0.26	0.17	1	0.55	0.17	0.22	0.6480
X_3^2	−20.19	1056.63	1	0.55	1056.63	1359.78	< 0.0001
X_4^2	2.21	12.60	1	0.55	12.60	16.21	0.0011
Residual error	11.66						
Lack of fit	9.98						
Mean	65.67						
C.V.%	1.34						
Adeq. precision	108.81						

Table 4 Settings for multi-criteria optimization.

Factor/response	Goal	Lower limit	Upper limit	Importance
X_1	In range	30	50	3
X_2	In range	20	40	3
X_3	In range	10.00	30.00	3
X_4	In range	1:10	1:20	3
TAC	maximize	3.17	10.16	3
TPC	Maximize	165.11	540.31	3
TFC	Maximize	32.1	99.31	3

Table 5 Predicted and experimental values of the responses at optimum conditions.

Optimal levels of process parameters	Optimized values (predicted values)			Experimental values		
	TAC (mg/100 g)	TPC (mg GAE/100 g)	TFC (mg RE/100 g)	TAC (mg/100 g)	TPC (mg GAE/100 g)	TFC (mg RE/100 g)
X_1 (°C) = 50 X_2 (W) = 20 X_3 (min) = 20 X_4 (g/ml) = 1:18.6	10.17	546.98	100.93	10.26 ± 0.39	552.64 ± 1.57	104 ± 1.13

conditions could be considered as optimum as well as feasible conditions.

The suitability of the model equations for predicting optimum response values was tested under the conditions (extraction temperature of 50 °C, ultrasound power of 20 W, extraction time of 20 min and solid–liquid ratio of 1:18.6 g/ml) as determined by Derringer's desired function methodology. Experiments were carried out under the optimal conditions in order to compare the experimental results with the predicted values of the responses using the developed empirical model equation (Eqs. (11)–(13)). The experiments were conducted in triplicates and the average values are reported in Table 5. The mean values of the TAC, TPC and TFC obtained were compared with the predicted values. The experimental values were found to be in agreement with the predicted values and clearly indicated the suitability of the developed quadratic models. The results obtained through confirmation experiments indicate the suitability of the developed quadratic models and it may be noted that these optimal values are valid within the specified range of process parameters.

4. Conclusions

Central composite face-centered design was successfully employed to optimize and study the individual and interactive effect of process variables such as extraction temperature, power of ultrasound, extraction time and solid–liquid ratio on the UAE of anthocyanin, polyphenols from rambutan fruit peel. The results showed that, the extraction conditions showed significant effects on the pigment and polyphenols' content. From this study, second-order models were developed to describe the relationship between the independent variables and the responses. The developed models could have the ability to predict the total anthocyanin, phenolic and flavonoid and was found to be comparable with that of the experimental values. Analysis of variance showed a high coefficient of determination values (R^2) of 0.998 for TAC, 0.999 for TPC and 0.998 for TFC, ensuring a satisfactory fit of the developed second-order polynomial models with the experimental data. The optimum conditions were found to be, extraction temperature of 50 °C, ultrasound power of 20 W, extraction time of 20 min and solid–liquid ratio of 1:18.6 and the predicted maximum TAC yield of 10.71 mg/100 g, TPC of 546.98 mg GAE/100 g and 100.93 mg RE/100 g respectively. Under the optimized conditions, the experimental values (total anthocyanin (10.26 ± 0.39 (mg/100 g)), phenolics (552.64 ± 1.57 (mg GAE/100 g)) and flavonoid content (104 ± 1.13 (mg RE/100 g)) closely agreed with the predicted values (10.17 mg/100 g of total anthocyanin, 546.98 mg GAE/100 g of total phenolics and 100.93 mg RE/100 g of total flavonoid content) and indicated the suitability of the developed models.

References

Anagnostopoulou, M.A., Kefalas, P., Papageorgiou, V.P., Assimopoulou, A.N., Boskou, D., 2006. Radical scavenging activity of various extracts and fractions of sweet orange peel *Citrus sinensis*. Food Chem. 94, 19–25.

Azargohar, R., Dalai, A.K., 2005. Production of activated carbon from Luscar char: experimental and modeling studies. Microporous Mesoporous Mater. 85, 219–225.

Chen, W., Huang, Y., Qi, J., Tang, M., Zheng, Y., Zhao, S., Chen, L., 2012. Optimization of ultrasound-assisted extraction of phenolic compounds from areca husk. J. Food Process. Preserv. http://dx.doi.org/10.1111/j.1745-4549.2012.00748.x.

Dash, K., Thangavel, Krishnamurthy, N.V., Rao, S.V., Karunasagar, D., Arunachalam, J., 2005. Ultrasound-assisted analyte extraction for the determination of sulphate and elemental sulphur in zinc sulphide by different liquid chromatography techniques. The Analyst 130, 498–501.

Derringer, G., Suich, R., 1980. Simultaneous optimization of several response variables. J. Qual. Technol. 12, 214–219.

Eren, İ., Ertekin, F.K., 2007. Optimization of osmotic dehydration of potato using response surface methodology. J. Food Eng. 79 (1), 344–352.

Giusti, M.M., Wrolstad, R.E., 2000. Current Protocols in Food Analytical Chemistry. John WileySons, NewYork.

Herbach, K.M., Stintzing, F.C., Carle, R., 2004. Thermal degradation of betacyanins in juices from purple pitaya [*Hylocereus polyrhizus* Weber, Britton Rose] monitored by high-performance liquid chromatography–tandem mass spectrometric analyses. Eur. Food Res. Technol. 219, 377–385.

Jayaprakasha, G.K., Selvi, T., Sakariah, K.K., 2003. Antibacterial and antioxidant activities of grape *Vitis vinisfera* seed extracts. Food Res. Int. 36, 117–122.

Jayaprakasha, G.K., Singh, R.P., Sakariah, K.K., 2001. Antioxidant activity of grape seed *Vitis vinifera* extracts on peroxidation models in vitro. Food Chem. 73, 285–290.

Kabuki, T., Nakajima, H., Arai, M., Ueda, S., Kuwabara, Y., Dosako, S., 2000. Characterization of novel antimicrobial compounds from mango *Mangifera indica* L. kernel seeds. Food Chem. 71, 61–66.

Khonkarn, R., Okonogi, S., Ampasavate, C., Anuchapreeda, S., 2010. Investigation of fruit peel extracts as sources for compounds with antioxidant and antiproliferative activities against human cell lines. Food Chem. Toxicol. 48, 2122–2129.

Ma, Y.-Q., Chen, J.-C., Liu, D.H., Ye, X.Q., 2008. Effect of ultrasonic treatment on the total phenolic and antioxidant activity of extracts from citrus peel. J. Food Sci. 73, T115–T120.

Nawawi, A., Nakamura, N., Hattori, M., Kurokawa, M., Shiraki, K., 1999. Inhibitory effects of Indonesian medicinal plants on the infection of herpes simplex virus type 1. Phytother. Res. 13, 37–41.

Negro, C., Tommasi, L., Miceli, A., 2003. Phenolic compounds and antioxidant activity from red grape marc extracts. Bioresour. Technol. 87, 41–44.

Okonogi, S., Duangrat, C., Anuchpreeda, S., Tachakittirungrod, S., Chowwanapoonpohn, S., 2007. Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. Food Chem. 103, 839–846.

Palanisamy, U., Cheng, H.M., Masilamani, T., Subramaniam, T., Ling, L.T., Radhakrishnan, A.K., 2008. Rind of rambutan, *Nephelium lappaceum*, a potential source of natural antioxidants. Food Chem. 109, 54–63.

Prakash Maran, J., Manikandan, S., 2012. Response surface modeling and optimization of process parameters for aqueous extraction of pigments from prickly pear (*Opuntia ficus-indica*) fruit. Dyes Pigm. 95, 465–472.

Prakash Maran, J., Mekala, V., Manikandan, S., 2013. Modeling and optimization of ultrasound-assisted extraction of polysaccharide from *Cucurbita moschata*. Carbohydr. Polym. 92, 2018–2026.

Prakash Maran, J., Manikandan, S., Thirugnanasambandham, K., Vigna Nivetha, C., Dinesh, R., 2013a. Box-Behnken design based statistical modeling for ultrasound-assisted extraction of corn silk polysaccharide. Carbohydr. Polym. 92, 604–611.

Prakash Maran, J., Sivakumar, V., Sridhar, R., Prince Immanuel, V., 2013b. Development of model for mechanical properties of tapioca starch based edible films. Ind. Crop. Prod. 42, 159–168.

Prakash Maran, J., Sivakumar, V., Sridhar, R., Thirugnanasambandham, K., 2013c. Development of model for barrier and optical properties of tapioca starch based edible films. Carbohydr. Polym. 92, 1335–1347.

Rodrigues, S., Pinto, G.A.S., 2007. Ultrasound extraction of phenolic compounds from coconut *Cocos nucifera* shell powder. J. Food Eng. 80, 869–872.

Rostagno, M.A., Palma, M., Barroso, C.G., 2003. Ultrasound-assisted extraction of soy isoflavones. J. Chromatogr. A 1012, 119–128.

Rostagno, M.A., Palma, M., Barroso, C.G., 2007. Ultrasound-assisted extraction of isoflavones from soy beverages blended with fruit juices. Anal. Chim. Acta 597, 265–272.

Singh, R.P., Murthy, K.N.C., Jayaprakasha, G.K., 2002. Studies on the antioxidant activity of pomegranate *Punica granatum* peel and seed extracts using in vitro models. J. Agric. Food Chem. 50, 81–86.

Sivakumar, V., Lakshmi Anna, J., Vijayeeswaree, J., Swaminathan, G., 2009. Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather. Ultrason. Sonochem. 16 (6), 782–789.

Thitilertdecha, N., Teerawutgulrag, A., Kilburn, J.D., Rakariyatham, N., 2010. Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities. Molecules 15, 1453–1465.

Tiwari, B.K., Donnell, C.P., Cullen, P.J., 2009. Effect of sonication on retention of anthocyanins in blackberry juice. J. Food Eng. 93, 166–171.

Tiwari, B.K., Patras, A., Brunton, N., Cullen, P.J., O'Donnell, C.P., 2010. Effect of ultrasound processing on anthocyanins and color of red grape juice. Ultrason. Sonochem. 17, 598–604.

Toma, M., Vinatoru, M., Paniwnyk, L., Mason, T.J., 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrason. Sonochem. 8, 137–142.

Vatai, T., Škerget, M., Knez, Z., 2009. Extraction of phenolic compounds from elder berry and different grape marc varieties using organic solvents and/or supercritical carbon dioxide. J. Food Eng. 90, 246–254.

Verma, Lan, Y., Gokhale, R., Burgess, D.J., 2009. Quality by design approach to understand the process of nanosuspension preparation. Int. J. Pharm. 377, 185–198.

Vilkhu, K., Mawson, R., Simons, L., Bates, D., 2008. Applications and opportunities for ultrasound assisted extraction in the food industry — a review. Innov. Food Sci. Emerg. Technol. 9, 161–169.

Wang, J., Sun, B., Cao, Y., Tian, Y., Li, X., 2008. Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. Food Chem. 106, 804–810.

Wu, H., Hulbert, G.J., Mount, J.R., 2001. Effects of ultrasound on milk homogenization and fermentation with yoghurt starter. Innov. Food Sci. Emerg. Technol. 1, 211–218.

Yang, B., Liu, X., Gao, Y., 2009. Extraction optimization of bioactive compounds crocin, geniposide and total phenolic compounds from Gardenia *Gardenia jasminoides Ellis* fruits with response surface methodology. Innov. Food Sci. Emerg. Technol. 10, 610–615.

Ying, Z., Han, X., Li, J., 2011. Ultrasound-assisted extraction of polysaccharides from mulberry leaves. Food Chem. 127, 1273–1279.

Zhishen, J., Mengchang, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555–559.