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Original Research Article

Optimization of extraction of polyphenols from Sorghum Moench using response surface methodology, and determination of their antioxidant activities

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Abstract

Purpose: *To employ response surface methodology (RSM) hinged on a central composite design (CCD) for the optimization of the extraction of polyphenols from Sorghum moench (Sorghum M).*
Methods: *The combined influence of independent variables were assessed with RSM. Total phenolic content (TPC) determination was carried out using Folin-Ciocalteu method. Derivative compounds of phenolic acid were assayed using high performance liquid (HPLC). Antioxidant potential was determined through 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging test.*
Results: *The optimized extraction conditions were: 60.37 % ethanol, temperature of 59.07 ^{o}C and 2.97 h of extraction duration, which resulted in the extraction of maximum amount of TPC, i.e., 313 mg GAE/100g dry weight. The interactions between temperature and ethanol concentration, and between extraction time and ethanol concentration had significant effects of TPC ($p < 0.05$). Under these conditions, there was a consistency between the projected and actual experimental levels of polyphenols. A positive correlation was found between TPC and DPPH radical scavenging activity ($r=0.67$, $p <0.05$). Furthermore, ferulic acid correlated positively with p-coumaric acid ($r = 0.54$, $p <0.01$).*
Conclusion: *These results underscore the usefulness of conditions for extraction in accurate quantification of antioxidants and phenolic compounds from Sorghum M, for possible application in large scale commercial extraction.*

Keywords: *Response surface methodology, Sorghum moench, Polyphenols, Antioxidants*

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INTRODUCTION

Sorghum moench ranks next to wheat as one of the very important cereal grains worldwide [1]. Sorghum is cultivated arid, tropical, and sub-tropical regions, and is very cost-effective to produce because of its ability to adapt to different stress in the environmental. Humans consume over 35 % of sorghum produced globally, while the remaining 65 % is used for industrial

production of alcohol and for making feed for lower animals [2]. Studies have revealed that sorghum is a good source of some beneficial antioxidant phytochemical compounds such as anthocyanins, polyphenols, sterols and tannins [3]. Thus sorghum can offer protection against free radical-induced oxidative damage. Sorghum is receiving a lot of interest in the US due to its desirable food qualities such as negligible gluten content, minimal digestibility, anti-carcinogenic potential and low cholesterol index [2].

The type, yield and stability of extracts are affected by factors such as duration of extraction, solvent used, and extraction temperature. Variations in these factors affect the levels and antioxidant potential of TPCs [4]. Thus, it is important to optimize extraction conditions to maximize extract yield.

Response surface methodology (RSM) is a useful approach for assessing the interactions between stipulated response variables and various factors that influence them [5]. In addition, RSM is an excellent statistical approach for optimizing variables, and if properly used, it identifies optimal conditions for process improvement. It is useful for defining the influence of single or combined independent variables on the process, being that interactions cannot be determined by considering the various factors one-at-a-time. .The most frequently used form of RSM is central composite design (CCD) [6]. Therefore, research methodology with CCDis employed in the elucidation of interactions between factors because it is an accurate procedure for process design and product.

A previous study reported the antioxidant activity of *Sorghum M* species [7]. However, not much is known about the optimization of the extraction of TPC of *Sorghum M* with RSM method. The objectives of this study were to (a) optimize the extraction of TPC, ferulic acid, catechin and *p*-coumaric acid from *Sorghum M* from CCD; (b) evaluate the extracts for their DPPH radical scavenging potential, and (c) determine Pearson's correlation coefficients between antioxidant potential and TPC.

EXPERIMENTAL

Chemicals and materials

The free radical DPPH was product of Sigma-Aldrich (St. Louis, USA). Other reagents were analytical in quality. The *Sorghum M* (Liaotian 1) sample was purchased from a market vendor in Wuxi, China. The sorghum grains were ground to powder, sieved through a 60-mesh screen and preserved at -20 ^{o}C prior to use.

Sample preparation

The sorghum flour was subjected to extraction with ethanol: water at different volume ratios (Table 1). Flour (1 g) was added to 10mL of appropriate ethanol: water ratio and subjected to extraction at different temperatures in line with the design in Table 1. The extraction process was twice on the residue. In each case, the homogenate was clarified by centrifugation for 20 min at 3000g, and the clear extracts were kept at 4 $^{\circ}$C prior to analyses.

Table 1: Matrix of the model and combination of variables

Treatment code	Level code			Levels of the variables		
	Solvent [b]	***Temperature (℃)***	***Time (h)***	***Solvent*** [b]	***Temperature (℃)***	***Time (h)***
1	0	-1.68	0	60	26.36	2.5
2	-1	-1	-1	80	40	4
3	1	-1	-1	40	40	1
4	-1	-1	1	80	40	1
5	1	-1	1	40	40	4
6	-1.68	0	0	60	60	5.02
7	1.68	0	0	60	60	0
8	0	0	-1.68	60	60	2.5
9	0	0	1.68	26.36	60	2.5
10	0	0	0	60	60	2.5
11	0	0	0	60	60	2.5
12	0	0	0	60	60	2.5
13	0	0	0	60	60	2.5
14	0	0	0	93.64	60	2.5
15	0	0	0	60	60	2.5
16	-1	1	-1	40	80	1
17	1	1	-1	80	80	4
18	-1	1	1	40	80	4
19	1	1	1	80	80	1
20	0	1.68	0	60	93.64	2.5

[b]volume of enthanol in 100 mL of H_2O

Experimental design

The simulataneous influence of two process variables on TPC content of sorghum extracts was determined with an orthogonal rotatable CCD for K=3 factors in a quadratic function, with ethanol concentration and temperature as variables, while TPC and DPPH radical scavenging ability formed the responses (Table 1).

Assay of TPC

The levels of TPC were estimated in a colorimetric reaction with Folin–Ciocalteu reagent [8]. The reaction mixture contained equal aliquots of TCP extract and saturated $NaCO_3$, and 1 mL of Folin-Ciocalteau reagent. After mixing and incubating for 30 min at room temperature, the absorbance of the solution was read at 765 nm. The TPC contents were calculated as equivalents of gallic acid (GAE) per gram of extract dry weight (DW).

Determination of phenolic acids

The phenolic acids contents of the various extracts were determined using HPLC according to the procedure of Guo *et al* [9], with some modifications. The HPLC system employed a separation mode using a Waters 1525 binary pump and Waters photodiode detector (Milford, MA,USA). Manual injection volume was 20 µL, and the sample components were separated at 35 ^{o}C in a 250 x 4.6, 5µm Agilent C_{18} column with gradient elution using methanol (solvent A) and 0.1% acetic acid–water solution (solvent B) (flow rate = 0.8 mL/min). For characterization of the peaks, the UV spectra were obtained in the range of 254 - 400 nm. Peak area at maximum absorption was employed in calculating the phenolic acid levels.

Assay of DPPH radical scavenging activity

This was carried out according to a method described previouly [10]. The DPPH radical scavenging activity (D) was calculated as in Eq 1.

D (%) = {(Ac – As)/Ac}100 …… (1)

Statistical analysis

The data are expressed as mean ± SD, and were analyzed by Students' t-test using Design Expert 7.0. Statistical significance was assumed at $p < 0.05$.

RESULTS

Table 2 shows DPPH radical scavenging activity and TPC levels of the extracts. The TPC and DPPH radical scavenging potential were influenced not only by the type of solvent used, but also by the conditions used for extraction.

The four models shown in Table 2 were obtained by using DDPH radical scavenging ability, TPCs, catechin, ferulic acid, and *p*-coumaric acid as response variables in RSM, while the fitted TPC model parameters are presented in Table 3. Statistical significance ($p< 0.05$) was seen in A, B and C (lines variables), and in A^2,B^2 and C^2 (quadratic variables), which indicated polyphenol extractability was strongly influenced by these factors. Two of the two-variable interactions AB and AC had significant influences ($p <0.05$) affected extraction yield, but BC interaction did not produce any significant influence ($p >0.05$). These are reflected in the regression equation:

Table 2: Response of the model to extracts

Code	TPC	DPPH	FA	PC	C
1	201.01±2.18	74.4±0.34	5.34± 0.22	15.29 ±0.51	9.27±0.78
2	277.24±3.02	85.5±0.36	7.29 ±0.32	21.70±2.11	12.33±1.12
3	227.52±2.01	77.2±0.79	6.12±0.43	19.15±1.06	13.65±1.21
4	226.79±1.98	85.9±0.76	13.87 ±1.21	32.32±2.78	17.51±1.07
5	262.82±2.03	77.9±0.16	22.65 ±2.12	34.69±2.89	7.42±0.32
6	282.64±2.11	89.3±0.08	33.84 ±3.09	7.91±0.88	11.68±1.09
7	212.96±2.08	92.4±0.10	16.55 ±1.12	38.64±2.06	8.20 ±0.29
8	308.44±2.49	77.0±0.85	7.32 ±0.72	3.46 ±0.21	13.58±1.32
9	287.99±2.76	92.08±2.3	20.47 ±1.08	31.37±1.21	7.27±0.21
10	307.77±2.22	88.63±1.0	10.34 ±1.56	28.68±2.14	10.13±1.03
11	307.17±3.02	90.5±3.19	9.70 ±1.02	15.37±1.02	26.28±2.01
12	309.11±2.01	92.5±1.23	7.91±0.77	3.05±0.21	7.89 ±0.97
13	307.08±2.11	93.2±0.82	11.97±1.11	8.08 ±0.89	14.93±1.26
14	298.52±3.01	89.0±1.34	6.63± 0.18	4.14±0.32	8.95±0.21
15	309.26±2.26	92.0±0.32	12.61±1.05	9.42±0.32	14.04±1.21
16	207.49±2.12	73.9±0.55	8.00 ±0.24	3.42±0.67	19.91±1.21
17	260.31±2.21	82.9±0.72	3.57 ±0.16	2.72±0.21	2.71 ±0.21
18	239.95±2.31	73.2±0.18	3.21±0.23	3.11±0.43	2.23 ±0.13
19	212.73±2.11	82.6±2.02	3.11±0.18	2.35±0.12	2.62 ±0.18
20	169.96±1.06	70.6±0.09	2.22±0.28	2.98±0.11	1.82 ±0.08

TPC = total phenolic content; FA = ferulic acid; PC = *p*-coumaric acid; C = catechin

Table 3: ANOVA for quadratic models of TPC

Source	Sum of squares	df	Mean Square	F-value	*P*-value
Model	38311.26	9	4256.807	2191.85	< 0.0001
A	237.898	1	237.898	122.4948	< 0.0001
B	1164.521	1	1164.521	599.6174	< 0.0001
C	5863.434	1	5863.434	3019.11	< 0.0001
AB	17.73047	1	17.73047	9.129501	0.0129
AC	114.5327	1	114.5327	58.97344	< 0.0001
BC	4.07525	1	4.07525	2.098366	0.1781
A^2	362.2464	1	362.2464	186.5224	< 0.0001
B^2	26790.51	1	26790.51	13794.56	< 0.0001
C^2	6406.555	1	6406.555	3298.765	< 0.0001
Residual	19.42107	10	1.942107		
Lack of Fit	14.93439	5	2.986877	3.328602	0.1065
Total Correlation	38330.68362	19			
R^2	0.9995		R^2_{adj}	0.9990	

Table 4: ANOVA for quadratic model of DDPH radical scavenging potential

Source	Sum of squares	df	Mean Square	F-value	*P*-value
Model	758.33	9	84.26	2.46	0.0883
A	63.95	1	63.95	1.87	0.2015
B	29.98	1	29.98	0.88	0.3713
C	2.05	1	2.05	0.06	0.8114
AB	0.60	1	0.60	0.02	0.8974
AC	0.00	1	0.00	0.00	0.9992
BC	0.06	1	0.06	0.00	0.9662
A^2	2.10	1	2.10	0.06	0.8093
B^2	658.44	1	658.44	19.25	0.0014
C^2	1.08	1	1.08	0.03	0.8627
Residual	342.12	10	34.21		
Lack of Fit	158.07	5	31.61	0.86	0.5643
Total Correlation	1100.45	19			
R^2	0.6891		R^2_{adj}	0.4093	

$Y=308.10 + 4.17 \times A - 9.23 \times B + 20.72 \times V + 1.49 \times AB + 3.78 \times AC - 0.71 \times BC - 5.01 \times A2 - 43.12 \times B^2 - 21.08 \times C^2$

(B variables with negative coefficients depict decreased response, while increase in response was due to positive coefficients of the variables A and C).

Table 4 depicts results of fitting models for DPPH radical scavenging activity. F-test and *p* value were used to assess the model significance. The *p* value was 0.0883, which suggests adequacy of the model for use as a predictor within the range of experimental variables.

Table 5 shows the design matrix and results of ANOVA, fitness and adequacy of the models for ferulic acid content. The determination coefficient (R^2) obtained from variance of quadratic regression model was 0.6952. This demonstrates the inadequacy of the model for explaining total variance. In addition, the adjusted R^2 value was 0.4209 which also showed that the model lacked significance. The model was also inadequate for making predictions over the range of variables

used in the experiment. The values of the regression coefficients (Table 5) indicate that C^2 was significantly different ($p< 0.05$). However, there were no significant differences between AB, AC and BC.

Table 6 shows that the multiple correlation coefficient (r) and determination coefficient (R^2) were not satisfactory to describe *p*-coumaric acid. The value of R^2 from the quadratic regression model was 0.4397, suggesting that the model cannot explain the total variance.

Table 7, the quadratic models lacked significance and fit ($p>0.05$) with respect to catechin. In addition, the regression model produced R^2 value of 0.5489. These results indicate that the model cannot explain total variance.

Figure 1 shows contour plot response surfaces of TPC. A significant influence was produced on maximization of TPC extract by the two-factor interaction between temperature and solvent concentration (AB, $p < 0.05$). From the results, it is clear that 50-70% (v/v) ethanol and temperatures above 45 °C yielded high extracts of TPC (Figures 1A and 1B).

Similarly, the interaction between solvent concentration and extraction time (AC) had significant effect ($p < 0.05$) on TPC extraction. Moreover, ethanol concentrations within the range 50 – 70 % (v/v), and extraction times above 2.5 h resulted in higher TPC yields (Figures 1C and 1D). However, no significant effect was produced on the extraction of TPC by the interaction between extraction temperature and extraction time (BC, $p > 0.05$).

Table 5: ANOVA for ferulic acid quadratic models

Source	Sum of squares	Df	Mean square	F-value	*P*-value
Model	809.79	9.00	89.98	2.53	0.0818
A	91.74	1.00	91.74	2.58	0.1390
B	101.80	1.00	101.80	2.87	0.1212
C	88.21	1.00	88.21	2.48	0.1460
AB	1.19	1.00	1.19	0.03	0.8584
AC	39.84	1.00	39.84	1.12	0.3143
BC	25.48	1.00	25.48	0.72	0.4167
A^2	0.00	1.00	0.00	0.00	0.9961
B^2	171.14	1.00	171.14	4.82	0.0528
C^2	245.12	1.00	245.12	6.90	0.0253
Residual	355.00	10.00	35.50		
Lack of Fit	332.54	5.00	66.51	14.80	0.0051
Total Correlation	1164.79	19.00			
R^2	0.6952		R^2_{adj}	0.4209	

Table 6: ANOVA for *p*-coumaric acid quadratic models

Source	Sum of squares	Df	Mean square	F-value	*P*-value
Model	1323.87	3.00	441.29	4.19	0.0229
A	162.37	1.00	162.37	1.54	0.2325
B	1001.72	1.00	1001.72	9.50	0.0071
C	159.78	1.00	159.78	1.52	0.2361
Residual	1687.02	16.00	105.44		
Lack of Fit	1224.92	11.00	111.36	1.20	0.4457
Total Correlation	3010.89	19.00			
R^2	0.4397		R^2_{adj}	0.3346	

Table 7: ANOVA for catechin quadratic models

Source	Sum of squares	Df	Mean square	F-value	*P*-value
Model	409.06	9.00	45.45	1.35	0.3214
A	1.99	1.00	1.99	0.06	0.8127
B	94.74	1.00	94.74	2.82	0.1242
C	39.23	1.00	39.23	1.17	0.3054
AB	81.78	1.00	81.78	2.43	0.1499
AC	44.24	1.00	44.24	1.32	0.2780
BC	4.79	1.00	4.79	0.14	0.7137
A^2	44.55	1.00	44.55	1.32	0.2765
B^2	102.38	1.00	102.38	3.04	0.1116
C^2	17.81	1.00	17.81	0.53	0.4834
Residual	336.24	10.00	33.62		
Lack of Fit	133.45	5.00	26.69	0.66	0.6713
Total Correlation	745.30	19.00			
R^2	0.5489		R^2_{adj}	0.1428	

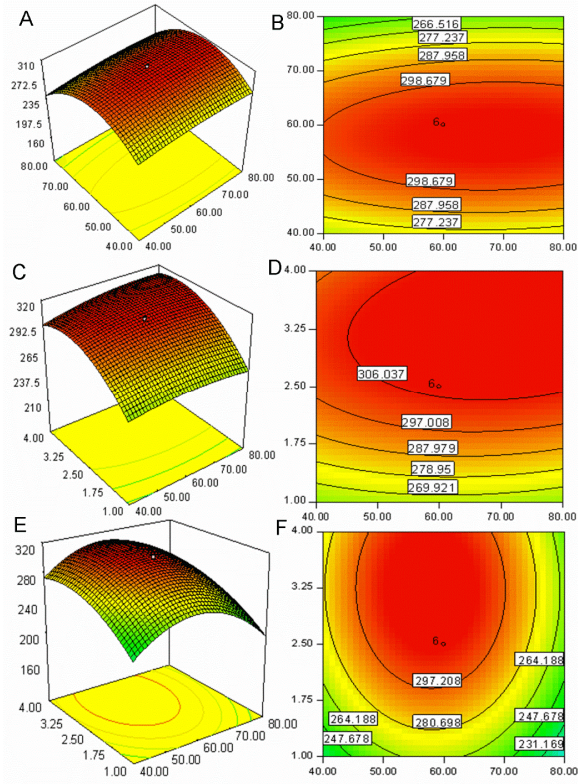

Figure 1: RSM plots and contour plots depicting effects of interaction between temperature, extraction time and solvent on TPC level. A, B: ethanol interaction with temperature; C, D: solvent interaction with solvent and extraction time; E, F: temperature interaction with extraction time

Correlations

Results on Table 8 indicate matrices of correlation coefficients amongst the four parameters determined. TPC was strongly associated with DPPH radical scavenging potential (r = 0.67), while between *p*-coumaric acid was strongly associated with ferulic acid (r = 0.54) ($p < 0.05$).

Table 8: Pearson's correlation coefficients between polyphenolics and antioxidant activity

Variable	*TPC*	*DPPH*	*Ferulic acid*	*p*-Coumaric acid	*Catechin*
TPC	1.00				
DPPH	0.67*	1.00			
Ferulic acid	0.28	0.44	1.00		
p-Coumaric acid	0.03	0.28	0.47*	1.00	
Catechin	0.32	0.26	0.19	0.15	1.00

*Correlation is significant at the $p < 0.05$ level

DISCUSSION

Sorghum M. is one of the most important cereal crops, commonly grown around the globe. It is consumed by humans in the form of alcohol (commercial product of *Sorghum*) and also used as animal feed. Natural antioxidants in *Sorghum* i.e. polyphenolics and phytosterols play significant roles against oxidative stress, and provide protection against degenerative diseases, cancers, ageing, cardiovascular disorders and diabetes. It is well established that various factors like type of solvent and extraction duration affect the stability and yield of phytochemicals.

Thus, in this study, RSM was used to optimize variables that appreciably affect the extraction of phytochemicals from *Sorghum* M. Total phenolic content and DPPH radical scavenging activity were 309.26 ± 2.26 mg GAE/100g DW and 93.20 ± 0.82, respectively, which are lower than values reported in previous studies [11]. This disparity may be attributed to differences in methods used for extraction. In RSM, the variables were catechin, TPC, ferulic acid, DPPH scavenging and *p*- coumeric acid. It has been demonstrated that values of R^2 close to unity indicate close fit between experimental error [12].

Overall, *Sorghum M* sample extracted for 3 hours at 60 °C using 60% ethanol resulted in maximum TPC. It was noted that long extraction time favored the extraction of polyphenols. This is so because, long time exposure of sample allows solvent molecules to penetrate in the plant tissues/cells to dissolve out more of the phytochemical compounds [13].

In the present study, extraction time also exhibited significant effect on the extraction of TPCs and phenolic acids. The maximum extraction of phenolic compounds at high temperature might be due to tissue softening, breakage of bonds linking polyphenolics and protein or polysaccharides, increases in the solubility of the phenolic compounds, and decreases in surface tension and viscosity of the solvent. These factors enhance diffusion and so result in high extraction of TPC [14]. However, this effect is limited because many phenolic compounds such as flavonols, flavonoids and flavonones are degraded at high temperatures, thereby resulting in lower yields. Temperature and concentration interacted in a manner that significantly influenced the yield of TPC, and DPPH scavenging potential showed positive correlation with TPC. The ability of the RSM model to predict TPC yield was tested at the optimal concentration of ethanol i.e. 60 % ethanol, optimal temperature for extraction i.e. 60 °C, and extraction duration of 3 h. Under these conditions, the yield of TPC was 315.87 mg GAE/100gDW, which indicate that the RSM model has very good and accurate predictive capacity for TPC yield.

CONCLUSION

The results of this study show that extraction time of 3 h, extraction temperature of 60 °C and use of 60 % ethanol were the best conditions for maximum extraction of TPCs from *Sorghum m*. These findings confirm that RSM is an important strategy for maximizing the extraction of natural antioxidants. Consequently, this approach may also helpful for maximum extraction of useful phytochemicals on commercial basis.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Awika JM, Dykes L, Gu LW, Rooney LW, Prior R. Effect of processing on molecular weight distribution and antioxidant properties of sorghum proanthocyanidins. Abstr Pap Am Chem Soc 2004; 228: U248-U248.*
2. *Awika JM, Rooney LW. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 2004; 65(9): 1199-1221.*
3. *Choi Y, Jeong HS, Lee J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem 2007; 103(1): 130-138.*
4. *Liu B, Ma Y, Liu Y, Yang Z, Zhang L. Ultrasonic-Assisted Extraction and Antioxidant Activity of Flavonoids from Adinandra nitida Leaves. Trop J Pharm Res 2013; 12(6): 1045-1051.*
5. *Yuan C, Liu B, Chen Y, Chen Y. Optimization of Preparation of Jujube Juice by Response Surface Methodology. In Future Material Research and Industry Application 2012; 455-456: 981-984.*
6. *Yasini P, Shemirani F, Khani R. Combination of In Situ Surfactant-based Solid Phase Extraction and Central Composite Design for Preconcentration and Determination of Manganese in Food and Water Samples. Food Anal Method 2012; 5(6): 1303-1310.*
7. *Liu MX, Wang YW, Han JG, Mao PS. Phenolic compounds from Chinese sudangrass, sorghum, sorghum-sudangrass hybrid, and their antioxidant properties. Crop Sci 2011; 51(1): 247-258.*
8. *Chen Y, Chen G, Fu X, Liu RH. Phytochemical profiles and antioxidant activity of different varieties of Adinandra tea (Adinandra Jack). J Agric Food Chem 2015; 63(1):169-176.*

9. Guo XD, Ma YJ, Parry J, Gao JM, Yu LL, Wang M. Phenolics Content and Antioxidant Activity of Tartary Buckwheat from Different Locations. Molecules 2011; 16(12): 9850-9867.
10. Chen Y, Zhang R, Liu C, Zheng X, Liu B. Enhancing antioxidant activity and antiproliferation of wheat bran through steam flash explosion. J Food Sci Tech 2016; 53(7): 3028-3034.
11. Dykes L, Rooney LW, Waniska RD, Rooney WL. Phenolic compounds and antioxidant activity of sorghum grains of varying genotypes. J Agric Food Chem 2005; 53(17): 6813-6818.
12. Lee WC, Yusof S, Hamid NSA, Baharin BS. Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). J Food Eng 2006; 73(1): 55-63.
13. Gan CY, Latiff AA. Optimisation of the solvent extraction of bioactive compounds from Parkia speciosa pod using response surface methodology. Food Chem 2011; 124(3): 1277-1283.
14. Kusic H, Koprivanac N, Papic S, Bozic AL. Influence of substituent type and position on photooxidation of phenolic compounds: Response surface methodology approach. J Photoch Photobio A Chem 2012; 242(31): 1-12.