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Optimization of extraction of phenolic compounds from wheat using response surface methodology

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Abstract

The optimum conditions for the extraction of crude phenolics from whole grain and bran of soft and hard wheat were determined using response surface methodology (RSM). A face-centered cubic design (FCD) was used to investigate the effects of three independent variables, namely solvent composition (%), extraction temperature (°C) and time (min) on the response, total antioxidant activity (TAA). The independent variables were coded at three levels and their actual values selected on the basis of preliminary experimental results. The FCD consisted of 14 experimental points and three replications at the center point. Data were analyzed using design expert and statistical analysis system software. A second-order polynomial model was used for predicting the response. Regression analysis showed that more than 89% of the variation was explained by the models. Canonical analysis of surface responses revealed that the stationary surface was a saddle. The optimal conditions forthe TAA obtained using ridge analysis were 54%, 61 °C, 64 min and 49%, 64 °C, 60 min, for whole grain and bran of soft wheat, respectively. Under the optimum conditions the corresponding predicted response values for TAA were 56.5 and 63 TE. The crude phenolics were extracted under optimum conditions to check the validity of the model. The values were 54.7 ± 3.2 and 61.3 ± 1.9 TE, for whole grain and bran of soft wheat, respectively; A similar trend was observed for TAA of hard wheat. The experimental values agreed with those predicted, thus indicating suitability of the model employed and the success of RSM in optimizing the extraction conditions.

Keywords: Response surface methodology; Extraction of phenolics; Optimization of extraction; Total antioxidant activity; Canonical and ridge analyses

1. Introduction

Phenolic compounds are common dietary phytochemicals found in fruits, vegetables and grains. Epidemiological evidences have suggested that food phenolics may have protective effects against degenerative diseases (Mazza, 2000). Most of the beneficial characteristics of phenolic compounds have been ascribed to their antioxidant activity which is a fundamental property important to life (Rice-Evans, Miller, & Paganga, 1997). Andreasen, Landbo, Christensen, Hansen, and Meyer (2000) have reported on the positive effects of higher intake of whole grain foods in lowering the risk of coronary heart disease. It has also been suggested that adults would gain appreciable protection from coronary heart disease by consuming the recommended three servings of whole grains daily (Andreasen, Christensen, Meyer, & Hansen, 2000). Thus, whole grains, rich in fiber and phytochemicals, are among the healthiest foods that individuals may consume and render a wide variety of health benefits (Andreasen et al., 2001). Plants and plant extracts have been used in traditional cures and herbal remedies for centuries throughout the world.

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Recently there has been a renewed interest in secondary plant metabolites because of their potential preventive effects on the chronic diseases such as cardiovascular disease and cancer (Rowland, 1999). Hence, isolation, identification and quantification of phytochemicals in foods and evaluation of their potential health benefits have been in focus: However, in vitro and animal studies have shown that the action of some chemicals are likely to be achieved only at doses much higher than those that can be obtained from eating plants (Rowland, 1999). Thus, the extraction of the active ingredient is essential if they are to be of prophylactic or therapeutic value in human subjects (Rowland, 1999).

Many factors such as solvent composition, extraction time, extraction temperature (Wettasinghe & Shahidi, 1999), solvent to solid ratio (Cacace & Mazza, 2003a) and extraction pressure (Cacace & Mazza, 2002), among others, may significantly influence the extraction efficacy. In general, optimization of a process could be achieved by either empirical or statistical methods; the former having limitations toward complete optimization. The traditional one-factor-at-a-time approach to process optimization is time consuming. Moreover, the interactions among various factors may be ignored hence the chance of approaching a true optimum is very unlikely. Thus, one-factor-at-a-time procedure assumes that various parameters do not interact, thus the process response is a direct function of the single varied parameter. However, the actual response of the process results from the interactive influence of various variables. Unlike conventional optimization, the statistical optimization procedures allows one to take interaction of variables into consideration (Haaland, 1989).

Response surface methodology (RSM), originally described by Box and Wilson (1951), enables evaluation of the effects of several process variables and their interactions on response variables. Thus, RSM is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing processes (Myers & Montgomery, 2002). Response surface methodology has been successfully used to model and optimize biochemical and biotechnological processes related to food systems (Cacace & Mazza, 2003b; Parajo, Santos, Dominguez, & Vazquez, 1995; Senanayake & Shahidi, 1999; Senanayake & Shahidi, 2002; Telez-Luis, Moldes, Alonso, & Vazquez, 2003; Vasquez & Martin, 1998) including extraction of phenolic compounds from berries (Cacace & Mazza, 2003a, 2003b) and evening primrose meal (Wettasinghe & Shahidi, 1999), anthocyanins from black currants (Cacace & Mazza, 2003a) and sunflower hull (Gao & Mazza, 1996) and vitamin E from wheat germ (Ge, Ni, Yan, Chen, & Cai, 2002), among others.

The extraction and purification of phytochemicals from natural sources is needed, since these bioactives are often used in the preparation of dietary supplements, nutraceuticals, functional food ingredients, food additives, pharmaceutical and cosmetic products (Gao & Mazza, 1996). In this study, optimization of experimental conditions that results in the highest antioxidant activity of crude wheat phenolic extracts was conducted. Whole grain and bran of soft and hard wheat were extracted with a number of polar solvents and their total antioxidant activity (TAA) determined and optimum experimental conditions derived using RSM.

2. Materials and methods

2.1. Materials

Whole grain and bran of commercial soft (70% Canadian eastern soft red spring and 30% Canadian eastern soft white winter) and hard (90% Canadian western hard red spring and 10% Canadian eastern hard red winter) wheat mixtures were obtained from the Robin Hood Multifoods Inc. plant in Saskatchewan through their head office in Markham, ON. The compounds 1,1-diphenyl-2-picrlthydrazyl (DPPH), 2,2′-azino-di[3-ethylbenzthiazoline sulfonate(ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferulic acid and Folin–Ciocalteu phenol reagent were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). All other chemicals and solvents were purchased from Fisher Scientific (Nepean, ON) and were of ACS grade or better.

2.2. Preparation of samples

Whole grain wheat and its bran were ground in an electric grinder (Black and Decker Canada Inc., Brockville, ON) to obtain a fine powder. All samples tested were defatted by blending the ground material with hexane (1:5 w/v, 5 min, ×3) in a Waring blender (Model 33BL73, Waring Products Division, Dynamics Corp. of America, New Hartford, CT) at ambient temperature. Defatted wheat samples were air dried for 12 h and stored in vacuum packaged polyethylene pouches at −20 °C until used for further analysis.

2.3. Selection of appropriate extraction conditions

The initial step of the preliminary experiment was to select an appropriate extraction medium for wheat phenolics. Three different solvent systems, namely ethanol, methanol and acetone were examined. Crude phenolic compounds from whole wheat and bran of soft and hard wheat were extracted using a series of extraction media varying in the range of 0–100% (v/v; water/ethanol, methanol or acetone). The crude phenolic ↑ extracts were prepared by extracting the ground wheat samples (6 g) with 100 ml of solvent for 20 min at 80 °C. Based on total antioxidant activity (TAA), determined

by Trolox equivalent antioxidant capacity (TEAC) assay and expressed as μM Trolox equivalents (TE), the best medium and its composition were chosen. The second step of the preliminary experiment was to determine the extraction temperature. Crude phenolics from wheat were extracted using the best solvent composition chosen in the previous step. The extraction temperature varied from 15 to 95 °C while holding the extraction time course constant at 20 min. Final step of the preliminary experiment was to select the appropriate extraction time course for extraction of phenolics. Using the solvent system from the first step, phenolics were extracted during various extraction times ranging from 15 to 105 min at a given temperature as determined from the second step. Based on the results the three levels (lower, middle, upper) of each process variable were determined for RSM.

2.4. Measurement of total antioxidant activity

Total antioxidant activity was determined according to the procedure described by van den Berg, Haenen, van den Berg, and Bast (1999) using the Trolox equivalent; antioxidant capacity (TEAC) assay. The extracts and reagents were prepared in a 0.1 M phosphate buffer (pH 7.4) containing 0.15 M sodium chloride (PBS buffer solution). A solution of 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) radical anion ($ABTS^{\bullet-}$) was prepared by mixing 2.5 mM 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) with 2.0 mM 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) ($ABTS^{\bullet-}$) in a 1:1 (v/v) ratio, and heating at 60 °C for 12 min. The radical solution was stored at room temperature and protected from light. A standard curve was prepared using different concentrations of Trolox. The reduction in the absorbance of the $ABTS^{\bullet-}$ solution (1960 μl) at different concentrations of Trolox (40 μl) over a period of 6 min was measured and plotted. The TEAC values of wheat extracts (5 mg/ml) were determined in the same way and expressed as μM Trolox equivalents.

2.5. Experimental design

Optimization of extraction of phenolics from whole grain and bran of soft and hard wheat in aqueous ethanol was carried out using RSM (Montgomery, 2001; Myers & Montgomery, 2002). A three-factor and a three level face-centered cube design (FCD) consisting of seventeen experimental runs was employed including three replicates at the center point. The effects of unexplained variability in the observed response due to extraneous factors were minimized by randomizing the order of experiments. The design variables were the solvent composition (X_1, %, v/v, water/ethanol), extraction temperature (X_2, °C) and extraction time (X_3, min) while response variable was total antioxidant activity (TAA).

2.6. Data analysis

The response surface regression (RSREG) procedure of statistical analysis system (SAS) and design expert (version 6.0.5) software were used to analyze the experimental data (Myers & Montgomery, 2002). Experimental data were fitted to a second-order polynomial model and regression coefficients obtained. The generalized second-order polynomial model used in the response surface analysis was as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j=1}^{3} \beta_{ii} X_i X_j,$$

where β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, and X_i, and X_j are the independent variables. The design expert software was used to generate response surfaces and contour plots while holding a variable constant in the second-order polynomial model. When the results showed a saddle point in response surfaces the ridge analysis of SAS RSREG procedure was used to compute the estimated ridge of the optimum response.

2.7. Verification of model

Optimal conditions for the extraction of phenolic compounds from wheat depended on solvent composition, extraction temperature and extraction time course were obtained using the predictive equations of RSM. The total antioxidant activity was determined after extraction of phenolic compounds under optimal conditions. The experimental and predicted values were compared in order to determine the validity of the model.

3. Results and discussion

3.1. Selection of lower, middle and upper levels of the design variables

In general, efficiency of the extraction of a compound is influenced by multiple parameters such as temperature, time and solvent polarity, among others, and their effects may be either independent or interactive (Montgomery, 2001). The influence of extraction variables such as solvent composition, temperature and time course on the recovery of phenolic compounds from wheat and its milling fractions has not yet been reported. The entire experiment was divided into three parts. The initial part included the determination of the lower, middle and upper levels of the three design variables employed in the RSM. These levels of independent variables were selected based on values obtained in preliminary experiments. Hence, the first step of the preliminary experiment was to select an appropriate

medium for the extraction of wheat phenolics. Selection of appropriate conditions is crucial in the extraction of antioxidant compounds from plant materials. The extraction conditions may not be generalized due to the diverse nature of natural antioxidants existing in different plant materials (Wettasinghe & Shahidi, 1999). Under these circumstances, RSM has shown to be a powerful tool in optimizing experimental conditions to maximize various responses (Cacace & Mazza, 2003a; Gao & Mazza, 1996; Liu, Ang, & Springer, 2000; Wettasinghe & Shahidi, 1999). Thus, the effect of various organic solvents (ethanol, methanol and acetone) on TAA of wheat phenolic ↑ extracts showed that the response behaved more as a quadratic ($r = 0.68$–0.95) function than a linear ($r = 0.004$–0.32) one. Hence, TAA of crude phenolic extracts started to increase with increased proportion of organic solvent in the extraction medium. Total antioxidant activity reached a maximum followed by a decrease with further increase in the proportion of the organic solvent in the extraction medium. A similar trend was observed for all three organic solvents employed. Furthermore, the trends were similar for all wheat extracts examined. Thus, the proportion of organic solvent in the extraction medium had a significant influence on the antioxidative properties of wheat extracts with respect to the organic solvent employed; A combination of the organic solvent with water at 1:1 (v/v) ratio produced the highest TAA for all wheat extracts. However, aqueous ethanol yielded extracts with higher TAA compared to other aqueous solvents from whole grain and bran of both soft and hard wheat and was considered as the most effective solvent. Subsequently, the lower, middle and upper levels of the solvent composition were selected based on the above results and the values were 30%, 50% and 70% ethanol, respectively, for each wheat fraction. Thus, a moderately polar solvent (50% ethanol) was chosen for determination of extraction temperature and extraction time.

With regard to extraction temperature, the TAA of wheat extracts increased with increasing temperature up to 60 °C and then began to decline. Results indicated that mobilization of active compounds from the substrate may occur up to a certain level followed by their possible loss due to decomposition at higher temperatures. According to Wettasinghe and Shahidi (1999), high temperatures may mobilize certain antioxidants while promoting possible concurrent decomposition of antioxidants which were already mobilized at lower temperatures. It was also stated that the rate of extraction of thermally stable antioxidants at elevated temperatures is higher than the rate of decomposition of less soluble antioxidants. This has been suggested by the relatively high antioxidant activities possessed by extracts prepared at higher temperatures. Increasing temperature may favor extraction by enhancing solubility of phenolic compounds in the solvent. A major effect of the increase of extraction temperature may be to increase in the rate of extraction thereby decreasing the extraction time (Cacace & Mazza, 2002). The regression analysis demonstrated that relationships between TAA and extraction temperature were more quadratic ($r = 0.82$–0.93) than linear ($r = 0.04$–0.27). All wheat extracts employed in RSM produced the highest TAA at about 60 °C, which led to the selection of 40, 60 and 80 °C as the lower, middle and upper levels, respectively, of the variable for optimization.

The selection of an appropriate extraction time was the final step in a series of preliminary experiments. Phenolic compounds were extracted from wheat samples by varying the time course of extraction using 50% aqueous ethanol while keeping the temperature constant at 60 °C. Relationships between TAA and extraction time demonstrated a quadratic function ($r = 0.69$–0.97) rather than a linear ($r = 8.73 \times 10^{-5}$–2.23×10^{-4}) one, hence indicating trends similar to those observed for the other two variables. The results showed that total antioxidant activity increased when extraction time increased from 15 to 60 min. Beyond 70 min, total antioxidant activity decreased sharply and reached a minimum at 105 min, possibly due to the decomposition of active compounds during the prolonged extraction time. Thus, the extraction time was another important parameter influencing the extraction of phenolic compounds and hence TAA. The best extraction time was approximately 60 min for whole grain and bran of both soft and hard wheat examined in this study. Hence, the lower, middle and upper levels of extraction time chosen for RSM were 45, 60 and 75 min, respectively.

3.2. Fitting the models

The three factors and lower, middle and upper design points for RSM in coded and natural/ uncoded values are shown in Table 1. In RSM, natural variables are transformed into coded variables that have been defined as dimensionless with a mean zero and the same spread or standard deviation (Myers & Montgomery, 2002). Multiple regression equations were generated relating response variable to coded levels of the independent variables. Multiple regression coefficients were determined by employing least squares technique (Myers & Montgomery, 2002) to predict quadratic polynomial

Table 1
Independent variables and their coded and actual values used for optimization

Independent variable	Units	Symbol	Coded levels		
			−1	0	+1
Solvent composition	% (v/v)	X_1	30	50	70
Temperature	°C	X_2	40	60	80
Time	min	X_3	45	60	75

models for TAA of wheat extracts. Analysis of variance (ANOVA) shows that the selected quadratic models adequately represented the data obtained for TAA. Table 2 shows the experimental design employed while Table 3 summarizes the data for TAA of all wheat extracts examined. The results of ANOVA for TAA with corresponding coefficients of multiple determination (R^2) for wheat fractions are shown in Table 4. The model was adequate and explained most of the variability for all wheat fractions. For the model fitted, software generates model coefficients, R^2-values; F-values and significant probabilities and hence one can justify the significance of each experimental variable. The maximum predictable response for TAA was obtained based on a total of 17 experiments required for determining 10 regression coefficients; of the model (Table 4). In general, proceeding with exploration and optimization of a fitted response surface may produce poor or misleading results unless the model exhibits an adequate fit (Myers & Montgomery, 2002). This makes the checking of model adequacy essential (Table 5). A plot of experimental and theoretical values indicated an excellent fit ($r \geqslant 0.94$, $p < 0.01$) for whole grain and bran of both soft and hard wheat.

A high proportion of variability was explained by the RSM models for TAA as indicated by R^2 with all wheat fractions (Table 4). The regression models were highly significant ($p < 0.001$ or $p < 0.05$) for all wheat fractions with satisfactory coefficient of determination (R^2) that varied from 0.89 to 0.94 for TAA. Moreover, coefficient of variation (CV) describes the extent to which the data were dispersed. The CV for TAA (Table 4) of each wheat fraction was within the acceptable range. Since CV is a measure expressing standard deviation as a percentage of the mean, the small values of CV give better reproducibility. In general, a high CV indicates that variation in

Table 2
Three-factor, three-level face-centered cube design used for RSM

Standard order[a]	Run order[b]	Factor 1 (X^1) Solvent composition (%)	Factor 2 (X^2) Temperature (°C)	Factor 3 (X^3) Time (min)
1	10	70 (+1)	60(0)	60(0)
2	14	50(0)	60(0)	75 (+1)
3	2	70 (+1)	40 (−1)	45 (−1)
4	13	50(0)	60 (0)	45 (−1)
5	12	50 (0)	80 (+1)	60 (0)
6	15	50(0)	60(0)	60 (0)
7	11	50 (0)	40 (−1)	60 (0)
8	8	70 (+1)	80 (+1)	75 (+1)
9	5	30 (−1)	40 (−1)	75 (+1)
10	6	70 (+1)	40 (−1)	75 (+1)
11	17	50 (0)	60 (0)	60 (0)
12	7	30 (−1)	80 (+1)	75 (+1)
13	4	70 (+1)	80 (+1)	45 (−1)
14	1	30 (−1)	40 (−1)	45 (−1)
15	3	30 (−1)	80 (+1)	45 (−1)
16	16	50 (0)	60 (0)	60 (0)
17	9	30 (−1)	60 (0)	60 (0)

[a] No randomized.
[b] Randomized.

Table 3
Experimental data for the response total antioxidant activity (μm TE) of soft and hard wheat extracts under different extraction conditions shown in Table 2

Standard order[a]	Soft wheat bran	Soft whole wheat	Hard wheat bran	Hard whole wheat
1	55.0	33.8	62.9	29.8
2	51.7	55.1	59.4	51.5
3	60.6	33.9	74.1	28.6
4	58.3	40.7	65.3	36.4
5	52.4	33.4	55.1	29.4
6	54.1	45.8	61.9	41.8
7	60.4	42.4	76.1	36.1
8	56.7	53.5	73.1	49.5
9	57.3	27.1	69.3	23.1
10	54.7	49.7	73.6	45.2
11	58.7	51.9	43.1	47.4
12	67.3	51.4	63.1	50.4
13	63.8	53.6	61.8	49.6
14	60.1	56.7	61.3	52.7
15	65.3	58.4	60.1	49.4
16	58.9	55.6	64.3	49.2
17	62.4	60.7	60.9	53.4

[a] Nonrandomized.

Table 4
Regression coefficients of predicted quadratic polynomial models for the response total antioxidant activity of whole grain and bran of soft and hard wheat

Coefficient	SWB[a]	SWW[b]	HWB[c]	HWW[d]
β_0	62.29**	55.17**	61.0***	49.54***
Linear				
β_1	−1.02	7.42***	−0.42	7.74***
β_2	3.14**	0.19	6.93***	0.11
β_3	−0.57	1.47	0.4	1.36
Quadratic				
β_{11}	−6.36***	−14.47***	11.03***	−14.54***
β_{22}	0.64	1.22	−7.32	0.21
β_{33}	−0.41	2.28	1.13	2.46
Crossproduct				
β_{12}	−0.55	1.97	−1.89	−1.61
β_{13}	0.45		2.01*	−0.46
β_{23}		3.88**	1.89	3.84**
R^{2e}	0.89	0.92	0.94	0.94
CV[f]	3.5	8.8	4.49	7.93

[a] Soft wheat bran.
[b] Soft whole wheat.
[c] Hard wheat bran.
[d] Hard whole wheat.
[e] Coefficient of multiple determination.
[f] Coefficient of variance.
* Significant at 10%.
** Significant at 5%.
*** Significant at 0.1%.

Table 5
Analysis of variance for the response surface quadratic model for total antioxidant activity

Source	DF[a]	Sum of squares	Mean square	*F*-value
Soft wheat bran		14.36	1.8	0.17
Lack of fit	8	20.54	10.27	
Pure error	2	34.9	3.49	
Total error	10			
Soft whole wheat				
Lack of fit	7	129.02	18.43	2.83[b]
Pure error	2	13.05	6.52	
Total error	9	142.06	15.78	
Hard wheat bran				
Lack of fit	5	47.62	9.52	1.91[b]
Pure error	2	9.95	4.97	
Total error	7	57.56	8.22	
Hard whole wheat				
Lack of fit	7	70.42	10.06	10.06[b]
Pure error	2	11.23	5.61	
Total error	9	81.64	9.07	

[a] Degrees of freedom.
[b] Insignificant.

Table 6
Analysis of variance of the regression parameters of the predicted response surface quadratic models

Regression	DF[a]	Sum of squares	R_2[b]	*F*-value[c]
Soft wheat bran				
Linear	3	112.24	0.36	7.85
Quadratic	3	161.39	0.51	11.29**
Cross product	3	4.36	0.01	0.3
Total model	9	278.0	0.89	6.48**
Soft whole wheat				
Linear	3	572.53	0.34	9.87**
Quadratic	3	813.67	0.49	14.02**
Cross product	3	153.97	0.09	2.65
Total model	9	1540.18	0.91	8.85**
Hard wheat bran				
Linear	3	483.61	0.47	19.6***
Quadratic	3	402.84	0.39	16.33**
Cross product	3	89.4	0.09	3.62*
Total model	9	975.86	0.94	13.19**
Hard whole wheat				
Linear	3	617.69	0.39	18.06**
Quadratic	3	732.71	0.47	21.42***
Cross product	3	140.32	0.09	4.1*
Total model	9	1490.73	0.95	14.53***

[c,***] Significant at 0.1%l.
[a] Degrees of freedom.
[b] Coefficient of multiple determination.
[*] Significant at 10%.
[**] Significant at 5%.

the mean value is high and does not satisfactorily develop an adequate response model (Daniel, 1991).

An ANOVA of the regression parameters of the predicted response surface quadratic models for TAA of wheat fractions is shown in Table 6. The results indicated that both linear and quadratic parameters were highly significant ($p < 0.001$ or $p < 0.05$) for all wheat extracts. However, interactions did not produce a significant effect in each case. Thus, linear and quadratic effects of independent variables were the primary determining terms that may cause significant effects in the response while the interaction terms were insignificant in; most cases. The positive coefficients for X_1, X_2

Table 7
Analysis of variance of the factors and the critical values obtained from ridge analysis of the response surface for total antioxidant activity (μM TE)

Source	Analysis of variance				Critical values	
	DF[a]	Sum of squares	Mean square	*F*-value[b]	Coded[c]	Uncoded
Soft wheat bran						
Solvent composition (X_1 %, v/v)	4	122.74	30.68	6.44**	−0.038	49
Temperature (X_2, °C)	4	102.44	25.61	5.37**	0.193	64
Time (X_3, min)	4	5.63	1.41	0.3	−0.034	60
Soft whole wheat						
Temperature (X_2, °C)	4	155.7	38.92	2.01	0.055	61
Time (X_3 min)	4	158.27	39.57	2.05	0.235	64
Hard wheat bran						
Solvent composition (X_1, %, v/v)	4	388.77	97.19	11.82**	−0.017	50
Temperature (X_2, °C)	4	680.72	170.18	20.69***	0.098	62
Time (X_3, min)	4	65.94	16.48	2.0	0.01	60
Hard whole wheat						
Solvent composition (X^1, %, v/v)	4	1187.75	296.94	26.04***	0.178	54
Temperature (X_2, °C)	4	138.86	34.71	3.04*	0.055	61
Time (X_3, min)	4	154.28	38.57	3.38*	0.235	65

[b,***] Significant at 1%.
[a] Degrees of freedom.
[c] Critical values obtained from ridge analysis.
[*] Significant at 10%.
[**] Significant at 5%.

and X_3 indicated linear effects that may increase the responses (Table 4). The quadratic effects of independent variables demonstrated both positive and negative effects. An ANOVA of independent variables shown in Table 7 indicates that solvent composition (X_1) was the most significant ($p < 0.001$ or $p < 0.05$) factor affecting TM of all wheat fractions. The model indicated that the proportion of ethandl had significant linear effects on TAA of whole grains of both soft and hard wheat (Table 4). Hence, ethanol concentration showed the largest positive linear regression coefficient. However, with respect to bran fraction of both soft and hard wheat extraction temperature was associated with the largest positive coefficients for the response TAA (Table 4). Thus, ethanol concentration and/or temperature contributed significantly to the response. On the other hand, the extraction time had no significant effect on TAA of wheat extracts. By considering the regression coefficients

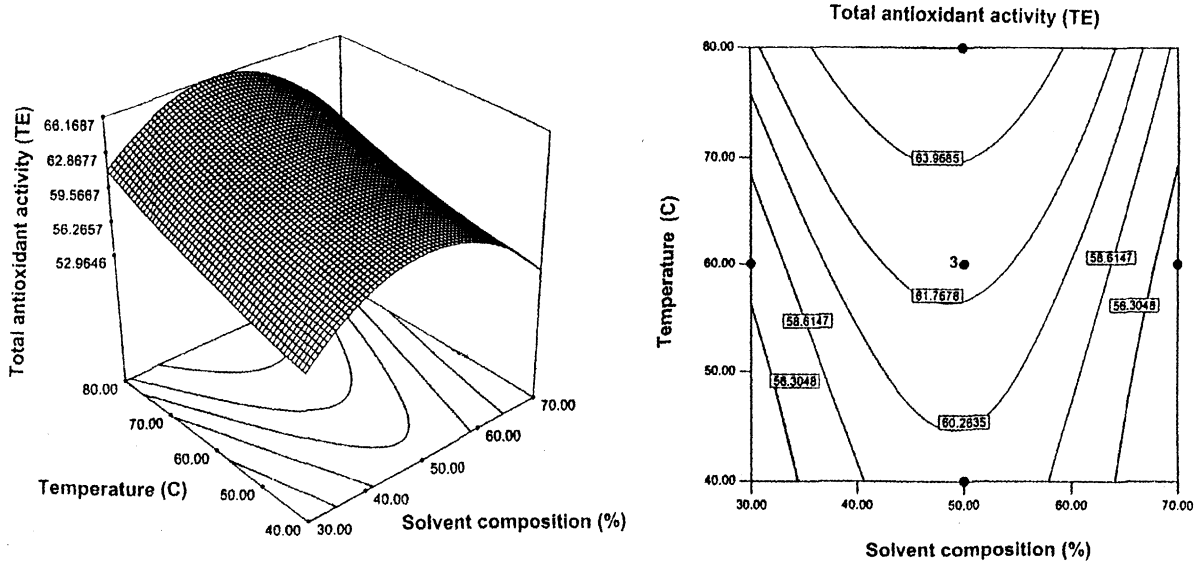

Fig. 1. Response surface and contour plots for the effects of solvent composition and temperature at a constant time course of 60 min on total antioxidant activity (μM TE) of soft wheat bran.

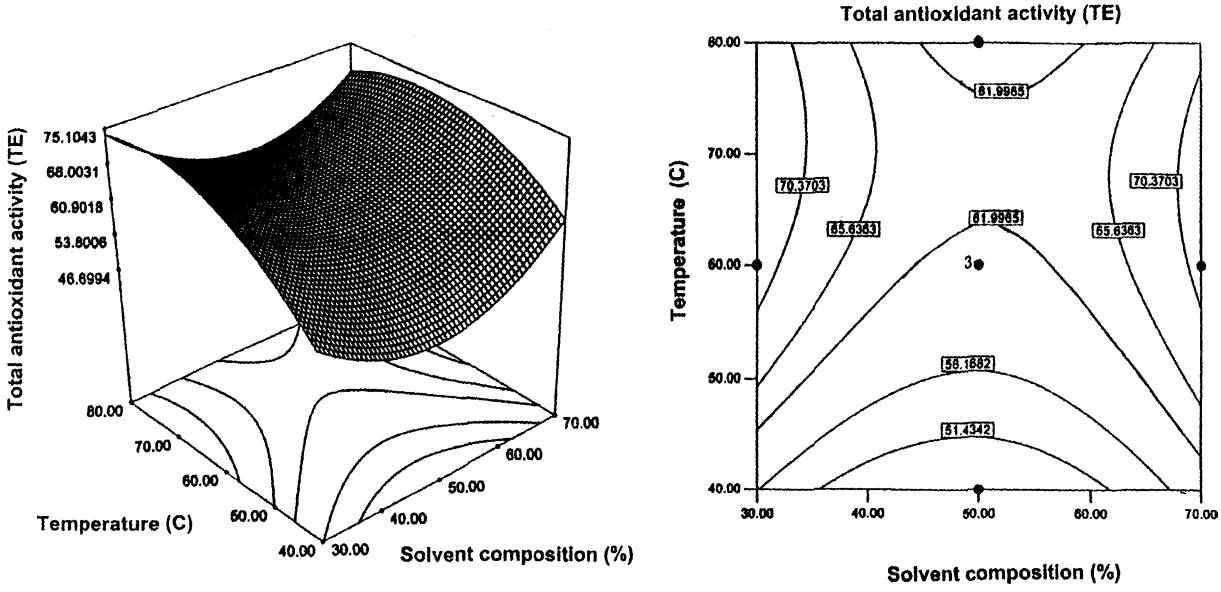

Fig. 2. Response surface and contour plots for the effects of solvent composition and temperature at a constant time course of 60 min on total antioxidant activity (μM TE) of soft whole wheat.

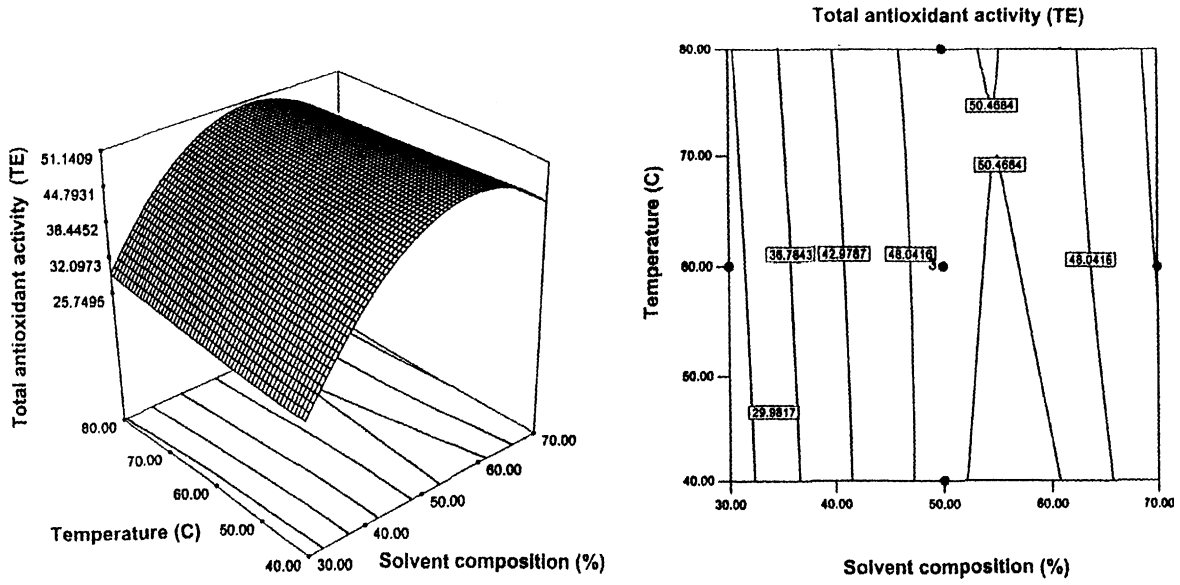

Fig. 3. Response surface and contour plots for the effects of solvent composition and temperature at a constant time course of 60 min on total antioxidant activity (μM TE) of hard wheat bran.

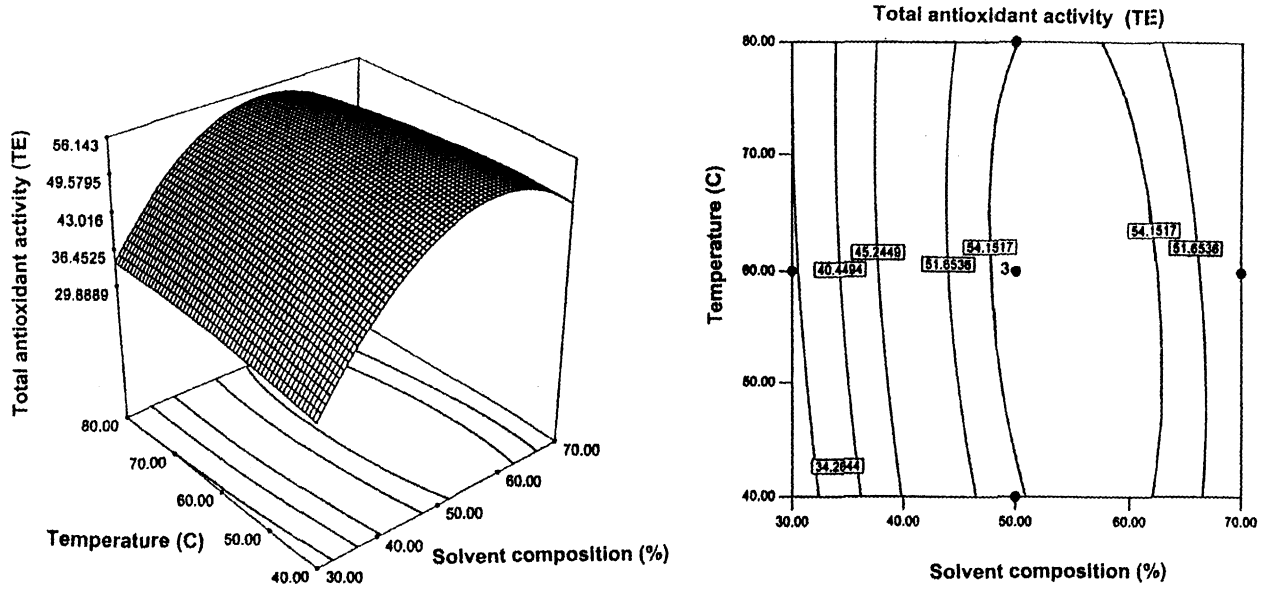

Fig. 4. Response surface and contour plots for the effects of solvent composition and temperature at a constant time course of 60 min on total antioxidant activity (μM TE) of hard whole wheat.

obtained for independent and dependent variables, ethanol concentration and temperature were perhaps the most important factors that may significantly influence TAA. Park, Lee, Jeong, and Kwon (1998) found that solvent concentration plays a critical role in the extraction of soluble solids from various natural products.; Similarly Kwon, Belanger, and Pare (2003) reported that solvent concentration was the most important factor contributing to the extraction of ginseng components using RSM.

3.3. Analysis of response surfaces

Since the models have shown lack of fit to be insignificant the responses were sufficiently explained by the regression equation. The regression models allowed the prediction of the effects of the three parameters on TAA of wheat fractions. The relationship between independent and dependent variables is illustrated in three-dimensional representation of the response surfaces and two-dimensional contour plots generated by the models

Table 8
Comparison of predicted and experimental values for the response variable, total antioxidant activity

Wheat fraction	Eigen values	Stationary point	Predicted value[a]	Observed value[b]
Soft wheat				
Bran	0.664, −0.411, −6.377	Saddle	63.0	61.3 ± 1.9
Whole grain	3.163, −2.035, −14.548	Saddle	56.5	54.7 ± 3.2
Hard wheat				
Bran	11.172, 1.156, −7.481	Saddle	61.6	58.2 ± 2.7
Whole grain	3.583, −0.861, −14.581	Saddle	50.9	51.6 ± 1.3

[a] Predicted using ridge analysis of response surface quadratic model.
[b] Mean ± standard deviation of triplicate determinations from different experiments.

for TAA. On the basis of coded data, canonical analysis for TAA demonstrated a saddle point as the stationary point for all wheat extracts examined.

Since analysis of the surface response revealed that the stationary point for TAA was a saddle, a ridge analysis was performed to determine the critical levels of the design variables that may produce the maximum response. The critical values in terms of coded and uncoded variables for TAA are given in Table 7.

Since time exhibited insignificant effects on TAA of wheat extracts under different circumstances the response surface and contour plots were generated as a function of solvent composition (30–70%) and temperature (40–80 °C) while keeping the time constant at 60 min. Fig. 1 depicts response surface and contour plots of the effects of the two variables, namely solvent composition and temperature on TAA of soft wheat bran extract. The solvent composition demonstrated quadratic effects on the response; hence TAA increased up to about 50% organic solvent in the medium followed: by a decline with its further increase. However, temperature demonstrated a linear effect on TAA. The effect of solvent composition and temperature on TAA of soft whole wheat extract is shown in Fig. 2. Solvent composition displayed a quadratic effect on the response yielding maximum between 50% and 60% ethanol concentration. When the time was kept constant at 60 min the temperature caused a linear increase in the response at least with low proportions of organic solvent in the medium. Moreover, the response and contour plots generated for hard wheat bran showed that at lower and upper levels of temperature the solvent content influenced the response in a quadratic manner and hence TAA decreased with increasing proportion of organic solvent up to a certain level (approximately 50%); but improved with further increase (Fig. 3). Further, at lower and upper levels of solvent composition, as the temperature increased the response also displayed an increase. The effect of solvent composition and temperature at 60 min on TAA of hard whole wheat is shown in Fig. 4. The solvent composition demonstrated quadratic effects on the response similar to those observed in soft wheat fractions, while the effect of temperature was linear regardless of the proportion of ethanol in the medium. Solvent compositions towards the upper design point of the variable produced a greater response compared to that produced at the lower design point.

3.4. Verification experiments

Verification experiments performed at the predicted conditions derived from ridge analysis of RSM demonstrated that experimental values were reasonably close to the predicted values confirming the validity and adequacy of the predicted models. Moreover, the verification experiments also proved that the predicted values of TAA for the model with each wheat fraction could be satisfactorily achieved within 95% confidence interval of experimental values (Table 8).

4. Conclusion

The high correlation of the model exhibited that: second-order polynomial model could be used to optimize extraction of phenolic compounds from wheat for maximizing the total antioxidant activity. Aqueous ethanol was found to be most effective in extracting phenolic compounds from whole grain and bran of both soft and hard wheat. Hence, the conditions for extraction of phenolics from whole grain and bran of soft wheat and whole grain and bran of hard wheat were 54%, 61 °C, 64 min; 49%, 64 °C, 60 min; 54%, 61 °C, 65 min and 50%, 62 °C, 60 min, respectively. Under optimized conditions the experimental values agreed with the values predicted by ridge analysis. The experimental conditions allow a fast, quantitative and maximum extraction of phenolic compounds from wheat.

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