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Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran

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Abstract

Wheat bran, an important by-product of the cereal industry, is rich in potentially health-promoting phenolic compounds. In this paper, the phenolic compounds from wheat bran were extracted by ultrasound-assisted extraction technology. The experiments were carried out according to a five level, three variable central composite rotatable design (CCRD), and the best possible combination of solvent concentration, extraction temperature and extraction time with the application of ultrasound, for maximum extraction of phenolic compounds from wheat bran, was obtained, through response surface methodology (RSM). The optimum extraction conditions were as follows: ethanol concentration, 64%; extraction temperature, 60 °C; and extraction time, 25 min; and the extraction time was the most significant parameter for the process. Under the above-mentioned conditions, the experimental total phenolic content was 3.12 mg gallic acid equivalents/g of wheat bran tested, which is well matched with the predicted content.

Keywords: Central composite rotatable design; Phenolic compounds; Ultrasound extraction; Wheat bran

1. Introduction

Grains contain unique phytochemicals. Epidemiological studies have strongly suggested that diets rich in cereals play a crucial role in the prevention of chronic diseases, such as cardiovascular disease and certain types of cancers (Aruoma, 2003; Slavin, 2000). The beneficial health effects derived from the intake of diets rich in cereals have mainly been ascribed to dietary fibre, or to some of the components associated with the fibre, including phenolic acids (Andreasen, Landbo, Christensen, Hansen, & Meyer, 2001; Zhao, Egashira, & Sanada, 2003). It is widely accepted that phenolic compounds, including ferulic, vanillic, *p*-coumaric, caffeic, and chlorogenic acids, are rich in the bran portion of cereal kernels and may contribute to the total antioxidant activities of grain (Baublis, Clydesdale, & Decker, 2000; Martínez-Tomé et al., 2004; Yu et al., 2002). These phenolic compounds exhibit *in vitro* chemoprotective and antioxidant properties, and are suggested to be mainly responsible for the beneficial effects of a diet rich in cereal bran (Andreasen, Kroon, Williamson, & Garcia-Conesa, 2001).

Wheat bran is produced worldwide in enormous quantities, as an important by-product of the cereal industry. Several investigations have been conducted to evaluate the antioxidant properties of wheat and wheat-based cereal extracts (Yu et al., 2002). However, to the best of our knowledge, there is limited literature on efficient extraction of phenolic compounds from wheat bran. In order to seek more environmentally friendly methods, decrease the solvent consumption, shorten the extraction time, increase the extraction yield, and enhance the quality of extracts, various novel extraction techniques have been developed for the extraction of nutraceuticals from plants, including ultrasound-assisted extraction, supercritical fluid

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extraction, microwave-assisted extraction, and accelerated solvent extraction (Wang & Weller, 2006). Among these, ultrasound-assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques. The enhancement in extraction obtained by using ultrasound is mainly attributed to the effects of acoustic cavitations produced in the solvent by the passage of an ultrasonic wave. Ultrasound also exerts a mechanical effect, allowing greater penetration of solvent into the sample matrix, increasing the contact surface area between solid and liquid phase; as a result the solute quickly diffuses from solid phase to the solvent (Rostagno, Palma, & Barroso, 2003). In addition, there is no chemical involvement in the ultrasound-assisted extraction, which could prevent possible chemical degradation of targeted compounds (Wang & Weller, 2006).

In the present study, ultrasound-assisted extraction parameters such as the solvent, extraction temperature and extraction time were optimised using response surface methodology (RSM), by employing a five-level, three-variable central composite rotatable design (CCRD), in order to obtain the optimal conditions for the extraction of phenolic compounds from wheat bran.

2. Materials and methods

2.1. Materials

Wheat bran was obtained from a milling plant in Beijing, China. The bran was milled and passed through a 0.5 mm sieve. The wheat bran was kept in a sealed plastic bag and stored at 4 °C until use. All chemicals and solvents used were of analytical grade.

2.2. Ultrasound-assisted extraction of phenolic compounds from wheat bran

Ultrasound-assisted extraction was performed in an ultrasonic cleaning bath (SB-5200D type, 40 kHz, 250 W, Ningbo Scientz Biotechnology Co. Ltd., China) with a useful volume of 10 l (internal dimensions: $300 \times 240 \times 150$ mm). Working frequency was fixed at 40 kHz. Samples were placed into a volumetric flask (100 ml), made up to volume with the extracting solvent and sonicated for different times at the required temperature. The temperature was controlled by circulating external water from a thermostatted water bath. After the extraction, the flask was removed from the bath and cooled to room temperature by cooling water. The wheat bran extracts were filtered through Whatman No. 1 paper under vacuum, and the solution was collected in a volumetric flask, and used for the determination of the total phenolic content.

2.3. Selection of extraction solvent

Five grams of accurately weighed wheat bran were extracted with 100 ml of 70% (v/v) methanol, 70% (v/v) ethanol and 70% (v/v) acetone, respectively, in a volumetric flask (100 ml) and kept for sonication at 50 °C. After 20 min, the supernatant and the sediment were separated by vacuum filtration. The extracts obtained were used for the determination of the total phenolic content. The procedure of ultrasonic extraction of material was repeated twice under the same conditions.

2.4. Effect of ethanol concentration on extraction of total phenolic compounds

Ethanol–water mixtures were used as extraction solvents. Phenolic compounds were extracted from wheat bran using different ethanol concentration, ranging from 20% (v/v) to 95% (v/v); the wheat bran (5.0 g) was macerated with the extracting solvents (100 ml), and sonicated for 20 min at 50 °C. The extract was filtered under vacuum and the filtrates were used for the determination of the total phenolic content.

2.5. Effect of extraction temperature on extraction of total phenolic compounds

Five grams of wheat bran were macerated with 70% (v/v) ethanol (100 ml), and sonicated for 20 min at different temperatures ranging from 25 to 75 °C. The extract was filtered under vacuum and the filtrates were used for the determination of the total phenolic content.

2.6. Effect of extraction time on extraction of total phenolic compounds

Wheat bran (5.0 g) was macerated with 70% (v/v) ethanol (100 ml), and sonicated for different times ranging from 10 to 50 min at 50 °C. The extract was filtered under vacuum and the filtrates were used for the determination of the total phenolic content.

2.7. Experimental design

A five level, three variable central composite rotatable design (Cochran & Cox, 1992) was applied to determine the best combination of extraction variables for the total phenolic content from wheat bran. The factorial design consisted of eight factorial points, six axial points (two axial points on the axis of each design variable at a distance of 1.68 from the design center) and four center points, leading to 18 sets of experiments. The variables X_i were coded as x_i according to the following equation:

$$x_i = (X_i - \overline{X_i})/\Delta X_i \quad (1)$$

where x_i is the coded value of an independent variable, $\overline{X_i}$ is the real value of an independent variable, is the real value of an independent variable at the center point, and ΔX_i is the step change value. The variables and their levels, with both coded values and natural values investigated in this study, are represented in Table 1. Table 2 lists the actual

Table 1
Variables and their levels employed in a central composite rotatable design for optimisation of wheat bran extracts

Variable	Coded levels				
	$-\alpha$ (−1.68)	−1	0	+1	$+\alpha$ (+1.68)
	Natural levels				
Ethanol concentration (%)	43	50	60	70	77
Extraction time (min)	11	15	20	25	29
Extraction temperature (°C)	33	40	50	60	67

experimental parameters corresponding to the designed levels, which were carried out for developing the model. Each experiment was performed in triplicate and the average total phenolic content was taken as the response, Y.

Regression analysis was performed based on the experimental data and was fitted into an empiric second order polynomial model as shown in the following equation:

$$Y = b_0 + \sum_{i=1}^{5} b_i x_i + \sum_{i=1}^{5} b_{ii} x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} b_{ij} x_i x_j \quad (2)$$

where Y is the response variable, b_0, b_i, b_{ii}, b_{ij} are the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively. x_i and x_j are independent variables.

The responses obtained from the experimental design set (Table 2) were subjected to multiple nonlinear regression using the software STATISTICA 6.0, to obtain the coefficients of the second polynomial model. The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2, and its statistical significance was checked by an F-test. The significances of the regression coefficient were tested by a t-test.

2.8. Determination of total phenolic content

The total phenolic content of the wheat bran extracts was determined with the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999). Briefly, an aliquot of 100 μl of appropriate dilution of the extracts was shaken for 1 min with 500 μl of the Folin–Ciocalteu reagent freshly prepared in our laboratory, and 6 ml of distilled water. After the mixture was shaken, 2 ml of 15% (w/v) sodium carbonate was added and the mixture was shaken again for 0.5 min. Finally, the solution was brought up to 10 ml by adding distilled water. After 2 h of reaction at ambient temperature, the absorbance at 750 nm was evaluated using glass cuvettes. Using gallic acid as standard, the total phenolic content of extracts was expressed as a gallic acid equivalent (mg gallic acid/g wheat bran). Data are reported as means ± SD for at least three replications.

2.9. Statistical analysis

All analyses were performed in triplicate. The experimental results obtained were expressed as means ± SD. Statistical analysis was performed using the software STATISTICA 6.0. Data were analyzed by analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range test.

Table 2
Experimental design of five-level, three-variable central composite rotatable design[a,b]

Test set	x_1, Ethanol concentration (%)	x_2, Extraction temperature (°C)	x_3, Extraction time (min)	Total phenolics content (mg GAE eq.[c]/g bran)	
				Experimental	Predicted
1	50 (−1)	40 (−1)	15 (−1)	2.07	1.98
2	50 (−1)	40 (−1)	25 (+1)	2.48	2.52
3	50 (−1)	60 (+1)	15 (−1)	2.26	2.26
4	50 (−1)	60 (+1)	25 (+1)	2.79	2.79
5	70 (+1)	40 (−1)	15 (−1)	2.38	2.20
6	70 (+1)	40 (−1)	25 (+1)	2.57	2.74
7	70 (+1)	60 (+1)	15 (−1)	2.39	2.48
8	70 (+1)	60 (+1)	25 (+1)	2.96	3.01
9	43 (−1.68)	50 (0)	20 (0)	2.37	2.41
10	77 (+1.68)	50 (0)	20 (0)	2.85	2.78
11	60 (0)	33 (−1.68)	20 (0)	2.29	2.34
12	60 (0)	67 (+1.68)	20 (0)	2.88	2.80
13	60 (0)	50 (0)	11 (−1.68)	1.83	1.95
14	60 (0)	50 (0)	29 (+1.68)	2.99	2.84
15	60 (0)	50 (0)	20 (0)	2.85	2.86
16	60 (0)	50 (0)	20 (0)	2.87	2.86
17	60 (0)	50 (0)	20 (0)	2.79	2.86
18	60 (0)	50 (0)	20 (0)	2.91	2.86

[a] Experimental total phenolic contents are average of triplicates.
[b] Average absolute relative deviation (%) = 2.69.
[c] Gallic acid equivalent.

3. Results and discussion

3.1. The total phenolic content of wheat bran extracted with various solvents

Aqueous alcohols and acetone, with different levels of water, have been widely used to extract phenolic components from botanical materials, especially herbs. An extraction solvent system is generally selected according to the purpose of extraction, polarity of the interested components, polarity of undesirable components, overall cost, and safety (Yu et al., 2002). In this paper, the efficiency of methanol, ethanol, and acetone on the extraction of the total phenolic content from wheat bran was compared, which was expressed as gallic acid equivalents (GAE). Fig. 1 shows the total phenolic contents of wheat bran extracted with various solvents. Significant difference in total phenolic content was observed among the various solvent extracts. Ethanol extracts contained the highest content of total phenolics, followed by methanol extracts and acetone extracts. In addition, ethanol is less toxic and can be easily recovered by reduced pressure distillation. Therefore, ethanol was used as the extraction solvent in the following study.

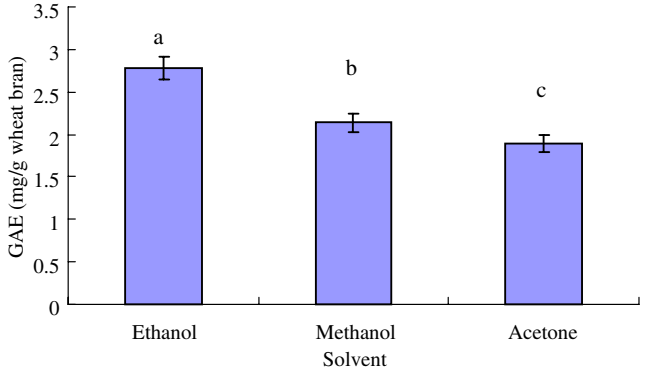

Fig. 1. The content of total phenolic compounds of wheat bran extracted with various solvents. The vertical bars represent the standard deviation ($n = 3$). Values marked by the same letter are not significantly different ($p < 0.05$). Extraction conditions under sonication: solvent concentration, 70%, extraction temperature, 50 °C; extraction time, 20 min.

3.2. Effect of ethanol concentration on extraction of total phenolic compounds

Adding a certain amount of water in ethanol might improve the extracting efficiency (Yu et al., 2002). Ethanol–water mixtures were used as the extraction solvent in the study. The effects of ethanol concentration in the extraction solvent on the content of phenolics in wheat bran extracts are shown in Fig. 2. When ethanol concentration increased from 20% to 60% (v/v), the total phenolic content of the extracts increased from 1.67 to 2.81 mg GAE/g of wheat bran tested. When ethanol concentration reached 80% (v/v), the total phenolic content in bran extracts decreased quickly, and at a concentration of 95% (v/v), the total phenolic content was 1.48 mg GAE/g of wheat bran. When 95% (v/v) ethanol was used to extract wheat bran, some lipid components were also extracted, which may limit the extraction of phenolics in wheat bran. A similar effect was reported in the extraction of phenolic antioxidants from peanut skins (Neptoe, Grosso, & Guzmán, 2005).

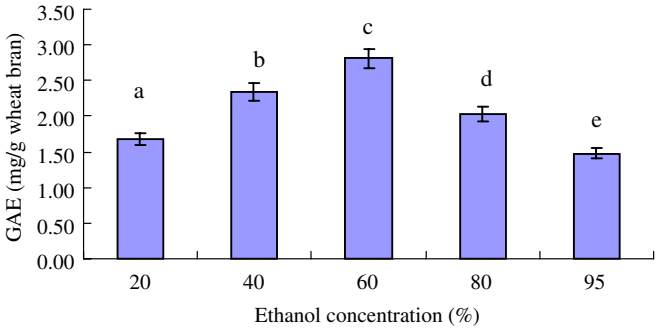

Fig. 2. Effect of ethanol concentration on the total phenolics content from wheat bran. The vertical bars represent the standard deviation ($n = 3$). Values marked by the same letter are not significantly different ($p < 0.05$). Extraction conditions under sonication: extraction temperature, 50 °C; extraction time, 20 min.

3.3. Effect of extraction temperature on extraction of total phenolic compounds

Fig. 3 shows the effect of extraction temperature under sonication on the contents of total phenolic compounds extracted from wheat bran. A significant increase of the total phenolic content was observed over the extraction temperature range (25–75 °C), and the total phenolic content reached a maximum of around 2.80 mg GAE/g of wheat bran at 65 °C. At a higher temperature, the solubility of phenolic compounds in wheat bran could be enhanced, and the viscosity of wheat bran extracts was decreased, accelerating the whole extraction.

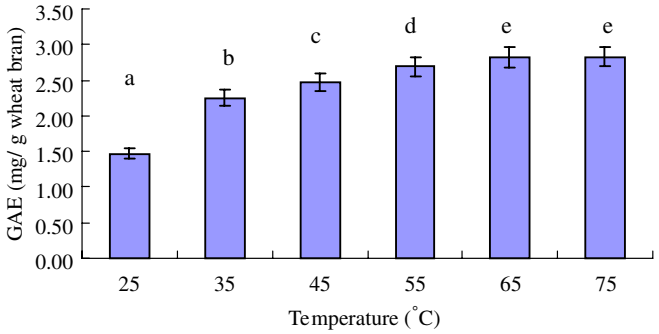

Fig. 3. Effect of extraction temperature on the total phenolics content from wheat bran. The vertical bars represent the standard deviation ($n = 3$). Values marked by the same letter are not significantly different ($p < 0.05$). Extraction conditions under sonication: ethanol concentration, 70%; extraction time, 20 min.

3.4. Effect of extraction time on extraction of total phenolic compounds

The contents of total phenolic compounds extracted from wheat bran at different times of sonication are presented in Fig. 4. A marked increase of the total phenolics content was observed up to 30 min, remaining constant until 50 min.

3.5. Optimisation of extraction conditions

Based on the investigations of the effects of ethanol concentration, extraction temperature and time on the content of phenolic compounds of wheat bran extracts, these variables were considered in the experimental design. To optimise the extraction process of the total phenolics from wheat bran, an ethanol concentration of 60% (v/v), an extraction temperature of 50 °C, and an extraction time of 20 min were chosen as the central condition of the CCRD.

Table 2 shows the experimental conditions and the results of extraction according to the factorial design. The maximum content of phenolic compounds (2.99 mg GAE/g bran) was recorded under the experimental parameters of an ethanol concentration of 60%, an extraction temperature of 50 °C and an extraction time of 29 min. The lowest content of phenolic compounds (1.83 mg GAE/g bran) was observed in test set No. 13, compared to the others. Statistical analysis revealed that the most relevant variable ($p < 0.001$) concerning the content of total phenolic compounds was the extraction time (Table 3). The total phenolic content was also high (2.96 mg GAE/g bran) with an ethanol concentration of 70%, an extraction temperature of 60 °C and an extraction time of 25 min, corresponding to test set No. 8. A higher content of phenolics from test set No. 14, compared to that from test set No. 13, could imply that an increase in extraction time was most favourable for phenolic compounds extraction.

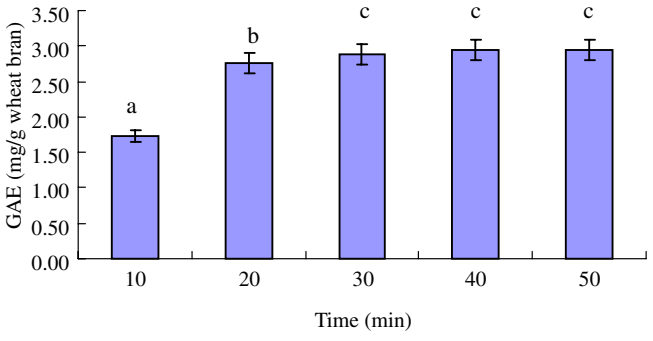

Fig. 4. Effect of extraction time on the total phenolics content from wheat bran. The vertical bars represent the standard deviation ($n = 3$). Values marked by the same letter are not significantly different ($p < 0.05$). Extraction conditions under sonication: ethanol concentration, 70%; extraction temperature, 50 °C.

Multiple regression analysis was performed on the experimental data and the coefficients of the model were evaluated for significance with a Student t-test. All the linear coefficients were significant ($p < 0.05$). All the cross-product coefficients were eliminated in the refined equation as their effects were not significant. The values of the coefficients are presented in Table 3. The analysis of variance for the CCRD is shown in Table 4. The coefficient of determination (R^2) of the model is 0.9492, which indicated that the model had adequately represented the real relationship between the parameters chosen. The average absolute relative deviation of the reduced model is 2.69%. Neglecting the non-significant terms, the final predictive equation obtained is as given below:

$$Y = 2.85637 + 0.110401x_1 + 0.138602x_2 + 0.267413x_3 - 0.091756x_1^2 - 0.101613x_2^2 - 0.163617x_3^2 \quad (3)$$

To determine optimal levels of the variables for the extraction of phenolic compounds from wheat bran, three-dimensional surface plots were constructed, according to Eq. (3). Fig. 5 shows the effect of ethanol concentration and extraction temperature on the content of total phenolic compounds. The total phenolic content increased slowly with the increase of ethanol concentration at a fixed extraction temperature, and nearly reached a peak at the highest ethanol concentration tested. Similarly, the increase in extraction temperature at a fixed ethanol concentration led to a gradual increase in the total phenolic content, and reached a maximum at the highest extraction temper-

Table 3
Estimated coefficients of the fitted second-order polynomial model for the phenolic content from wheat bran[a]

Term	Coefficients estimated	Standard error	t Value	p Value
b_0	2.85637	0.055803	51.19	<0.0001
b_1	0.110401	0.030261	3.65	0.0065
b_2	0.138602	0.030261	4.58	0.0018
b_3	0.267413	0.030261	8.84	<0.0001
b_{11}	−0.091756	0.031476	−2.95	0.0185
b_{22}	−0.101613	0.031476	−3.23	0.0121
b_{33}	−0.163617	0.031476	−5.2	0.0008

[a] Only terms with $p < 0.05$ were included.

Table 4
Analysis of variance of the second-order total phenolic contents of wheat bran model[a]

	Degree of freedom	Sum of squares	Mean square	F Value	p Value
Total model	9	1.868688	0.949200	16.62	0.0003
Linear	3	1.40417	0.713300	37.46	<0.0001
Quadratic	3	0.427768	0.217300	11.41	0.0029
Cross-product	3	0.03655	0.018600	0.98	0.451
Total error	8	0.099962	0.012495		
Lack of fit	5	0.092462	0.018492	7.4	0.0651
Pure error	3	0.0075	0.0025		

[a] Coefficient of determination (R^2) = 0.9492.

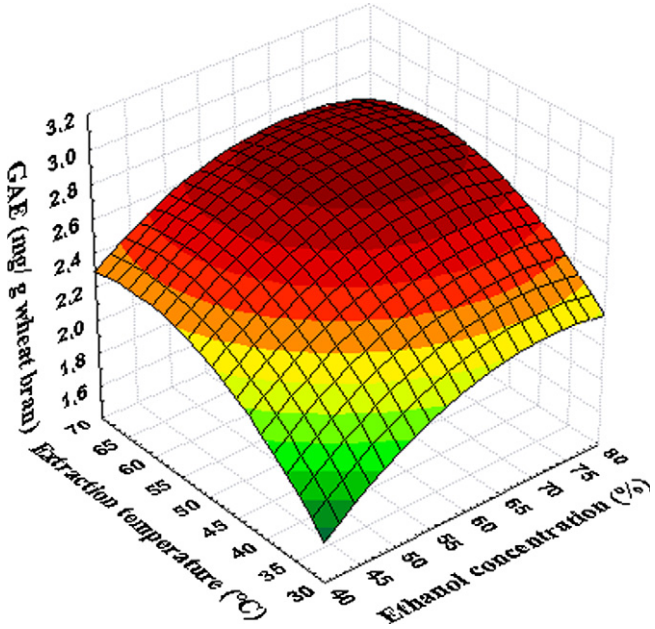

Fig. 5. Response surface plot showing the effect of ethanol concentration (%) and extraction temperature (°C) on the total phenolic content from wheat bran. Extraction time is constant at the zero level, 20 min.

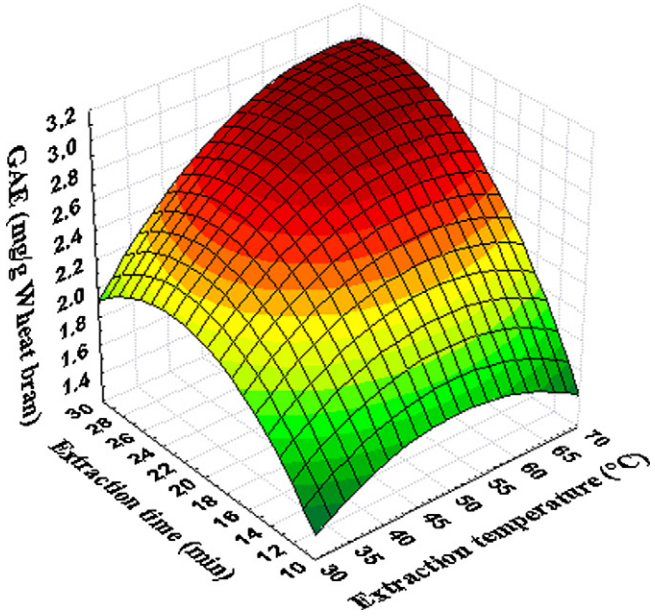

Fig. 7. Response surface plot showing the effect of extraction temperature (°C) and extraction time (min) on the total phenolic content from wheat bran. Ethanol concentration is constant at the zero level, 60%.

ature tested. The effect of ethanol concentration and extraction time shown in Fig. 6 demonstrated that the total phenolic content increased rapidly with the increase of ethanol concentration at a fixed extraction time, while an increase in extraction time at a fixed ethanol concentration also led to a marked increase in total phenolic content. Fig. 7 reflects the effect of extraction temperature and extraction time on the extraction of phenolic compounds from wheat bran. A similar linear increase in the total phenolic content with increase in extraction temperature at a fixed extraction time, while an obvious quadratic effect of extraction time were both observed, and the total phenolic content reached its highest value at an extraction time of about 25 min at a fixed extraction temperature.

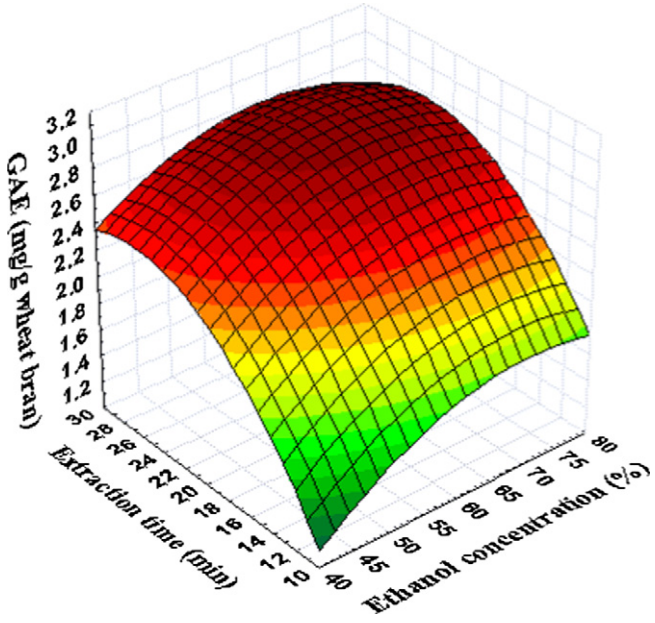

Fig. 6. Response surface plot showing the effect of ethanol concentration (%) and extraction time (min) on the total phenolic content from wheat bran. Extraction temperature is constant at the zero level, 50 °C.

The optimal conditions obtained using the model were as follows: ethanol concentration, 64%; extraction temperature, 60 °C; and extraction time, 25 min. Under optimal conditions, the model predicted a maximum response of 3.08 mg GAE/g bran. To compare the predicted result with the practical value, experimental rechecking was performed using this deduced optimal condition. A mean value of 3.12 ± 0.03 mg GAE/g bran ($n = 3$) obtained from real experiments validated the RSM model. The good correlation between these results confirmed that the response model was adequate to reflect the expected optimisation (Table 5).

It has been reported that some statistically significant differences in total phenolic content were obtained among

Table 5
Optimum conditions, predicted and experimental value of response under those conditions

Optimum conditions			Total phenolics content (mg GAE eq.[a]/g bran).	
Ethanol concentration (%)	Extraction temperature (°C)	Extraction time (min)	Experimental[b]	Predicted
64	60	25	3.12 ± 0.03	3.08

[a] Gallic acid equivalent.
[b] Means ± standard deviation ($n = 4$).

test wheat varieties extracted with traditional methods, such as Soxhlet extraction and mix-stirring extraction (Adom, Sorrellls, & Liu, 2003), and wheat grain and its fractions, such as aleurone, bran, and micronised aleurone, may significantly differ in their total phenolic contents (Zhou, Laux, & Yu, 2004). The total phenolic contents of the bran extracts from a hard red winter wheat grown at four different locations were 2.29–3.24 mg GAE/g bran, using Soxhlet extraction with absolute ethanol for 15 h (Yu, Perret, Harris, Wilson, & Haley, 2003). The total phenolic contents of the bran extracts from a hard white winter wheat grown at five different locations were 2.29–3.05 mg GAE/g bran, using Soxhlet extraction with absolute ethanol for 15 h (Zhou & Yu, 2004). This indicated that the application of ultrasonication for the extraction of phenolic compounds from wheat bran was as effective as Soxhlet extraction, and the ultrasound-assisted extraction could greatly shorten the extraction time. The efficiency of ultrasonication could be explained by the fact that sonication simultaneously enhanced the hydration and fragmentation process while facilitating mass transfer of solutes to the extraction solvent, without significant decomposition of the solvent (Toma, Vinatoru, Paniwnyk, & Masom, 2001).

4. Conclusions

This present study indicates that wheat bran can be considered a good source of phenolic compounds. The extraction time strongly affects the phenolic content of the extracts. The optimal conditions obtained by RSM for the extraction of phenolic compounds from wheat bran under ultrasonication include the following parameters: ethanol concentration, 64%; extraction temperature, 60 °C; and extraction time, 25 min.

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