Extraction optimization and *in vitro* antioxidant properties of phenolic compounds from Cumin (*Cuminum cyminum* L.) seed

AL Juhaimi, F. Y. and *Ghafoor, K.*

*Department of Food and Nutrition Sciences, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia*

**Abstract**

Extraction of phenolic compounds from cumin (*Cuminum cyminum* L.) seed was optimized by using a designed experiment including three process variables i.e. temperature (35-50°C), time (1-4 h) and ethanol concentration (40-70%). The extraction temperature and time were found significant (P < 0.05) variables for the recovery of phenolic compounds and maximum of 25.17 mg GAE/g of total phenolic compounds were obtained by extraction at 50°C for 4 h using 40% ethanol. Response surface methodology was also used to predict optimum levels of process variables i.e. 49°C temperature, 2.8 h time and 53.6% ethanol for the maximum amount of total phenolic compounds (24.66 mg GAE/g) from cumin seed. The $R^2$ value of the model was 0.9866 and it matched with the experimental validation. The extracts from cumin seed showed good antiradical (34.25-39.25%) and antioxidant (8.25-11.24 mg/mL) activities which were determined by DPPH radical scavenging and phosphomolybdenum complex methods, respectively.

**Keywords**

Cumin seed  
Total phenolic compounds  
Extraction optimization  
Antiradical activity  
Antioxidant activity

**Introduction**

Epidemiological and *in vitro* studies indicate that food products containing phytochemicals such as phenolic compounds have potential protective effects against different diseases. These phytochemicals can be used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial, agents (Senevirathne *et al*., 2006). Strong evidence exist which emphasize that the consumption of different types of phenolic compounds from natural foods may decrease the risk of serious health problems due to their antiradical and antioxidant activities (Surh, 2002). Antioxidants minimize rancidity of foods, restrict the formation of toxic oxidation products, maintain nutritional quality and increase shelf life. There have been various studies on the phenolic compounds and antioxidant activities of different parts of plants such as their leaves, bark, roots fruits, peels and seeds (Al-Juhaimi and Ghafoor, 2011; Ghafoor and Choi, 2009; Ghafoor *et al*., 2010).

Cumin (*Cuminum cyminum* L.) is annual herb from Apiaceae family. It has significance as a spice and particularly used due to it flavoring effects in foods. It is considered to be one of the most important spices and ranks second to black pepper. The cultivation of this herb is reported in Arabian Peninsula, India, China and in the countries neighboring Mediterranean Sea (Thippeswamy and Naidu, 2005). Cumin seeds are used as a spice for their special aromatic effect, commonly in cuisines of India, Pakistan, North Africa, Middle East, Sri Lanka, Cuba, Northern Mexico and the Western China. Cumin seeds have been reportedly used for traditional treatment of toothache, dyspepsia, diarrhoea, epilepsy and jaundice (Nostro *et al*., 2005). The proximate analysis of the seeds reveals that they contain fixed oil (approximately 10%), protein, cellulose, sugar, mineral elements and volatile oil (1.5%) (Li and Jiang, 2004). Cumin seeds volatile oil imparts the characteristic aroma to the seeds. Rebey *et al.* (2012) studied cumin seed grown in Tunisia a reported they also contain good amounts of phenolic compounds which show considerable radical scavenging, carotene/linoleic acid chelating and reducing power activities.

The extraction and purification of phytochemicals from natural sources is gaining considerable attention, since these bioactive substances are often used in the preparation of dietary supplements, functional food ingredients, nutraceuticals, food additives, pharmaceuticals and cosmetic products. Various solvent systems and methods have been tested for extraction of polyphenols from plant materials (Chavan *et al*., 2001). Yield of bioactive compounds according to the method of extraction (Goli *et al*., 2005). The extraction method must enable maximum extraction of the compounds of interest and must avoid their chemical modifications (Zuo *et al*., 2002). Water, aqueous mixtures of ethanol, methanol and acetone are commonly used (Sun and Ho, 2005).
There are various other process variables, depending on the method, which have high significance and impact on the yield of bioactive compounds and may also include temperature, time, particle size etc. (Ghafoor et al., 2010). Statistical techniques such as response surface methodology are frequently applied for optimizing different processing conditions in food manufacture (Farah et al., 2012). Such approach is important for enhancing recovery of biologically important components from different plant matrices along with selection of suitable extraction method.

The objective of carrying out this research was to optimize the extraction of total phenolic compounds from cumin seed using response surface methods and also to evaluate these extracts for their biological activities using DPPH radical scavenging and phosphomolybdenum complex assays.

Materials and Methods

Materials

Seeds of cumin (*Cuminum cyminum* L.) originated from Syria were purchased from local market in Riyadh, Saudi Arabia. Dried cumin seeds were ground to a powdered form using an electrical grinder and passed through a 0.5 mm sieve. Ethanol (96% v/v) was purchased from BDH Laboratory Supplies (Poole, England) and all other chemicals were from Sigma Chemical Co. (St. Louis, MO).

Preparation of extracts

Seed extracts were prepared in a shaking water bath (Model 1083, Ollman & Co KG, Friedberg, Germany) at a continuous speed of 30 rpm, by mixing 2 g of cumin seed powder with 100 mL ethanol followed by filtration through Whatman No. 4 filter paper and evaporation to dryness. The yield (%) of evaporated dried extracts was calculated as 100xDW$_{\text{dried}}$/DW$_{\text{sample}}$, where DW$_{\text{dried}}$ is the weight of extract after evaporation of solvent, and DW$_{\text{sample}}$ is the dry weight of original sample. The yield of extracts ranged from 8-11%. For analysis dried extract was stored at 4°C before analyses. It was re-dissolved in ethanol to make a total volume of 100 mL to carry on analytical work.

Experimental design

The extraction process was carried out according to an orthogonal array design, in order to optimize the temperature, time and ethanol concentration for the extraction of phenolic compounds from cumin seed powder. An L16 orthogonal matrix with three factors, each factor containing 4 levels was selected to arrange the experiments. Extraction temperatures were 35, 40, 45 and 50°C, ethanol concentration was 40, 50 and 60, 70% and the extraction time was 1, 2, 3 and 4 h. These conditions were selected based on preliminary trials and extensive literature review for safe ranges of these variables. Regression analysis was performed on the data of the response variable (total phenolic compounds) obtained by triplicate observations of extracts as affected by the extraction conditions, and it was fitted into an empirical second-order polynomial model (Eq. 1) as shown:

\[ Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 + b_12 X_1 X_2 + b_13 X_1 X_3 + b_23 X_2 X_3 + b_11 X_1^2 + b_22 X_2^2 + b_33 X_3^2 \]  

where $Y$ represents the response variable (total phenolic compounds), and $X_1$, $X_2$ and $X_3$ correspond to the independent variables, namely, extraction temperature, extraction time and ethanol concentration respectively. The $b_n$ values represent corresponding regression coefficients.

Total phenolic compounds

The total phenolic compounds of the ethanolic extracts were determined by using Folin-Ciocalteu reagent, according to the procedure described by Dewanto et al. (2002). Briefly, 200 μL of properly diluted sample extract or standard solution were dissolved in 400 μL of Folin-Ciocalteu reagent. The total volume of the solution was made 4.6 mL with the addition of deionized water. The mixture was shaken, kept for 10 min at room temperature. Afterwards 1 mL of 10% Na$_2$CO$_3$ was added and the mixed thoroughly. After incubation in the dark for 90 min, the absorbance was measured at 760 nm using a spectrophotometer (Ultrospec II 4050; LKB Biochrom, Cambridge, England). Total phenolic amounts were expressed as mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW), using a calibration curve with gallic acid.

Antioxidant activity by phosphomolybdenum complex method

The antioxidant activity of the cumin seed extracts was evaluated by the phosphomolybdenum complex method (Ghafoor and Choi, 2009). Briefly, 0.4 mL of sample solution (100 μL of cumin seed extract dissolved in 1 mL of methanol) was combined with 4 mL of reagent solution containing 0.6 M sulfuric acid, 2 mM sodium phosphate, and 4 mM ammonium molybdate. The blank solution contained 4 mL of reagent solution and 1 mL of methanol. Test tubes were capped and placed in hot water for 90 min at 95°C. After samples were cooled to room temperature, absorbance was measured at 695 nm against a blank and the antioxidant activity was expressed as mg/mL.
2, 2-diphenyl-1-picrylhydrazyl radical scavenging assay

Radical scavenging activity was determined according to Hanato et al. (1998). One ml of the extract at different concentrations was added to 2 mL of a 2 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol. After shaking, the mixture was incubated at room temperature in the dark for 10 min, and then the absorbance was measured at 517 nm. Methanol (1 mL mixed in 2 mL of DPPH solution) was used as control. DPPH radical-scavenging activity or antiradical activity was expressed as percentage and was calculated using the following formula:

\[
\text{Antiradical activity (\%) = 100 \times \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right)}
\]

where \(A_{\text{control}}\) is the absorbance of the control at 10 min reaction and \(A_{\text{sample}}\) is the absorbance of the sample.

Statistical analysis

All the analysis was carried out in triplicate, and the experimental results obtained were expressed as means ± standard deviation (n = 3). The responses obtained from the experimental design set were subjected to multiple nonlinear regression analysis to obtain the coefficients of the second polynomial model. The quality of the fit of polynomial model was expressed by the coefficient of determination \(R^2\), and its statistical significance was checked using an F-test. The optimal extraction conditions were estimated through three-dimensional response surface analyses of the significant independent variables and dependent variable. Statistical analysis was performed by using the Statistical Analysis System (SAS, version 9.1, SAS Institute, Cary, NC). Data were analyzed by analysis of variance, and the mean values were considered significantly different when \(p < 0.05\).

Results and Discussions

Modeling of the extraction process from cumin seed

In order to optimize the extraction of total phenolic compounds from cumin seed, an orthogonal array design (OAD) was developed as represented in Table 1. Table 1 also presents the experimental values of percent extract yields and total phenolic compounds of cumin seed extracts at various experimental conditions. The values of coefficients are presented in Table 2. The results of analysis of variance, goodness of fit and the adequacy of the models are summarized in Table 3. The significance of regression coefficients was estimated by using Student’s t-test and P values as presented in Table 2.

The data showed a good fit with Eq. (1), which was statistically acceptable at \(p < 0.05\) and adequate with satisfactory \(R^2\) values.

Effect of process variables on total phenolic compounds

Solid–liquid extraction is a mass transport phenomenon in which solids contained in a matrix migrate into solvent brought into contact with the matrix. This mass transport phenomenon can be enhanced with changes in diffusion coefficients induced by extraction temperature (Ghafoor et al., 2011). Solvent concentration and extraction time can also play a significant role in the extraction of functional components from plant materials (Wang et al., 2008). Total phenolic contents from cumin seed obtained under different conditions (temperature, time and solvent concentration) are presented in Table 1. The maximum output of total phenolic compounds (25.17 mg GAE/g DW) was obtained in experimental run 16 which included extraction at 50°C for 4 h using 40% ethanol. Multiple regression analysis was performed on the experimental data, and the coefficients of the model were evaluated for significance. The effects of temperature (35-50°C) and time (1-4 h) of extraction on the total phenolic compounds were significant (\(p < 0.05\)); however, that of ethanol concentration (40-70%) was non-

Table 1. Orthogonal array experimental design and total phenolic compounds of cumin seed extracts

<table>
<thead>
<tr>
<th>Run</th>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Solvent (%)</th>
<th>Yield (%)</th>
<th>Total phenolics (mg GAE/g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>1</td>
<td>10</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>2</td>
<td>10</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>3</td>
<td>10</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>4</td>
<td>10</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>1</td>
<td>20</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>2</td>
<td>20</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>3</td>
<td>20</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>4</td>
<td>20</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>1</td>
<td>30</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>10</td>
<td>50</td>
<td>2</td>
<td>30</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>11</td>
<td>50</td>
<td>3</td>
<td>30</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
<tr>
<td>12</td>
<td>50</td>
<td>4</td>
<td>30</td>
<td>11.05 ± 0.3</td>
<td>23.84 ± 0.32</td>
</tr>
</tbody>
</table>

* Analytical results represented by means (n = 3) ± SD.

Table 2. Regression coefficients and analysis of the model for total phenolic compounds from cumin seed

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>Estimate</th>
<th>Standard error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>b0</td>
<td>-0.261182</td>
<td>0.093568</td>
<td>-2.80</td>
<td>0.005</td>
</tr>
<tr>
<td>b1</td>
<td>3.229068</td>
<td>0.142504</td>
<td>2.30</td>
<td>0.030</td>
</tr>
<tr>
<td>b2</td>
<td>0.027100</td>
<td>0.127466</td>
<td>0.26</td>
<td>0.795</td>
</tr>
<tr>
<td>b3</td>
<td>-0.007641</td>
<td>0.002178</td>
<td>-3.56</td>
<td>0.002</td>
</tr>
<tr>
<td>b4</td>
<td>-0.009808</td>
<td>0.002180</td>
<td>-4.60</td>
<td>0.000</td>
</tr>
<tr>
<td>b5</td>
<td>0.004691</td>
<td>0.002180</td>
<td>2.18</td>
<td>0.035</td>
</tr>
<tr>
<td>b6</td>
<td>0.000432</td>
<td>0.002180</td>
<td>0.20</td>
<td>0.838</td>
</tr>
</tbody>
</table>

DF = degree of freedom.

Table 3. Analysis of variance of the second order total phenolic compounds of the cumin seed model

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>Sum of squares</th>
<th>Mean square</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>8</td>
<td>43.158441</td>
<td>0.006726</td>
<td>49.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Linear</td>
<td>3</td>
<td>42.66840</td>
<td>0.007306</td>
<td>49.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Quadratic</td>
<td>3</td>
<td>0.140151</td>
<td>0.000048</td>
<td>0.065</td>
<td>0.774</td>
</tr>
<tr>
<td>Cross-product</td>
<td>3</td>
<td>0.059451</td>
<td>0.000198</td>
<td>0.190</td>
<td>0.360</td>
</tr>
<tr>
<td>Residual</td>
<td>6</td>
<td>0.586559</td>
<td>0.065599</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The antiradical activity (%) was calculated using the following formula:

\[
\text{Antiradical activity (\%) = 100 \times \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right)}
\]
significant. By increasing the contact time of the solvent with solids may improve the diffusion of the compounds and similarly temperature also plays main role in increasing the solubility of compounds in extraction solvent (Wang and Weller, 2006). The values of the coefficients as presented in Table 2 were used for the final predictive equation (Eq. 3), neglecting the non-significant quadratic and cross product terms as given below:

$$Y = 0.60350X_1 + 3.220568X_2$$

(3)

where $Y$ represent the response variable (total phenolic compounds) $X_1$ and $X_2$ are significant process variables, i.e. temperature and time for extraction, respectively. Based on this model the response surface plot showing the linear relation between temperature and time of extraction is presented in Figure 1. This shows a linear increase in total phenolic compounds by increasing temperature while keeping the time constant or vice versa. Based on our findings, the predicted extraction conditions were 49°C temperature, 2.8 h time and 53.6% ethanol for the maximum amount of total phenolic compounds (24.66 mg GAE/g DW) from cumin seed. The $R^2$ value of the model was 0.986, $R^2$ adjusted value was 0.981, F value was 49.05 and $p$ value was 0.0001, which represent that the model had adequately represented the real relation between the parameters chosen. In order to compare the predicted results with experimental values, experimental rechecking was performed using the optimum conditions, and the mean values of 24.66 mg GAE/g DW of total phenolic compounds were obtained from cumin seed. The comparison of experimental values with predicted values was done by using Student’s t-test, which showed non-significant differences. The good correlation between these results validated that the response model was adequate in reflecting the expected optimization.

Time and temperature of extraction are important parameter to be optimized even in order to minimize energy cost of the process. Many authors agree in the fact that an increase in the working temperature favors extraction by enhancing both the solubility of solute and the diffusion coefficient, but also that beyond a certain value phenolic compounds can be denatured (Yilmaz and Toledo, 2006). The significance of temperature of extraction for other plant materials has also been previously documented. In one of the study on grape marc (Spigno and De Faveri, 2007), it was observed that phenols yield was higher at 60°C than at 28°C. However, it should be considered that beyond certain temperatures, the extraction yield of polyphenolic compounds may decrease due to thermal degradation and/or polymerization which may also effect the analytical quantification (Pinelo et al., 2005). Considering these results, we performed our study for optimization of phenolic compounds from cumin seed at a temperature range of 35 to 50°C. The effect of temperature can not be generalized since it strongly depends on typology of compounds. For example, Cacace and Mazza (2003) suggested a maximum temperature of 30-35°C for extraction of anthocyanins from ribes with 85% ethanol; while Herodez et al. (2003) indicated 20°C, 0°C and 0°C for the highest yields of ethanolic extraction of carnosic acid, ursolic acid, and oleanolic acid, respectively, from Balm leaves. Hence, we can conclude that the temperature of extraction is a very significant factor for recovery of bioactive compounds from plant materials and should be carefully optimized considering the nature of compounds and the type of raw material. Extraction time (1-4 h) was observed to be other significant variable for the extraction of phenolic compounds from cumin seed which is in agreement with findings of other researchers (Spigno et al., 2007), who observed that the yield of phenolics from plant matrix such as grape marc increased significantly with increasing the time of extraction up to 5 h, however beyond that and upto 25 h, the effect of time was non-significant.

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). Adding a certain amount of water in ethanol might improve the extraction efficiency, besides the fact that it is safe alcohol comparing with other organic solvents. Ethanol–water mixtures were used as the extraction solvent in our study and the concentration was varied from 40%
to 70%, however the effect was observed to be non-significant in this range. Therefore we suggest a lower concentration of ethanol for extraction purposes. Also due to the fact that higher concentration of ethanol such as above 90% may not be feasible for the extraction of phenolic compounds due to the simultaneous extraction of lipid fractions, which reduces the yield of target phenolics (Wang et al., 2008).

Free radical scavenging activities of cumin seed extracts

The antiradical activities of the cumin seed extracts obtained in total phenolic compounds optimization trials were assessed using DPPH radical scavenging assay. It is quick, reliable and reproducible method to search in vitro general antiradical activities of plant extracts and pure compounds (Koleva et al., 2002). This method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine and the remaining DPPH. The DPPH radical scavenging activities of 16 cumin extracts, according to OAD as listed in Table 1, are shown in Figure 2. All the extracts showed good mean DPPH radical scavenging activities (34.25-39.25%) and the maximum activity (39.27%) was observed for the extract 15 obtained at 50°C, after 4 h of extraction in ethanol. Food materials rich in bioactive compounds with higher free radical scavenging abilities are protective against certain types of cancer and may also reduce the risk of cardiovascular and cerebrovascular disorders (Miraliakbari and Shahidi, 2008).

Antioxidant activities of cumin seed extracts

Extracts of cumin seed were analyzed by a spectrophotometric method for the quantitative determination of antioxidant capacity. The assay is based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH. The antioxidant activity of cumin seed extracts (Figure 3) ranged from 8.25 to 11.24 mg/mL, the highest being in the extract prepared at 50°C after 3 h of extraction with 50% ethanol. We observed from the results of DPPH radical scavenging and phosphomolybdenum complex assays, that these bioactivities of cumin seed extracts increased with increase of phenolic compounds. Such a correlation between total phenolic content and antioxidant capacity assays have been reported by others too (Shan et al., 2005). Our results also suggest that phenolic compounds are key contributors to the antioxidant capacity of the cumin seed extracts.

Numerous physiological and biochemical processes in human body may produce oxygen-centered free radicals and other reactive species of oxygen as byproducts. Higher production of such free radicals can cause oxidative damage to important biomolecules (e.g. lipids, proteins, DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans (Poulson et al., 1998). Phenolic compounds from fruits, vegetables, spices, and medicinal herbs might prevent cancer through antioxidant action and/or the modulation of several protein functions. Phenolics may inhibit carcinogenesis by affecting the molecular events in the initiation, promotion, and progression stages (Yang et al., 2001). Phenolics demonstrated agonism and/or antagonism of carcinogenesis-related receptors such as arylhydrocarbon receptor, epidermal growth factor, and estrogen receptor β. They modulated the secretion of protein kinases in tumor cell proliferation, and induced the expression of anticarcinogenic enzymes or inhibited induction of cancer-promoting enzymes (Cai et al., 2004). Due to these various highly important bioactivities, the optimum recovery of phenolic compounds from plant materials is crucial for getting maximum benefits for the improvement of human health.

Conclusions

Our study demonstrates that cumin seed is a good source of these bioactive compounds which...
also possess good antioxidant activities against free radicals. Response surface methodology was also used to predict optimum levels of process variables which were 49°C temperature, 2.8 h time and 53.6% ethanol for the recovery of 24.66 mg GAE/g total phenolics from cumin seed. We can use designed experiments and statistical procedures to maximize the recovery of important phenolic compounds from cumin seed using safe organic solvent such as ethanol and lower extraction temperatures to ensure the recovery of high quality extracts rich in antioxidant compounds and health promoting properties.

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