Optimization studies on ultrasonic assisted extraction of the capsaicinoids from sweet-to-superhot chilli samples using response surface methodology

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Abstract
This study was aimed to imply the response surface methodology (RSM) for an extraction of capsaicinoids in chilli using ultrasonic assisted extraction (UAE) method prior to analysis by HPLC. The UAE conditions were optimized with common parameters: type of extraction solvent (methanol, ethanol and acetonitrile), methanol and water ratio (50-100%), solvent volume to sample mass ratio (5-25 ml/0.5 g), and extraction temperature (30-60°C) and time (10-60 min). The optimum yield of capsaicinoids of 16.48 mg/g was obtained using 0.5 g sample with 20 ml of 80% (v/v) methanol in water at 50°C for 20 min. The analytical figures of merit were validated and applied to determine capsaicin and dihydrocapsaicin in real samples. For comparison, the experiment was statistically optimized for the solvent extraction using a central composite design (CCD) based on RSM, emphasizing the effects of solvent to sample ratio, and extraction temperature and time on their yields. A quadratic regression model that relates to the yield (response) with three basic factors was developed using the Design-Expert software. The contents of both capsaicinoids in sweet-to-superhot chilli peppers were widely found between 573.44 and 15,219.60 mg/g DW. Regression analysis revealed that maximum yield of the capsaicinoids (16.73 mg/g) could be reached under the optimum conditions of 24.83 ml of the solvent used per 0.5 g sample at 48.48°C for 20 min. The high value of the adjusted R-square of the regression (0.8762) demonstrates that the regression equation provides a good model to fit the data obtained.

Introduction
Chilli peppers are the fruits of plants from the genus Capsicum and belong to the family Solanaceae. There are several domesticated species of chili peppers, among them C. annuum, C. frutescens and C. chinense, which include many common varieties. Hot or spicy peppers are savory food additives that are widely utilized in many parts of the world, and are highly valued for their attributes of color, pungency and aroma. Capsaicinoids are the compounds responsible for the hot, spicy flavor imparted by many peppers. Two major components of capsaicinoids present in most varieties of the chilli peppers are capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillyl nonanamide), which comprise over 90% of the total present in the fruit (Barbero et al., 2008). However, all of the major and minor identified capsaicinoids in the Capsicum fruits are vanillyl amides of branched fatty acids, with 9–11 carbons (Topuz et al., 2004). The capsaicinoids have several properties and applications that make them important in the food and pharmaceutical industries. Among their pharmacological applications, there are many uses for the treatment of pain and inflammation in different diseases, such as rheumatoid arthritis, migraine and diabetic neuropathic (Garcia-Salas et al., 2010).

Numerous extraction techniques for capsaicinoids from hot chilli pepper have been used including an accelerated solvent extraction (Chanthai et al., 2012), supercritical fluids extraction, enzymatic extraction, maceration, magnetic stirring extraction, microwave assisted extraction (Barbero et al., 2006a), pressurized liquid extraction (Barbero et al., 2006b) and ultrasound assisted extraction (Pena-Alvarez et al., 2012). These extractions need longer extraction time and require large volume of solvent. In this regard, the green extraction method still be adopted and conducted by using ultrasound assisted extraction (UAE) because the application of UAE offers many advantages including the reduction of solvent, temperature and the extraction time, which
is very useful for the extraction of thermolabile and unstable compounds. The UAE technique is based on the use of the energy derived from ultrasounds (sound waves with frequencies higher than 20 kHz) to facilitate the extraction of analytes from the solid sample by the organic solvent, which is selected in conjunction with the nature of solutes to be extracted. This technique has been successfully employed to extract a variety of compounds from fruits, vegetables, and other biological tissues. Ultrasound is attributed to the cavitation phenomenon produced in the solvent by the passage of ultrasonic waves. There are some previous applications of UAE in the determination of capsaicinoids in hot chilli pepper. UAE was optimized with various extraction parameters, particularly the appropriate solvent, temperature, extraction time and quantity of sample (Barbero et al., 2008). This developed method was used to quantify the capsaicinoids present in several varieties of hot peppers cultivated in Spain. The developed and optimized method was applied for the determination of capsaicinoids in eleven varieties of Mexican hot peppers using UAE followed by GC-MS (Pena-Alvarez et al., 2012).

The capsaicinoids could be quantified by various techniques including TLC and column chromatography and spectrophotometry (Perucka et al., 2002). These traditional techniques are not a separation and identification method of an individual capsaicinoid. In recent years, the most reliable and accurate techniques for analysis of capsaicinoids are HPLC and GC because there are common types of the chromatography used in analytical chemistry for separating and analyzing compounds (Collins et al., 1995). RP-HPLC is currently the most popular and reliable techniques for the analysis of capsaicinoids. The improved method using HPLC was validated and determined the content of eight capsaicinoids in different parts of the pepper plants, fresh peppers, freeze-dried whole peppers, and dry and liquid pepper-containing foods sold commercially (Kozukue et al., 2005). The capsaicin contents in different fruit parts of different chilli pepper varieties using HPLC had been reported (Supalkova et al., 2007). The determination of capsaicin in Habanero pepper using HPLC was also reported (Davis et al., 2007).

The objective of this research was to investigate the optimum conditions for capsaicinoids extraction from sweet-to-superhot chilli samples using ultrasonication as a green extraction method prior to analysis by HPLC. In the present study, the response surface methodology based on a central composite design (CCD) was comparatively used to find mathematically those optimal conditions.

**Materials and Methods**

**Chemicals and instruments**

Capsaicin and dihydrocapsaicin standard were used without further purification as obtained from Sigma-Aldrich (USA). Acetonitrile, methanol and ethanol were of HPLC grade from Merck (Germany). The instruments and other equipments included rotary evaporator, R-200 (Buchi, Switzerland), high-performance liquid chromatograph with photodiode array detector/LC-20A (Shimadzu, Japan) and ultrasonic sonicator, RF103H (Bandelin Sonorex, Germany) were also used.

**Plant materials**

Seven varieties of chilli pepper (*Capsicum annuum* L.) samples of which their relative spicy taste and local types were subject to study including KKU-p31065 (sweet taste, S1), MS4 (sweet taste, S2), PC2-1 (hot taste, S3), YTP16-98-37-1-1 (hot taste, S4), Yodson Khem 80 (hot taste, S5), Akkanee Piroth (superhot taste, S6) and PB8-1-1 (superhot taste, S7). These samples were obtained from Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kean University. The sample was dried in an oven at 60°C for 48 h and ground using a kitchen grinder to pass a 100-mesh sieve. The ground samples were kept storing in desiccator before use.

**Determination of two major capsaicinoids by HPLC**

Standard solutions of capsaicin and dihydrocapsaicin were prepared by dissolving of 0.025 g in 25 mL methanol, transferred into vial and kept cool at 4°C prior to use. The calibration curves of both capsaicinoids were ranged of 10.0-100 µg/ml.

**Optimization of HPLC separation**

The optimum conditions for capsaicinoids analysis in the extract of chilli by RP-HPLC-PDA were preliminarily investigated by varying the composition of mobile phase and found to be the ratio of acetonitrile and DI water (70:30, v/v), whereas other conditions used throughout the study included column ODS-C18 RP (4.6 mm i.d.×250 mm), photodiode array detector at 280 nm, flow-rate of 1 ml/min at ambient temperature with 20 µl injection volume and about 20 min analysis time.

**Method validation**

To determine whether the proposed method provided suitable aspects for both quantitative and qualitative analyses of the target analytes, the
following validation data were deduced. A series of standard solutions were prepared covering a concentration range of 10.0-100 µg/ml by serial dilution of stock solutions containing 1,000 µg/ml of capsaicin and dihydrocapsaicin. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as the analyte concentration giving a signal to noise ratios (S/N) of 3 and 10, respectively. The precision of the proposed method was presented as the repeatability and reproducibility of retention time and peak area. The repeatability (intra-day precision) was deduced from ten replicates in one day (n = 10) and reproducibility (intra-day precision) was calculated from the experiments carried out in 3 consecutive days (n = 3×5) using both standard capsaicinoids solutions of 10.0 µg/ml. The accuracy of the proposed method was expressed in terms of recovery. The recovery was studied by spiking a known concentration of standard into sample before extraction and then analyzed in triplicate.

The optimization study of capsaicinoids extraction using UAE method

The optimum conditions for the extraction of capsaicinoids in chilli sample were studied including extraction solvent, ratio of solvent to water, ratio of sample to solvent, extraction temperature and extraction time. All experiments were performed in triplicate.

Three kinds of organic solvents including methanol, ethanol and acetonitrile were used. The test was performed with 0.5 g sample in 20 ml of the solvent used at 50°C for 20 min. For the effect of different ratio of methanol and water, various ratios of methanol in water expressed as percentage of methanol including 50-100% were investigated. The test was performed with 0.5 g sample in 20 ml methanol in water at 50°C for 20 min.

The effect of solvent volume to sample mass ratio was carried out using a series of that ratio with different solvent volumes (5, 10, 15, 20 and 25 ml/0.5 g). The test was performed with 0.5 g sample in methanol at 50°C for 20 min. In addition, the effects of extraction temperature and time were performed with 0.5 g sample in 20 ml 80% methanol for 20 min by varying both of temperature range of 30, 40, 50 and 60°C and the extraction time for 10, 20, 40 and 60 min.

The extraction procedure for the capsaicinoids in chilli samples was slightly modified (Barbero et al., 2008). Sample (0.5 g) was sonicated by UAE method with 20 ml solvent. The extract was centrifuged and then filtered through Whatman No. 42 filter paper. The extract solvent was evaporated to dryness using a rotary evaporator. The residue was dissolved with the solvent to make up final volume of 5.0 ml. The capsaicinoids extract was filtered through 0.45 µm filter membrane prior to analysis by HPLC.

The optimization study of capsaicinoids extraction using UAE based on CCD-RSM method

Experimental design

The response surface methodology based on a central composite design (CCD) was used to optimize the extraction conditions to obtain the highest concentration of capsaicinoids. The ratio of solvent volume to sample mass (A), extraction temperature (B) and extraction time (C) were chosen as the independent variables. The extraction experiments were designed using the Design-Expert 6.0.10 software package (Stat-Ease Inc., Minneapolis, MN, USA). The range and levels of the variables investigated are given in Table 1.

A three-level, three-factor factorial CCD consisting of 8 factorial points, 6 axial points and 3 replicates at the central point leading to 17 runs were employed for extraction (data not shown). For statistical calculation, the variables were coded according to the following Eq. (1):

$$X_i = (A_i - A_0) / \Delta A_i \quad (1)$$

where $X_i$ is the coded value of the independent variables.

$A_i$ is the actual values of the independent variable.

$A_0$ is the actual value of $A_i$ at the central point.

$\Delta A_i$ is meant the step change of the independent variables.

For predicting the optimal point, a second-order polynomial function was fitted to correlate the relationship between the independent variables and the response. The quadratic model for predicting the optimal point was expressed as Eq. (2):

$$Y_i = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (2)$$

where $Y_i$ is the predicted response (extraction yield, mg/g of capsaicinoids).

$\beta_0$ is the intercept coefficient.

$\beta_1, \beta_2, \beta_3$ are the linear coefficients.

$\beta_{11}, \beta_{22}, \beta_{33}$ are the quadratic coefficients.
$\beta_{12}, \beta_{13}, \beta_{23}$ are the cross-product coefficients. A, B, C are the independent variables studied.

**Model fitting and statistical analysis**

All extraction experiments were carried out in triplicate and the results expressed as mean values. The results obtained from CCD were used to determine the regression coefficients of the second-order multi regression model. The analysis of variance (ANOVA) was evaluated using Design-Expert 6.0.10. The quality of the fit of the polynomial model equation was assessed by determining the $R^2$ coefficient and the adjusted $R^2$ coefficient; its statistical and regression coefficient significance were checked with F-test and P-value, respectively. Three-dimensional (3D) surface plot and corresponding contour plots were drawn to illustrate the effect of the independent variables on the responses (total yield of capsaicinoids). The optimum values for the selected variables were obtained by solving the regression equation.

**Results and Discussion**

**Determination of two major capsaicinoids by HPLC**

The chromatographic conditions were optimized with mobile phase compositions of organic solvents. In this study, the mobile phase containing various percent of acetonitrile in deionized water was investigated. Using the optimal isocratic elution, capsaicin ($t_R$ 5.9 min) and dihydrocapsaicin ($t_R$ 7.2 min) were separated within 10 min using the mobile phase containing acetonitrile and deionized water (70:30, v/v). In the present study, the software was used to identify compounds from their absorbance spectra obtained from PDA.

The quantitative features of the method were studied under the optimum conditions. The linearity was obtained covering a concentration range of 10.0-100 µg/ml. The calibration curve was plotted between peak area of capsaicinoids standard and their concentration, giving $y = 12194x - 22386$ ($r^2 0.9987$) and $y = 9188x - 16793$ ($r^2 0.9985$) for capsaicin and dihydrocapsaicin, respectively. LOD and LOQ were found to be 0.125 and 0.25 µg/ml, respectively, for both capsaicinoids. The repeatability and reproducibility of retention time for the two capsaicinoids were in good precision which their relative standard deviations (RSD) were lower than 1% while the RSD of the peak area were lower than 2%.

**Optimization conditions of ultrasonic assisted extraction**

The optimum conditions for the extraction of capsaicinoids in the sample code S06 were studied. The effectiveness of the ultrasound-assisted extraction depends on the extraction solvent’s capacity and the capsaicinoids should be soluble in the solvent used (Barbero et al., 2008). The three organic solvents, except water, are methanol, ethanol and acetonitrile.

The extraction was performed with 0.5 g sample in 20 ml of each solvent at 50°C for 20 min (data not shown). The contents of the capsaicinoids were found rather different when using three kinds of the solvents extracted. It is noted that both methanol and ethanol extracts gave similar amounts of the capsaicinoids. Acetonitrile was a fairly efficacious solvent, but is less efficacious than methanol and ethanol. So, in this case, methanol was used as the extraction solvent.

The different ratios of methanol in deionized water are an important factor that must be optimized to increase the extraction yield of capsaicinoids. The test was performed with 0.5 g sample in 20 ml methanol at 50°C for 20 min (data not shown). As percentage of deionized water in methanol decreases, there is an increase in the quantity of capsaicinoids. It is due to the good solubility of capsaicinoids in methanol, which diminishes as the polarity of the solvent increases by the addition of more water. The contents of the capsaicinoids were different depending on the mixed ratios of methanol in deionized water. It was found that the polarity of the solvent mixture increased, resulted in an increase in the capsaicinoids contents when using it up to 80-100% MeOH/H$_2$O. Thus, 80% methanol could be used as the extraction.
The ratio of solvent volume (ml) to sample mass (g) is a factor that must be studied to increase the extraction yield of capsaicinoids. To evaluate the effect of solvent volume on the extraction, a series of that ratio was carried out with different solvent volumes (5, 10, 15, 20 and 25 ml/0.5 g). The test was performed with 0.5 g sample in 20 ml of 80% methanol at 50°C for 20 min (data not shown). The contents of capsaicinoids did not show much different with the differences in the sample/solvent ratios. In this test, the volume of the extraction solvent was varied with fixed 0.5 g sample. Therefore, the varying of solvent volume will not be a determining factor when extracting of capsaicinoids under this conditions. The solvent of 20 ml gave rather higher amount of the capsaicinoids. Thus, the solvent/sample ratio of 20 ml/0.5 g was selected.

In this study, the extraction temperature was also evaluated ranging from 30 to 60°C to increase the extraction yield of capsaicinoids. The test was performed with 0.5 g sample in 20 ml 80% methanol at 50°C for 20 min (data not shown). The contents of the capsaicinoids were different when varying the extraction temperature between 30 and 60°C. It is noted that the extraction temperature around 40-60°C gave similar amounts. It was found that temperature did not have any clearly defined effect on the extraction of capsaicinoids. Thus, the extraction was performed at 50°C.

The effect of extraction time on the extraction yield of capsaicinoids was performed between 10 and 20 min. The test was performed with 0.5 g sample in 20 ml 80% methanol at 50°C for 20 min (data not shown). The contents of the capsaicinoids were also different when varying the extraction time. It was found that the extraction times longer than 20 min gave no difference in the amounts of the capsaicinoids. Thus, the extraction was performed at 50°C for 20 min.

### Determination of capsaicinoids in real samples

The developed procedures were applied for the determination of capsaicin and dihydrocapsaicin in sweet-to-hot chilli peppers. These chilli samples are described with their codes and pungent levels including sweet chilli pepper (S1, S2), hot chilli pepper (S3, S4, S5) and very hot chilli pepper (S6, S7). The total yields of the capsaicinoids were widely found in the range of 573.44-15,219.60 µg/g DW (Table 2).

### RSM optimization of the capsaicinoids extraction conditions

The quantitative features of the proposed method were studied under the optimum conditions. The design matrix and the corresponding results of RSM experiments to determine the effect of the three independent variables including ratio of solvent volume to sample mass (A), extraction temperature (B) and extraction time (C) are shown in Table 3.

By employing multi-regression analysis on the experimental data, the predicted response Y for the total yield of capsaicinoids can be obtained using the following second-order polynomial equation:

\[
Y = 16.46 + 1.07A - 0.10B - 0.02C - 1.08A^2 - 1.11B^2 - 1.12C^2 + 0.032AB + 0.035AC - 0.023BC
\]

(3)

where \(Y_i\) is the predicted extraction yield (mg/g of capsaicinoids), A, B and C are the code values of solvent volume to sample mass, extraction temperature and extraction time, respectively.

ANOVA which is carried out to test the significance of the fit of the second-order polynomial equation for the extraction yield is presented in Table 4. The ANOVA is used to estimate the statistical significant of the factors and interactions between terms. A model F-value of 13.58 and a low probability value [(Prob > F) less than 0.05] implying significant model fit of the model was investigated. The quality
of the fit of the quadratic regression model equation is evaluated by the coefficient of determination ($R^2$). The value of $R^2$ was 0.9458, indicating that 94.58% of the variability in the response could be explained by the statistical model. The model is stronger and the predicted response is better as the $R^2$ value becomes closer to 1.0000. A regression model, with a $R^2$ value greater than 0.9000, is considered to have a very high correlation (Jaya et al., 2010).

The adjusted $R^2$ value corrected the $R^2$ value for the sample size and the number of terms. The value of the adjusted $R^2$ was 0.8762, which is also high. These results show that the regression model provides a well fit to the data. The coefficient of variation (CV) indicates the degree of precision with which the treatments were compared (Cao et al., 2009). A very low value of CV (4.24%) clearly indicates a high degree of precision and a good deal of reliability for the experimental data. The signal to noise ratio was measured by “Adequate Precision”. A ratio greater than 4 is desirable. The ratio of 10.784 obtained indicates an adequate signal, which implies this model can be used to navigate the design space. Furthermore, the results of the error analysis indicated that the Lack of Fit F-value of 1.52 implying the Lack of Fit is not significant [(Prob > F) more than 0.05], which indicated the well fit model. The model equation is adequate to predict the extraction yield within the range of experimental variables.

The significance of each coefficient was determined by P-value (Table 4). Value of “Prob > F” less than 0.05 indicates that the model terms are significant whereas value greater than 0.100 indicates that model terms are not significant (Diler and Ipek, 2012). In this case, linear terms (A) and quadratic terms ($A^2$, $B^2$, $C^2$) are significant model terms ($P < 0.05$), whereas linear terms ($B$, $C$) and two-way interaction terms ($AB$, $AC$, $BC$) are not significant model terms ($P > 0.05$). This suggests that the ratio of solvent volume to sample mass have a significant effect on the extraction yield. To determine the optimum levels of the variables for the total yield of capsaicinoids, the 3D response surface and contour plots were determined using Eq. (3) and then an overlay contour plot was created to select the optimum extraction conditions.

The RSM analysis is indeed a useful technique to simultaneously study and optimize the extraction of capsaicinoids. Normally, this methodology is important to check the adequacy of the second degree polynomial model to ensure that it provides maximum approximation on the relationship between independent variables and dependent variable (response). The residuals from the least squares are important tool for evaluating the model adequacy (Qi et al., 2009).

Normal probability was checked by plotting the normal probability plot of residual. The normal assumption is satisfactory as normal residuals fall along a straight line (data not shown). There is the plot of regression standardized residuals versus the predicted response values (data not shown). The residual plot of model is randomly distributed without any trends, indicating that good predictions

Table 3. Experimental design with three independent variables and the results obtained from total capsaicinoids (mg/g DW)

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Optimization for the extraction of capsaicinoids

The effects of variables and their interactions on the capsaicinoid yield are described by the 3D response surface plots and 2D contour plots. These plots were obtained by plotting the response (total yield of capsaicinoids) on the Z-axis against any two variables while keeping the other variable constant at its ‘0’ level. Figure 1a shows the response surface and contour lines for the ratio of solvent volume to sample mass and extraction temperature on the capsaicinoids yield.

The results revealed that total yield of capsaicinoids increases from 13.61-16.11 mg/g when ratio of solvent volume to sample mass and extraction temperature increase and decrease, respectively. Then, the value gradually decreases from 16.11-13.61 mg/g with decrease in the ratio of solvent volume to sample mass (from 20-10 ml/0.5 g) and increase extraction temperature (from 50-70°C). The increasing trends in their total yield at too high ratio of solvent volume to sample mass and decrease in the extraction temperature are due to the occurrence of the fact that more solvent can enter cells while more capsaicinoids compounds can permeate into solvent under the higher solvent to material ratio and solvent volatilization. Some degradation processes may occur at high temperature. Their maximum total yield was achieved at the ratio of solvent volume to sample mass of 20 ml/0.5 g at 50°C.

From the results (Figure 1b), it is revealed that the total yield of capsaicinoids increases from 13.61-16.11 mg/g when the ratio of solvent volume to sample mass and the extraction time are increase and decrease, respectively. The increasing trends in the total yield at too high ratio of solvent volume to sample mass and decrease in the extraction time are due to the occurrence of the longer the extraction time, the more extraction solvent will be volatile. Their maximum total yield was achieved at the ratio of solvent volume to sample mass of 20 ml/0.5 g for 20 min.

In addition, the effects of the interactions between the extraction temperature and extraction time on total yield of capsaicinoids were carried out as shown in Figure 1c. The 3D response and contour lines reveal that the total yield continuously increases as the extraction temperature and extraction time decrease. The highest total yield was achieved using the extraction temperature and the extraction time at 50°C for 20 min. The optimum region obtained by overlaying plots of the response evaluated (total yield of capsaicinoids) as a function of ratio of solvent volume to sample mass, extraction temperature and extraction time.

To optimize the extraction of capsaicinoids, an overlay contour plot was created using Design-Expert software. The optimum extraction conditions

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<td>4.050×10³</td>
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<td>0.9164</td>
</tr>
<tr>
<td>Residual</td>
<td>2.40</td>
<td>7</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>1.90</td>
<td>5</td>
<td>0.38</td>
<td>1.52</td>
<td>0.4451</td>
</tr>
<tr>
<td>Pure Error</td>
<td>0.50</td>
<td>2</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Core Total</td>
<td>44.22</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C.V. = 4.24; R² = 0.9458; Adjusted R² = 0.8762; Adequate Precision = 10.784
to achieve high total yield of capsaicinoids were defined with the following criterion: total yield > 15 mg/g. The non-shaded area in the overlay plot is the region that meets the proposed criterion. Based on the overlay plot, the optimum extraction parameters were found to be at the ratio of solvent volume to sample mass of 24.83 ml/0.5 g with the extraction temperature at 48.48°C and 20 min extraction time. The predicted maximum yield of total capsaicinoids was recorded to be 16.73 mg/g.

Validation of the extraction model

The validity of the results predicted by the RSM model was confirmed by carrying out the repeated experiments under optimum extraction conditions including the ratio of solvent to sample mass (24.83 ml/0.5 g), extraction temperature (48.48°C) and 20 min extraction time. The results obtained from ten replications demonstrated that the maximum total yield (16.48 mg/g) was closed to that of the predicted values. This result indicates that there is excellent correlation between experimental and predicted data with the error of 1.49% and in turn proves the validity of the extraction model.

Conclusion

The HPLC separation of two major capsaicinoids using PDA detector and their separation conditions were described and validated. The suitable conditions were obtained when the extraction was done in 80% (v/v) methanol at 50°C for 20 min using the solvent/sample mass ratio of 20 ml/0.5 g DW. The optimum conditions for the highest extraction yield of capsaicinoids were comparatively determined based on the three level three factorial CCD of RSM method. A quadratic regression model equation for the extraction yield was developed, with $R^2$ and adjusted $R^2$ determined to check the quality of fit of the equation. Both high value of $R^2$ greater than 0.9000 and the adjusted $R^2$ greater than 0.8000 have revealed that the model provides a good fit to the data. The optimum conditions that results in the maximum yield of capsaicinoids were obtained as follows: the ratio of solvent volume to sample mass of 24.83 ml/0.5 g with the extraction temperature at 48.48°C and 20 min extraction time. Under these optimum conditions, the predicted value from the model of the capsaicinoids gave excellent correlation with experimental value and in turn proved the validity of the extraction model because of the small deviation error between them. Therefore, the model could be successfully used to identify the optimum conditions and to predict maximum yield of capsaicinoids.

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