Optimization of Ultrasound-Assisted Extraction of Natural Pigments from Annatto Seeds (Bixa Orellana) Using Response Surface Methodology

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Abstract

Dyes derived from natural source using “green” extraction technology have emerged recent years due to the public concern about safety of synthetic dyes. In the present study, ultrasound technology was applied to extract bixin from the annatto seeds, using absolute ethanol as solvent. Response surface methodology, using Box-Behnken design was employed to optimize and investigate the effect of three independent variables: seed/solvent ratio (1/15 - 1/25 g/mL), sonication time (30 - 50 min) and extraction temperature (50 - 70°C) on the bixin yield. The results showed that, the experimental data were fitted to a second order polynomial equation using multiple regression analysis with high coefficient of determination value (R^2 = 0.9781). The model F-value of 34.76 with low p-value (p < 0.0001) indicated that the model was highly significant and could be employed to optimize the extraction process. Optimal extraction conditions for the bixin yield were determined as follows: seed/solvent ratio of 1/15 g/mL, sonication time of 50 min and extraction temperature of 54.6°C. Under these conditions, the highest bixin yield was predicted to be 0.912%, which was in good agreement with the experimental value of 0.899 ± 0.021%.

Keywords: Annatto; Bixin; Optimization; Response Surface Methodology; Ultrasound-Assisted Extraction

Abbreviations

BBD: Box-Behnken Design; RSM: Response Surface Methodology; ANOVA: Analysis Of Variance; C.V.: Coefficient Of Variance

Introduction

Nowadays, the use of color additives to make food products more attractive is very popular, and the public concern about safety of synthetic colorants also increased. Therefore, applications of natural pigments get more attention in recent years. The annatto (Bixa orellana) is a shrub native to tropical America, where it has been a traditional part of food culture for centuries. Annatto extract refers to a series of pigments extracted from the outer coating of the annatto seeds. In which, cis-bixin occupies more than 80% of the total pigments. The remaining constituents include trans-bixin, cis-norbixin and trans-norbixin [1,2]. Annatto pigments, whose colors vary between yellow and red, ranks the second among the most economically important natural colors in the world and have huge importance in pharmaceutical, cosmetic and food industries [2,3]. In food industries, annatto pigments are used in many kinds of products such as cheeses, sausages, meats, fishes, snack foods, candies, soups, etc. [4-6].

Ultrasound is a special type of sound wave beyond human hearing. It can pass through a medium by creating expansion and compression. The process produces a phenomenon called cavitation, which is the production, growth and collapse of bubbles. The implosion of cavitation bubbles can hit the surface of the solid matrix, break the cells and release the compounds [7-9]. In comparison with other extraction techniques, the ultrasonic-assisted extraction is less expensive and easier in practice. The advantages of ultrasound-assisted extraction include reduction of extraction time, energy and use of solvent [10,11]. Ultrasound-assisted extraction has been employed for several studies of plant materials, antioxidants and natural dyes (e.g. Yolmeh et al. [2], Santos et al. [12] and Sivakumar et al. [13]).

Response surface methodology (RSM) is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experiment data that describe the behavior of a data set with the objective of making statistical previsions. It is useful for analyzing the effects of several independent variables on the response. The main objective of RSM is to simultaneously optimize the levels of these variables, determine the optimum operational conditions to obtain the desirable response [14]. One of the advantages of RSM is the reduction in number of experimental trials required to evaluate multiple parameters and their interactions [15].

Box-Behnken designs (BBD) are a class of rotatable or nearly rotatable second-order designs based on three level incomplete factorial designs. BBD suggest how to select points from the three level factorial arrangements, which allows the efficient estimation of the first and second order coefficients of the mathematical model. In comparison with other response surface designs such as central composite and three-level full factorial design, the BBD are slightly more efficient [14,16].

Annatto seeds are grown in many regions of Vietnam. The extract efficiency of traditional method is very poor and material consuming. Ultrasonic extraction is a “green” extraction technology that has been employed in many studies with good outcomes. However, the studies of ultrasound-assisted extraction of bixin from annatto seeds and the optimization of bixin yield using RSM are still very limited. Therefore, it is essential to carry out this research. The specific objectives of this research including: to examine the effects of three independent extraction parameters (ratio of solvent to raw material, sonication time and extraction temperature) on the bixin yield; to apply a three factor, three level Box-Behnken design combining with RSM for maximizing the bixin yield and to verify the validity of the RSM model.

Materials and Methods

Materials

All the chemicals used were of analytical grade. Annatto seeds were obtained from local market (Ho Chi Minh city, Vietnam). The seeds were sieved to remove soil and broken seeds, using a mesh with 2.5 mm diameter of opening. After sieving, the seeds were washed with distilled water to remove any adhering soil and dust, followed by drying at 60°C for 48h. The seeds were stored in dark and kept in dry environment prior to the experiments.

Ultrasound-assisted extraction (UAE)

The UAE was performed in an ultrasonic cleaning bath (DH.WUCA10H, witeg Labortechnik GmbH, Germany) at a frequency of 40 kHz. Absolute ethanol was chosen to be the solvent for the extraction process. Ten grams of annatto seeds were extracted with absolute ethanol in a 250-mL Erlenmeyer flask submerged in an ultrasonic bath. After the extraction, annatto seeds were removed and the extract was filtered by filter paper to remove any insoluble materials. Finally, the solvent was removed by magnetic stirrer and vacuum pump, and then dried at 60°C for 24h to produce the solvent free extract.

Determination of bixin content in the extract

The bixin content (C%) in the extract was determined by using spectrophotometer method according to Joint FAO/WHO Expert Committee on Food Additives Monographs [17]. The absorbance was measured in a 1 cm cuvette at 487 nm, using an UV-VIS spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA). The percentage of bixin in solution was calculated according to the Lambert-Beer law, using $E_{1%1cm}^{10} = 3090$, in the following equation:

$$C% = \frac{A \times V_i \times ... \times V_n}{W \times E_{1%1cm}^{10} \times V_i \times ... \times V_n}$$

In which:
A: average absorbance of the sample at 487 nm
Optimization of Ultrasound-Assisted Extraction of Natural Pigments from Annatto Seeds (Bixa Orellana) Using Response Surface Methodology

\[ V_i: \text{dilution volume (i = 1, 2 \ldots n)} \]
\[ W: \text{sample mass, in gram} \]
\[ E_{1cm}^{th}: \text{absorptivity coefficient (equal to 3090 for bixin)} \]
\[ V_i': \text{volume of aliquot for dilution (i = 1, 2 \ldots n)} \]

**Determination of bixin yield**

The yield of bixin (Q%) was calculated using the following equation:

\[
Q\% = \frac{\text{mass of bixin obtained from the extract (g)}}{\text{mass of seeds (g)}} \times 100
\]

**Screening of variables values**

Screening test was used to examine the effect of each factor on the extraction and to determine the range of the extraction variables including seed/solvent ratio, sonication time and extraction temperature. The values of 3 variables were screened by varying single variable at a time and keeping others variables constant. The optimal value of each parameter was chosen and applied for the rest of the treatments. The experiments were performed in duplicate and the average values of bixin yield were taken as response.

**Box-Behnken experimental design**

In this study, the extraction process was optimized using RSM. A three level, three variable BBD was used to evaluate the main and interaction effects of the factors: seed/solvent ratio, sonication time and extraction temperature on the bixin yield.

After studying the effects of three independent factors including seed/solvent ratio, sonication time and extraction temperature on the bixin yield, three values in the proper range of each factor were chosen and used in the subsequent Box-Behnken experiments. Three factors chosen for this study was designated as \(X_1\) (Seed/solvent ratio), \(X_2\) (Sonication time) and \(X_3\) (Sonication temperature), and presented into three levels, coded -1, 0 and +1 for low, middle and high value, successively.

The number of experiments required to investigate the above three parameters using BBD was 17. In which, 12 were factorial experiments and 5 were zero point tests performed to estimate the pure errors. Each experiment was performed in duplicate and the average bixin yield was taken as the response.

In a system involving three significant independent variables \(X_1, X_2\) and \(X_3\), the mathematical relationship of the response on these variables can be approximated by the quadratic (second order) polynomial equation:

\[
Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2
\]

In which, \(Y\) is the estimated response; \(\beta_0\) is the constant, \(\beta_1, \beta_2\) and \(\beta_3\) are linear coefficients; \(X_1, X_2\) and \(X_3\) are independent variables; \(\beta_{12}, \beta_{13}\) and \(\beta_{23}\) are interaction coefficients between the three factors; \(\beta_{11}, \beta_{22}\) and \(\beta_{33}\) are quadratic coefficients.

**Statistical Analysis**

Statistical comparisons between means of bixin yield at different levels of single factor were conducted using one-way ANOVA. Duncan’s multiple-range test was performed if differences were identified between the groups. The data were expressed as means ± S.D setting \(P < 0.05\) as criterion of statistical significance. These statistical analyses were performed using the SPSS program (Version 20.0, IBM Corp., USA).

Multiple regression analysis and analysis of variance (ANOVA) were conducted for fitting the mathematical model using the statistical package Design-Expert software (Version 9.0.6.2, Stat-Ease Inc., USA). The modeling was started with a quadratic model including

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linear, squared, and interaction terms. Significant terms in the model for each response were found by analysis of variance (ANOVA) and significance is judged by the F-statistic calculated from the data. The experimental data was evaluated with various descriptive statistical analyses to reflect the statistical significance of the developed quadratic mathematical model. After fitting the data to the models, the generated data were used for plotting response surfaces.

The optimization of the extraction process was done by using the Design-Expert software. Under the optimal conditions obtained by desirability function methodology, triplicate confirmatory experiments were performed. The average value of the experiments was compared with the value predicted from the model to determine the validity of the developed response model.

Results and Discussion

Effects of process variables on bixin yield

Effects of seed/solvent ratio

Solvent extraction is a crucial step in the extraction of bixin. In this study, absolute ethanol was employed as extraction solvent. The effect of different ratios of seed/solvent on the bixin yield was examined at 1:5, 1:10, 1:15, 1:20, 1:25 and 1:30 at 50°C for 10 min in the process of the ultrasound-assisted extraction.

As shown in Figure 1, the bixin yield increased from 0.099% to 0.593% with the ratio of seed/solvent varied from 1/1 to 1/20 (g/mL). This result is reasonable and consistent with the mass transfer principle. The driving force that drives the mass transfer is the concentration gradient between the solid and solvent (i.e., the concentration difference between the interior plant cell and the exterior solvent), which is greater when a higher ratio of solvent to seed increase [18,19]. However, the bixin yield changed very little when the ratio of solvent to seed increased from 20/1 to 30/1 (mL/g). Therefore, three levels of seed/solvent ratio selected for BBD were 1/15, 1/20 and 1/25 (g/mL).

![Figure 1: Effect of seed/solvent ratio on bixin yield](image)

(Data is expressed as mean ± S.D. Data points with different superscripts are significantly different (one-way ANOVA) at the level of P < 0.05 by Duncan’s multiple comparison test).

Effects of sonication time

To examine the influence of sonication time on the bixin yield, series of experiments were carried out for 10, 20, 30, 40, 50, 60, 70 min at 50°C and the ratio of seed to solvent was 1/20 (g/mL). The three levels of the sonication time variable were determined according to the results presented in Figure 2.

An increase in bixin yield from 0.595% to 0.900% was observed when the sonication time varied from 10 to 50 min. Due to the effects of ultrasound on the extraction process mentioned in the study of Joana Gil-Chavez et al. [9] and Maran et al. [18], the cell wall of the seeds
could be disrupted that facilitated the release of bixin and others compounds from the seed’s outer coating to the exterior solvent. Beyond 50 min, there was a little difference observed in the bixin yield. Therefore, 30, 40 and 50 min were chosen for the coded levels at -1, 0 and +1, respectively.

**Figure 2: Effect of sonication time on bixin yield**
(Data is expressed as mean ± S.D. Data points with different superscripts are significantly different (one-way ANOVA) at the level of P < 0.05 by Duncan’s multiple comparison test).

**Effects of temperature**

In this study, the influence of extraction temperature on the bixin yield under the sonication was investigated at 30, 40, 50, 60, 70 and 80°C. The seed/solvent ratio and sonication temperature were fixed at 1/20 (g/mL) and 50 min, respectively. The results of the experiments are presented in Figure 3. The bixin yield increased from 0.765% to 0.911% with the increasing of temperature from 30 to 60°C. This phenomenon could be due to the improvement of mass transfer rate at higher temperature. When the temperature was increased from 30 to 60°C, the viscosity and density of the extracts decreased; the solubility of pigments and diffusion coefficient increased which facilitated the penetration of solvent deeper into the cell membrane and permitted a higher extraction rate. Similar observations were reported by several authors (e.g. Maran et al. [18], Maran et al. [20] and Elksibi et al. [21]). However, when the temperature continued to increase over 70°C, the bixin yield reduced significantly. It was due to the degradation of bixin at elevated temperature (>70°C) as reported in the study of Giridhar et al. [6] and Smith [22]. Therefore, 50, 60 and 70°C were three levels of temperature selected for BBD.

**Figure 3: Effect of extraction temperature on bixin yield**
(Data is expressed as mean ± S.D. Data points with different superscripts are significantly different (one-way ANOVA) at the level of P < 0.05 by Duncan’s multiple comparison test).
Experiments of BBD

In order to study the combined effects of independent variables on the extraction of bixin, a total number of 17 runs of experiment, including 5 center points were performed according to the BBD. Three experimental variables (seed/solvent ratio, sonication time and extraction temperature) with their levels chosen from the previous screening test are presented in Table 1. All the experiments were performed in duplicate and the average bixin yield was taken as the response, as given in Table 2.

Table 1: Symbols and coded levels of three variables chosen for BBD

<table>
<thead>
<tr>
<th>Variables</th>
<th>Symbol</th>
<th>Coded levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed/solvent ratio (g/mL)</td>
<td>X₁</td>
<td>1/15 1/20 1/25</td>
</tr>
<tr>
<td>Sonication time (min)</td>
<td>X₂</td>
<td>30 40 50</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>X₃</td>
<td>50 60 70</td>
</tr>
</tbody>
</table>

Table 2: BBD matrix for three variables and the observed response

<table>
<thead>
<tr>
<th>No.</th>
<th>Independent variables</th>
<th>Response (Q%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X₁</td>
<td>X₂</td>
</tr>
<tr>
<td>1</td>
<td>1/25</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>1/15</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>1/15</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>1/15</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>1/25</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>1/20</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>1/20</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
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<td>30</td>
</tr>
<tr>
<td>9</td>
<td>1/20</td>
<td>30</td>
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<tr>
<td>10</td>
<td>1/25</td>
<td>40</td>
</tr>
<tr>
<td>11</td>
<td>1/20</td>
<td>40</td>
</tr>
<tr>
<td>12</td>
<td>1/20</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
<td>1/20</td>
<td>40</td>
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<tr>
<td>14</td>
<td>1/20</td>
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<tr>
<td>15</td>
<td>1/25</td>
<td>50</td>
</tr>
<tr>
<td>16</td>
<td>1/20</td>
<td>40</td>
</tr>
<tr>
<td>17</td>
<td>1/15</td>
<td>30</td>
</tr>
</tbody>
</table>

*Average value of duplicate experiments

Regression analysis

Based on the Box-Behnken experimental design model, an empirical relationship between the input variables and obtained experimental results were expressed by a second order polynomial equation with interaction terms. The model obtained in terms of coded factors is given below:

Where $Q\%$ is the predicted bixin yield, $X_2$ and $X_3$ are the coded terms for two independent test variables, sonication time and extraction temperature, respectively.

The statistical significance and fitness of the model was evaluated by the analysis of variance (ANOVA) as presented in Table 3. The model F-value of 34.76 with low p-value ($p < 0.0001$) indicated that the model was highly significant. The lack of fit F-value of 2.15 and the associated p-value of 0.2370 was insignificant due to relative pure error which indicated the model equation was adequate for predicting the bixin yield. The goodness of fit of model was evaluated by the determination coefficient ($R^2$), adjusted determination coefficient ($R^2_{Adj}$), predicted determination coefficient ($R^2_{Pred}$) and coefficient of variance (C.V.); the results are given in Table 3. The $R^2 = 0.9781$ indicated that only 2.19% of the total variations was not explained by the model. The value of $R_{2 Adj}$ was also very high (0.9500), confirmed that the model was highly significant. The higher the $R^2_{Adj}$, the better the degree of correlation between the values observed from the experiments and the values predicted from the model. Predicted determination coefficient ($R^2_{Pred}$) is a measure of how good the model predicts a response value. The difference between $R^2_{Adj}$ and the $R^2_{Pred}$ should be less than 0.20; otherwise there may be a problem with either the data or the model. In this case, the $R^2_{Pred}$ was 0.7709 which was in reasonable agreement with the $R^2_{Adj}$ of 0.9500. Furthermore, a high degree of precision and a good deal of reliability in conducted experiments were indicated by the low coefficient variance (C.V.% = 1.68).

\[
Q\% = 0.840 + 0.045X_2 - 0.048X_3 - 0.022X_2X_3 + 0.018X^2_2 - 0.070X^2_3
\]

The p-values were used as a tool to check the significance of each factor and the interaction effects between factors on the bixin yield; the results are given in Table 4. With very small p-values ($p < 0.05$), it could be concluded that two linear coefficients ($X_2, X_3$), two quadratic coefficients ($X^2_2, X^2_3$) and one interactive coefficient ($X_2X_3$) significantly affected the bixin yield. Others coefficients were not significant ($p > 0.05$).

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>DF</th>
<th>Mean square</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0.059</td>
<td>9</td>
<td>$6.535 \times 10^{-3}$</td>
<td>34.76</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>$1.316 \times 10^{-3}$</td>
<td>7</td>
<td>$1.880 \times 10^{-4}$</td>
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<td></td>
</tr>
<tr>
<td>Lack of fit</td>
<td>$8.118 \times 10^{-4}$</td>
<td>3</td>
<td>$2.706 \times 10^{-4}$</td>
<td>2.15</td>
<td>0.2370</td>
</tr>
<tr>
<td>Pure error</td>
<td>$5.040 \times 10^{-4}$</td>
<td>4</td>
<td>$1.260 \times 10^{-4}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cor total</td>
<td>0.060</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9781</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2_{Adj}$</td>
<td>0.9500</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2_{Pred}$</td>
<td>0.7709</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.V.%</td>
<td>1.68</td>
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</tr>
</tbody>
</table>

The p-values were used as a tool to check the significance of each factor and the interaction effects between factors on the bixin yield; the results are given in Table 4. With very small p-values ($p < 0.05$), it could be concluded that two linear coefficients ($X_2, X_3$), two quadratic coefficients ($X^2_2, X^2_3$) and one interactive coefficient ($X_2X_3$) significantly affected the bixin yield. Others coefficients were not significant ($p > 0.05$).

The 3D response surface is the graphical representations of regression equation that show the relative effects of any two variables when the remaining variable is kept constantly at zero level. The relationship between seed/solvent ratio, sonication time, temperature and bixin yield is illustrated in Figure 4.

![3D response surface plots](image)

**Figure 4:** Response surface plots for the effect of (a) solvent/seed ratio and sonication time; (b) solvent/seed ratio and temperature; (c) sonication time and temperature on the bixin yield

**Table 4:** Test of significance for regression coefficient

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>DF</th>
<th>Mean square</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$X_1$</td>
<td>$4.651 \times 10^{-4}$</td>
<td>1</td>
<td>$4.651 \times 10^{-4}$</td>
<td>2.47</td>
<td>0.1597</td>
</tr>
<tr>
<td>$X_2$</td>
<td>0.016</td>
<td>1</td>
<td>0.016</td>
<td>85.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$X_3$</td>
<td>0.018</td>
<td>1</td>
<td>0.018</td>
<td>98.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>$X_1X_2$</td>
<td>$3.600 \times 10^{-5}$</td>
<td>1</td>
<td>$3.600 \times 10^{-5}$</td>
<td>0.19</td>
<td>0.6748</td>
</tr>
<tr>
<td>$X_1X_3$</td>
<td>$2.250 \times 10^{-6}$</td>
<td>1</td>
<td>$2.250 \times 10^{-6}$</td>
<td>0.01</td>
<td>0.9159</td>
</tr>
<tr>
<td>$X_2X_3$</td>
<td>$1.980 \times 10^{-3}$</td>
<td>1</td>
<td>$1.980 \times 10^{-3}$</td>
<td>10.54</td>
<td>0.0141</td>
</tr>
<tr>
<td>$X_1^2$</td>
<td>$9.474 \times 10^{-6}$</td>
<td>1</td>
<td>$9.474 \times 10^{-6}$</td>
<td>0.05</td>
<td>0.8288</td>
</tr>
<tr>
<td>$X_2^2$</td>
<td>$1.441 \times 10^{-3}$</td>
<td>1</td>
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<td>7.67</td>
<td>0.0277</td>
</tr>
<tr>
<td>$X_3^2$</td>
<td>0.021</td>
<td>1</td>
<td>0.021</td>
<td>110.55</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

The 3D response surface is the graphical representations of regression equation that show the relative effects of any two variables when the remaining variable is kept constantly at zero level. The relationship between seed/solvent ratio, sonication time, temperature and bixin yield is illustrated in Figure 4.

Figure 4(a) shows the effect of solvent/seed ratio and sonication time on the bixin yield at fixed temperature of 60°C. The bixin yield increased with the increasing of sonication time and solvent/seed ratio due to effect of ultrasound and enhanced mass transfer rate, respectively. However, the increase of bixin yield due to solvent/seed ratio was not significant whereas the effect of sonication time on bixin yield was significant. Furthermore, the sonication time curve did not level off at 50 min, which may indicate that a slightly higher sonication time is required to achieve the maximum and maintainable bixin yield. The interaction between solvent/seed ratio and temperature at the fixed sonication time of 40 min is shown in Figure 4(b). The effect of solvent/seed ratio on the bixin yield was not significant which was mentioned previously. The increase of bixin yield was recorded with the increasing of temperature from 50 to 60°C, which was in agreement with the mass transfer principle. However, a decline of bixin yield was observed with further increase of extraction temperature due to the degradation of bixin at elevated temperature. Figure 4(c) shows the effect of temperature and sonication time on the bixin yield at fixed seed/solvent ratio of 1/20 (g/mL). It was obvious that two variables of the sonication time, temperature and their interactions had positive impact on the bixin yield.

Optimization

Desirability function methodology was employed to optimize the extraction process. In the numerical optimization, desired goal for each factor and response was chosen. The possible goals are: maximize, minimize, target and in range. Level of sonication time within range of 30 - 40 min, extraction temperature within range of 50 - 70°C and minimum solvent/seed ratio due to insignificant effect were set for maximum bixin yield. By seeking from 113 starting points in the response surface changes, the highest bixin yield was found to be 0.912% at seed/solvent ratio of 1/15 (g/mL), sonication of 50 min and extraction temperature of 54.6°C with an overall desirability of 0.972.

In order to validate the adequacy of the model equation, verification experiments were carried out under optimal conditions. However, considering the operability in actual production, the optimal conditions are modified as follow: ratio of seed/solvent of 1/15 (g/mL), sonication time of 50 min and extraction temperature of 55°C. Triplicate confirmatory experiments were performed and the average value is presented in Table 5.

Table 5: Predicted and experimental values of the bixin yield under optimal conditions

<table>
<thead>
<tr>
<th>Ratio (g/mL)</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Bixin yield (Q%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>1/15</td>
<td>50</td>
<td>54.6</td>
</tr>
<tr>
<td>Modified</td>
<td>1/15</td>
<td>50</td>
<td>55</td>
</tr>
</tbody>
</table>

*Predicted value using response surface quadratic model

The mean value of 0.899 ± 0.021% (n = 3), obtained from the confirmatory experiments, demonstrated the validity of the RSM model, as there was no significant difference between 0.912% and 0.899 ± 0.021% (n = 3; p > 0.05). The strong correlation between the practical and predicted results demonstrated the validation of the developed quadratic model.

In comparison with the study of Taham et al. [3], in which the extraction of bixin from annatto seeds was investigated using combined technologies; the bixin yield obtained from this study, using ultrasound technology was higher. These results have demonstrated the efficiency of ultrasound technology on the extraction of bixin from annatto seeds.

Conclusion

In the present study, ultrasound-assisted extraction (UAE) was employed to extract bixin from the annatto seeds. Response surface methodology (RSM) and Box-Behnken design (BBD) were used to estimate and optimize three experimental variables: seed/solvent ratio (g/mL), sonication time (min) and extraction temperature (°C). The results of the ANOVA test showed that two independent variables and quadratic of sonication time and extraction temperature had significant effect on the bixin yield, followed by the significant interaction effect between sonication time and extraction temperature. A high correlation of the quadratic polynomial mathematical model was gained and could be employed to optimize bixin extraction from annatto seeds by ultrasonic technology. The optimal extraction conditions for the bixin yield were determined as follows: seed/solvent ratio of 1/15 (g/mL), sonication time of 50 min and extraction temperature of 54.6°C. Under these conditions, the experimental bixin yield was 0.899 ± 0.021%, which was in agreement with the predicted value, 0.912%.

Bibliography

4. Van Chuyen H., et al. "Improvement of bixin extraction yield and extraction quality from annatto seed by modification and combination of different extraction methods". International Journal of Food Science and Technology 47.7 (2012): 1333-1338.


