Enzymolysis-Assisted Treatments for Improved Phytochemical Contents and Bioactivities Levels from Unfertilized Corn Silk Extracts

Kim HS1, Kim SL2, Kang HJ, Kim WK3 and Kim MH**
1Department of Food Engineering, Dankook University, Chungnam, Republic of Korea
2Crop Foundation Division, National Institute of Crop Science, Jeonbuk, Republic of Korea
3Department of Food Science and Nutrition, Dankook University, Chungnam, Republic of Korea

Abstract

Corn silk is an excellent source of many bioactive compounds, which is well known functional food and traditional herbal medicine. The aim of this study was to evaluate the effects of Novozym 33095 treatment to improve the phytochemical contents and bioactivities in unfertilized corn silk ethanol extracts. Furthermore, the treatment conditions were simultaneously optimized using a response surface methodology with a central composite design. The influence of Novozym 33095 concentration, reaction temperature, and reaction time on total polyphenol contents, total flavonoid contents, maysin contents, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities, and tyrosinase inhibition was analyzed. The following optimal conditions were determined by simultaneous optimization of several responses with the Derringer’s desirability function using the numerical optimization function of the Design-Expert program: Novozym 33095 concentration 0.11 ml/L, reaction temperature 20 °C, and reaction time 120 min. Under these conditions, the predicted values of total polyphenol contents, total flavonoid contents, maysin contents, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, and tyrosinase inhibition were 5462.26 µg GAE/g dried sample, 3932.03 µg QUE/g dried sample, 3213.64 mg/100 g dried sample, 87.57%, and 75.78%, respectively, and the overall desirability (D) was 0.73. Those values of unfertilized corn silk ethanol extracts were 2921.32 µg GAE/100 g dried sample, 1703.69 µg QUE/g dried sample, 3213.64 mg/100 g dried sample, 62.34%, and 48.21%, respectively, demonstrating significant improvement from Novozym 33095 treatment.

Keywords: Unfertilized corn silk; Enzymolysis; Optimization; Phytochemical contents; Bioactivities

Introduction

Corn silk (Zea mays L.) is a traditional herb that contains many bioactive compounds such as carbohydrates, proteins, vitamins, calcium, potassium, sodium salts, steroids, alkaloids, flavonoids, and other phenolic compounds [1]. Corn silk has been used for the treatment of several diseases with multiple pharmacological activities reported, such as antioxidant [2], antidiabetic [3], antitumor [4], immune enhancement [5], antifatigue [6], antiobesity [7], and neuroprotective effects [8]. Heat reflux, organic solvent and ultrasound assistance are common methods for extracting polysaccharides from plant tissues; however, these processes generally require long extraction times, high extraction temperatures, and/or result in substantial environmental pollution. Enzyme-assisted extraction is an emerging technology in the food industry owing to its advantages such as a high extraction yield, shorter extraction times, lower extraction temperatures, lower investment costs, environmentally friendly technology, and simple manipulation compared with conventional extraction methods [9]. Carbohydrate-hydrolyzing enzymes such as cellulase, hemicellulase, and pectinase disrupt the cell wall through hydrolysis of its components, leading to increase permeability that facilitates the release of metabolites from the plant [10]. Enzyme-assisted extraction uses enzyme preparations either alone or as mixtures, which catalyze the hydrolysis of the cytoderm, and can enhance the release of bioactive substances by disrupting plant cell walls [11].

In the present study, unfertilized corn silk extracts (UCSE) were treated with Novozym 33095 (NOV) to improve the phytochemical concentrations and bioactivities. A response surface methodology (RSM) with a central composite design (CCD) was employed to determine the optimal NOV treatment conditions. The three independent variables considered were NOV concentration, reaction temperature, and reaction time, and were optimized according to the responses of the following dependent variables: total polyphenol contents, total flavonoid contents, maysin contents, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities, and tyrosinase inhibitions.

Materials and Methods

Materials

Unfertilized corn silk powder (UCS) with a mean diameter of 28.28 ± 0.96 micrometre was obtained from Rural Development Administration (Jeonju, Korea) in 2016, and stored at -70°C until use.

Sample preparation

According to a previous study on the optimal conditions for the ethanol extraction of UCS, UCS was extracted using 80.45% ethanol at 53.49°C for 4.95 hr, and then freeze-dried. This ethanol-extracted unfertilized corn silk powder (UCSE) was used for the enzyme (Novozym 33095, Bagsvaerd, Denmark) treatment.

NOV treatment

NOV exhibits broad-spectrum pectolytic enzyme activities, and is to break down the cell wall of fruits with application in fruit processing.

*Corresponding author: Kim MH, Department of Food Engineering, Dankook University, Chungnam 31116, Republic of Korea, Tel: +82415503563; E-mail: kmh1@dankook.ac.kr

Received August 04, 2017; Accepted August 24, 2017; Published August 31, 2017


Copyright: © 2017 Kim HS, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
reaching an activity level of 10,000 PECTU/ml. NOV is produced by Aspergillus aculeatus and Aspergillus niger fermentation. The tested extraction conditions were selected based on the manufacturer’s recommendations as follows: concentration of 0.25%-0.75%, reaction temperature of 20°C to 60°C, and reaction time of 40 min-120 min.

**Experimental design**

The optimization experiment was carried out using an RSM with a CCD experimental design for NOV treatment to UCSE. The three independent variables were NOV concentration (X1), reaction temperature (X2), and reaction time (X3). In addition, the low, middle, and high levels of each variable were designed as coded terms –1, 0, and +1, respectively (Table 1). The experimental data were fitted to second-order polynomial models to express the responses as a function of the independent variables according to the following equation:

\[ Y = b_0 + \sum_{i=1}^{3} b_{i}X_i + \sum_{i=1}^{3} b_{ij}X_i^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} b_{ij}X_iX_j \]  

(1)

Where Y is the measured response variable, b0 is a constant, b1, b2, and b3 are the linear, quadratic, and interaction coefficients, respectively, and X1, X2, and X3 are the levels of the independent variables.

**Total phenol contents**

The total phenolic content of the sample was analyzed by the Folin-Ciocalteu colorimetric method with slight modification, using gallic acid as a standard [12]. An aliquot (0.5 ml) of the sample was transferred to test tubes with 5 ml distilled water. After the addition of Folin-Ciocalteu reagent (5 ml) and 10% aqueous Na2CO3 solution (2 ml), the tubes were vortexed. After 60 min, the absorbance was recorded at 750 nm. The total phenolic content was determined using the standard gallic acid calibration curve and expressed as microgram gallic acid equivalents per gram dry mass of sample (µg GAE/g dried sample).

**Total flavonoid contents**

The 0.5 ml sample was placed in a volumetric flask. Ethanol (1.5 ml) was added followed by 10% aluminium nitrate (0.1 ml), 1 M potassium acetate (0.1 ml), and distilled water (2.8 ml). The solution was mixed and the absorbance was measured at 415 nm. Calculations were based on a standard curve obtained with quercetin. The total flavonoid content was expressed as micrograms of quercetin equivalents per gram of dry mass sample (µg QUE/g dried sample).

**Maysin contents**

Maysin contents were analyzed by high-performance liquid chromatography; the operation conditions are described in Table 2. Standard maysin was obtained from Rural Development Administration, which was confirmed by electrospray ionization-mass spectrometry using Micromass Electrospray Interface ZMD 4000 (Micromass, Manchester, UK). The maysin contents of the samples were measured by comparison of the retention time of standard maysin, and calculated based on the peak area of standard maysin [14].

**Antioxidant activity assay**

A 0.2 ml sample was added to 1 ml of a 0.2 mm DPPH methanol solution, mixed, and allowed to stand for 30 min in the dark. Distilled water was used as a blank control. The percent inhibition activity of absorbance at 517 nm was calculated and plotted as a function of the concentration of the standard for samples to determine the ascorbic acid equivalent antioxidant concentration [15]. The percentage of DPPH radical scavenging activity of the sample was calculated according to the following equation:

\[ \text{DPPH radical scavenging activity} \% = (1 - A/B) \times 100 \]  

(2)

Where, A is the absorbance of the sample and B is the absorbance of the control.

**Tyrosinase inhibition**

The tyrosinase inhibition of the samples was analyzed using the method reported by Kwon et al. [16]. A 0.2 ml sample was added to 2.3 ml of a 0.2 M potassium phosphate buffer (pH 6.5) and 0.4 ml of a 2 mm tyrosinase solution. The solution was mixed and 0.1 ml mushroom tyrosinase (220 unit/ml) was added. After 20 min of reaction in a 37°C water bath, the absorbance was recorded at 470 nm (A). The absorbance of 0.1 ml of distilled water instead of the enzyme solution (B) and that of 0.2 ml of distilled water instead of the sample (C) were also measured. Tyrosinase inhibition was then calculated according to the following equation:

\[ \text{Tyrosinase inhibition} \% = (1 - (A - B)/C) \times 100 \]  

(3)

**Statistical analysis**

Each experiment was performed in triplicate, and statistical analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA) software.

**Results and Discussion**

**Fitting the RSM**

In a previous study, we attempted NOV-assisted ethanol extraction, but the results were not satisfactory, showing an improvement of less than 5% for the dependent variables. Therefore, in the present study, we attempted NOV-assisted treatments as a second extraction step after ethanol extraction from UCS which contains over 20 times more maysin than commercial corn silk (unpublished data).

According to the CCD, 15 experiments were performed in triplicate and the obtained results are presented in Table 3. The experimental results of investigated responses (total polyphenol contents, total flavonoid contents, maysin contents, DPPH radical scavenging activity, and tyrosinase inhibition) obtained under different NOV concentrations, reaction temperatures, and reaction times were fitted to a second-order polynomial model (Eq. 1), and multiple regression
coefficients were generated for all responses using a least-squares approach in Table 4. Their statistical significance based on coefficients of determination and probability values for each investigated response are summarized in Table 5. For each term in the model, a large linear coefficient was statistically significant (P<0.05) for all investigated responses, it was possible to conclude that the applied mathematical model provides a proper representation of the experimental results. All the linear coefficients were statistically significant (P<0.05) for all responses. Among the quadratic coefficients, total polyphenol and DPPH radical scavenging activity were not statistically significant (P>0.05). Interactive coefficients of total flavonoid contents and DPPH radical scavenging activity were also not statistically significant (P>0.05).

Effects of NOV treatments on total polyphenol contents

Corn silk is rich in phenolic compounds that are known to significantly benefit human heath, such as anthocyanins, p-coumaric acid, vanillic acid, proto-catechuic acid, derivatives of hesperidin, and quercetin, and bound hydroxycinnamic acid forms composed of 4-deoxy-6-O-methyl-alpha-d-galact-4-enuronosyl groups at their non-reducing ends. NOV reacts as a pectin lyase catalyzing the eliminative cleavage of (1,4)-alpha-d-galacturonan methyl ester, giving oligosaccharides with 4-deoxy-6-O-methyl-alpha-d-galact-4-enuronosyl groups at their non-reducing ends.

Use of surface response plots of the model would allow for visualizing the effect of the independent variables on the dependent variables by adjusting two factors simultaneously and keeping the third variable constant at the -1, 0, and 1 level. The contour plot for the total polyphenol contents following NOV treatment to UCSE as a function of NOV concentration, reaction temperature, and reaction time is shown in Figure 1. The total polyphenol content of UCSE (control) was 2921.32 µg GAE/g dried sample, which increased to 5258.49-5575.89 µg GAE/g dried sample, representing a 180%-191% improvement after NOV treatment. In addition, increasing the reaction time of the NOV treatment decreased the total polyphenol contents of UCSE (Figures 1D-1I). This suggested that the minimum reaction time (60 min) was sufficient for the NOV treatment, as a prolonged reaction time increases the chance of the decomposition of phenolics [19].

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>X1</th>
<th>X2</th>
<th>X3</th>
<th>Total polyphenol contents (µg GAE/g dried sample)</th>
<th>Total flavonoid contents (µg QUE/g dried sample)</th>
<th>Maysin contents (mg/100 g dried sample)</th>
<th>DPPH radical scavenging activity (%)</th>
<th>Tyrosinase inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td>5258.49</td>
<td>3992.64</td>
<td>3017.69</td>
<td>87.63</td>
<td>80.06</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>1</td>
<td>-1</td>
<td>5558.64</td>
<td>3933.49</td>
<td>2985.59</td>
<td>87.20</td>
<td>78.50</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5482.74</td>
<td>3958.14</td>
<td>3120.79</td>
<td>87.26</td>
<td>72.27</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
<td>5499.99</td>
<td>3956.90</td>
<td>3192.48</td>
<td>87.66</td>
<td>76.01</td>
</tr>
<tr>
<td>5</td>
<td>-1</td>
<td>0</td>
<td>1</td>
<td>5488.94</td>
<td>3887.71</td>
<td>3039.31</td>
<td>87.39</td>
<td>78.50</td>
</tr>
<tr>
<td>6</td>
<td>-1</td>
<td>-1</td>
<td>0</td>
<td>5444.79</td>
<td>3869.42</td>
<td>3037</td>
<td>87.34</td>
<td>76.32</td>
</tr>
<tr>
<td>7</td>
<td>-1</td>
<td>0</td>
<td>-1</td>
<td>5430.99</td>
<td>3907.62</td>
<td>3089.78</td>
<td>87.37</td>
<td>72.59</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5479.29</td>
<td>3944.58</td>
<td>3128.89</td>
<td>87.63</td>
<td>71.34</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>5575.89</td>
<td>3910.08</td>
<td>3201.82</td>
<td>87.29</td>
<td>68.22</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5499.99</td>
<td>3947.05</td>
<td>3098.35</td>
<td>87.61</td>
<td>72.90</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>0</td>
<td>-1</td>
<td>5410.29</td>
<td>3783.17</td>
<td>3158.87</td>
<td>87.42</td>
<td>69.16</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>5299.89</td>
<td>3929.80</td>
<td>3164.28</td>
<td>87.63</td>
<td>70.09</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>-1</td>
<td>0</td>
<td>5317.14</td>
<td>3823.83</td>
<td>3158.71</td>
<td>87.90</td>
<td>62.93</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>5361.99</td>
<td>4102.30</td>
<td>3213.69</td>
<td>87.39</td>
<td>71.96</td>
</tr>
<tr>
<td>15</td>
<td>-1</td>
<td>1</td>
<td>0</td>
<td>5420.64</td>
<td>4077.65</td>
<td>2968.85</td>
<td>87.20</td>
<td>70.09</td>
</tr>
</tbody>
</table>

Table 3: Central composite design of Novozym 33095 treatments and its dependent variables.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total polyphenol contents</th>
<th>Total flavonoid contents</th>
<th>Maysin contents</th>
<th>DPPH radical scavenging activity</th>
<th>Tyrosinase inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5117.615</td>
<td>4066.958</td>
<td>3731.677</td>
<td>87.478</td>
<td>93.729</td>
</tr>
<tr>
<td>X1</td>
<td>470.063</td>
<td>1475.519</td>
<td>2916.288</td>
<td>3.525</td>
<td>48.325</td>
</tr>
<tr>
<td>X2</td>
<td>-1.459</td>
<td>-6.782</td>
<td>1.092</td>
<td>-0.004</td>
<td>0.013</td>
</tr>
<tr>
<td>X3</td>
<td>9.444</td>
<td>-4.076</td>
<td>-18.161</td>
<td>-0.003</td>
<td>-0.509</td>
</tr>
<tr>
<td>X1 X1</td>
<td>-3248.75</td>
<td>-6196.688</td>
<td>-5685.875</td>
<td>-4.375</td>
<td>-341.458</td>
</tr>
<tr>
<td>X2 X2</td>
<td>35.363</td>
<td>-15.248</td>
<td>-36.093</td>
<td>-0.061</td>
<td>1.441</td>
</tr>
<tr>
<td>X3 X3</td>
<td>-0.058</td>
<td>0.016</td>
<td>0.06</td>
<td>0</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Table 4: Estimated regression coefficients of five responses.
contrast, elevated temperature increased the total polyphenol contents (Figures 1A-1C, 1H and 1I) and thus reduced the reaction time to reach the maximum polyphenolic content recovery [20]. The most significant factor affecting the total polyphenol content was the NOV concentration, as shown in Table 6. The second and third important factors were reaction temperature and reaction time, respectively.

**Effects of NOV treatment on total flavonoid contents**

The flavonoid compounds of corn silk are the major constituents for scavenging DPPH radicals owing to the presence of hydroxyl groups in their structure and their electron donating ability [19]. The flavonoid compounds of corn silk are mainly composed of luteolin, formononetin, and apigenin [21]. The contour plot for the total flavonoid content of NOV-treated UCSE as functions of NOV concentration, reaction temperature, and reaction time is shown in Figure 2. The total flavonoid content of the control UCSE was 1703.69 µg QUE/g dried sample, which increased to 3783.17 µg to 4102.30 µg QUE/g dried sample, representing a 222% to 241% improvement compared to the control after NOV treatments. Reaction time did not have a significant effect on the total flavonoid contents (P>0.05). With an increase in the reaction temperature, the total flavonoid contents increased (Figures 2A-2C and 2G-2I), and the optimal NOV concentration was in the range of approximately 0.10-0.15 ml/L (Figures 2A-2F). The most significant factor influencing the total flavonoid content was NOV concentration, as shown in Table 6, followed by reaction temperature and reaction time. The effect of reaction time on total flavonoid contents did not show significant differences (P>0.05).

**Effects of NOV treatment on maysin contents**

The predominant phenolic compounds in corn silk extracts have been identified as maysin, apimaysin, 3′-methoxymaysin, iso-orientin, and luteolin derivatives. Among these compounds, maysin [rhamnosy-6-C-(4-ketofusyl)-5,7,3′,4′tetrahydroxyflavone], a flavonoid glycoside containing a rhamnose residue, is the most abundant flavonoid [22].

<table>
<thead>
<tr>
<th>Response</th>
<th>Regression</th>
<th>R²</th>
<th>P&gt;F</th>
<th>Total regress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenol contents</td>
<td>R²</td>
<td>0.4446</td>
<td>&lt;.0001</td>
<td>0.7602</td>
</tr>
<tr>
<td>Total flavonoid contents</td>
<td>R²</td>
<td>0.5103</td>
<td>&lt;.0001</td>
<td>0.7576</td>
</tr>
<tr>
<td>DPPH activity radical scavenging</td>
<td>R²</td>
<td>0.5526</td>
<td>&lt;.0001</td>
<td>0.6768</td>
</tr>
<tr>
<td>Tyrosinase inhibition</td>
<td>R²</td>
<td>0.321</td>
<td>&lt;.0001</td>
<td>0.8824</td>
</tr>
</tbody>
</table>

Table 5: Determination coefficients and probabilities of five responses.
Maysin from corn silk has shown anticancer [23], neuroprotective [24], immunomodulating [23], and antiobesity activities [7]. The contour plot for maysin contents of NOV-treated UCSE as functions of NOV concentration, reaction temperature, and reaction time is shown in Figure 3. The maysin content of the UCSE control was 801.40 mg/100 g dried sample, which increased to 2801.82-3213.69 mg/100 g dried sample, representing a 350% to 401% improvement compared to the control after NOV treatments. With increased reaction temperature, the maysin contents decreased (Figures 3A-3C and 3I). The most significant factor influencing the maysin content of UCSE was the NOV concentration, as shown in Table 6, followed by reaction temperature and reaction time. Reaction time did not have a significant effect on maysin contents (P>0.05).

Effects of NOV treatment on DPPH radical scavenging activity

DPPH radical scavenging activity, based on the reduction of DPPH solution in the presence of a proton-donating substance, has been extensively employed to evaluate the radical scavenging ability of samples [25]. The DPPH radical scavenging activity of corn silk was shown to be related to the contents of total polyphenol compounds (r=0.9415) and total flavonoids (r=0.9546) [18]. The contour plot for the DPPH radical scavenging activity of NOV-treated UCSE as functions of NOV concentration, reaction temperature, and reaction time is shown in Figure 4. The DPPH radical scavenging activity of the control was 62.34%, which increased to 87.20% to 87.90% after NOV treatments. DPPH radical scavenging activity increased with decreased reaction temperature and increased NOV concentrations (Figures 4A-4C). The most significant factor affecting DPPH radical scavenging activity was NOV concentration, as shown in Table 6, followed by reaction temperature and reaction time. Reaction time did not have a significant effect on DPPH radical scavenging activity (P>0.05).

Effects of NOV treatment on tyrosinase inhibition

There has been increased interest in finding natural tyrosinase inhibitors from herbs and applying them as skin care products, which have become potential sources of skin whiteners. Antioxidants are good...
Figure 3: Contour plot for maysin contents (mg/100 g dried sample) of Novozym 33095 treatments. (Reaction time, A: 60 min, B: 90 min, C: 120 min; Reaction temperature, D: 20°C, E: 40°C, F: 60°C; Novozym 33095 concentration, G: 0.05 ml/L; H: 0.15 ml/L; I: 0.25 ml/L).

Figure 4: Contour plot for DPPH radical scavenging activity (%) of Novozym 33095 treatments. (Reaction time, A: 60 min, B: 90 min, C: 120 min; Reaction temperature, D: 20°C, E: 40°C, F: 60°C; Novozym 33095 concentration, G: 0.05 ml/L; H: 0.15 ml/L; I: 0.25 ml/L).
Figure 5: Contour plot for tyrosinase inhibition (%) of Novozym 33095 treatments. (Reaction time, A: 60 min, B: 90 min, C: 120 min; Reaction temperature, D: 20°C, E: 40°C, F: 60°C; Novozym 33095 concentration, G: 0.05 ml/L; H: 0.15 ml/L; I: 0.25 ml/L).

Figure 6: Response optimization for multi-response surfaces of Novozym 33095 treatments.
inhibitors of tyrosinase activity and melanin production, and certain antioxidants have been applied as melanogenesis inhibitory agents [26]. The contour plot for the tyrosinase inhibition of NOV-treated UCSE as functions of NOV concentration, reaction temperature, and reaction time is shown in Figure 5. The tyrosinase inhibition of the control UCSE was 48.21%, which increased to 62.93% to 80.06% after NOV treatments. As the reaction temperature decreased, tyrosinase inhibition increased (Figures 5A-5C). The most significant factor for tyrosinase inhibition was NOV concentration, as shown in Table 6, followed by reaction temperature and reaction time.

Multiple responses optimization

It is relatively simple to find the optimal conditions for a single response using RSM; however, in this study we sought to optimize five responses. Consequently, a Derringer’s desirability function was utilized to optimize several responses simultaneously. The procedure involved constructing individual desirability (d) and then obtained an overall desirability function, D. The function D is then used to maximize the ability of choosing the best conditions for the designated variables. The overall desirability function D is defined as the weighted geometric average of the individual desirability (di), according to following equation [27].

$$D = (d_1 \times d_2 \times d_3 \times \ldots \times d_n)^{1/m}$$

Where, d1, d2, d3, ..., dm correspond to the individual desirability functions for each response.

Therefore, the optimal NOV treatment conditions for improving the phytochemical levels and bioactivities in UCSE were simultaneously optimized with the Derringer’s desirability function using the numerical optimization function of the Design-Expert program. Optimized conditions for multi-response surfaces of NOV treatment from UCSE are shown in Figure 6. The optimal conditions given by the model were as follows: NOV concentration 0.11 mL/L, reaction temperature 20°C, and reaction time 120 min. Under these conditions, the model predicted the values of total polyphenol, total flavonoids, maysin, DPPH radical scavenging activity, and tyrosinase inhibition were 5462.26 μg GAE/g dried sample, 3932.03 μg GAE/g dried sample, 3213.64 mg/100 g dried sample, 87.57%, and 75.78%, respectively, and the overall desirability (D) was 0.73.

Conclusion

Using an optimized Novozym 33095 treatment to improve the phytochemical contents and bioactivities in unfertilized corn silk ethanol extracts, we successfully obtained high total polyphenol contents, total flavonoid contents, maysin contents, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities, and tyrosinase inhibition. For all response variables, the most significant factor among the independent variables was Novozym 33095 concentration, followed by reaction temperature and reaction time in this experimental design. We assumed that Novozym 33095 treatment was disrupted the cell wall through hydrolysis of its components, leading to increase permeability that facilitates the release of metabolites from the unfertilized corn silk.

Acknowledgment

This research was supported by the Cooperative Research Program for Agriculture Science & Technology Development (PJ011305022017) of the Rural Development Administration, Republic of Korea.

References


