Optimization of extraction of pigment from chestnut shell using response surface methodology

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Abstract. Chestnut shell was selected as the raw material for extracting brown pigment, and the response surface methodology was utilized for optimizing the extraction conditions. In this paper, the effects of ethanol concentration, extraction temperature and time on the color value were studied and analysed by response surface experiments. The results showed that the quadratic regression model had ideal fitting effect to the experimental results. When the pH value of extraction system was 13, the optimum conditions for color value were consisted of an ethanol concentration of 28wt%, a reaction temperature of 83°C, and a reaction time of 2.3h. The extraction temperature had the greatest influence on the color value of the pigment. The interaction between ethanol concentration and extraction temperature had significant effects on the color value. And the interaction between ethanol concentration and extraction time had no significant effects. The color value of chestnut shell pigment can reach 31.25 ± 0.50 with the response surface optimization.

1.Introduction

Chestnut belongs to Fagaceae, and it is mainly distributed in Vietnam and China [1]. Chestnut foods of various flavors have been developed, such as canned chestnuts, chestnut roasted with sugar and chestnut sauce. Natural brown pigment can be extracted from chestnut shell which is the waste in chestnut production [2]. The characteristics of the pigment are steady, and it has antioxidant activity and bacteriostatic effect [3-7]. Therefore, the pigment can be used as a food additive with a triple effect of colorants, antioxidants and preservatives [8-10]. In short, the pigment has a promising development prospect [11-12].

The response surface methodology is one of the most commonly methods in process parameter analysis in recent years. It can analyze the regression relationship between dependents and multiple independent variables to establish a relationship model between one or more measured indicators and key factors. The main effects of the factors and their interactions are quantitatively analyzed and evaluated, and the required measurement values can be calculated within the levels of the influencing factors of the design. Because it uses a more reasonable experimental design, it can effectively and quickly determine the optimal conditions of multi-factor systems in an economical way [13-15].

In this paper, the response surface methodology was utilized for optimizing the extraction conditions of chestnut shell pigment to obtain the pigments with a high color value.

2.Materials and methods

2.1 Samples and chemicals

The chestnut shells used in this research were the waste provided by chestnut production factory in Beijing. Through the shells washing, drying, crushing and finally sifting with a 40-mesh sieve, the chestnut shell powder was made.

Sodium hydroxide, absolute ethyl alcohol and citric acid was analytical grade.

2.2 Extraction of chestnut shell pigment

Pre-experiments found that the factors affecting the extraction of chestnut shell pigment mainly included the pH value of the extraction system, ethanol concentration, extraction temperature and time, while the solid-liquid ratio had little effect on the color values of the pigment. Furthermore, with the increase of the pH value, the color value of the pigment increased significantly. However, when the pH value was higher than 13, a large amount of cellulose and lignin were dissolved as impurities because of the reduction of the color value [16].

On this basis, 5.0g chestnut shell powder and 62.5mL alkaline solutions with different ethanol concentrations were added in 250 mL Erlenmeyer flasks separately, and reflux extraction was used with a certain time at different temperatures. After vacuum filtration, the pH value of extracting solution was adjusted to neutral with citric acid. Then the solutions were concentrated to a viscous state by a rotary evaporator, and finally dried in a vacuum to obtain the solid pigments. The color value of the pigments were measured as an indicator.

2.3 Response surface optimization experiment

Table 1. Experimental factors and levels in analysis of response surface methodology.

Level	Factor		
	Ethanol concentration(A)/%	Temperature (B)/°C	Time(C)/h
-1	20	70	2.0
0	30	80	2.5
1	40	90	3.0

Based on the results of the single-factor experiment, the response surface methodology was used to further optimize the extraction conditions of chestnut shell pigment. According to the experimental design principle of Box-Behnken in Design-Expert software, the factors including ethanol concentration, extraction temperature and time were selected for experimental design. The levels and codes in the experimental design are shown in Table 1.

2.4 Determination of color value

The determination method of cocoa shell pigment in national standard (GB1886.30-2015) was referred. 0.100g pigment powder was dissolved with water and diluted to 100mL. After the shaking, 20mL solutions were moved in a new volumetric flask and diluted to 100mL again (0.02% diluent was made). Distilled water was used as a reference solution, and the absorbance was measured with a spectrophotometer at a wavelength of 400 nm. The color value was estimated by the following formula:

$$Y=50\times Abs \qquad (1)$$

Y in the equation is the color value of the 1% concentration of the chestnut shell pigment, and Abs is the absorbance of the actual sample.

3.Results and Discussions

3.1 Establishment of regression model and analysis of variance

Based on the experimental design principle of Box-Behnken in the Design-Expert 8.0.6 software and the results of the single-factor experiment, the extraction conditions of chestnut shell pigments were optimized by the response surface methodology with 3 factors and 3 levels. The response values were the color values of the pigments. The experimental factors and levels were shown in table 1, and the experimental design and results of response surface were shown in table 2.

The data in table 2 were analyzed by Design-Expert 8.0.6 software and the regression model was obtained. The multiple regression equation was $Y = -205.463 + 2.61917A + 4.99205B - 6.1425C - 0.02185AB - 0.0305AC + 0.269BC - 0.013167A^2 - 0.030092B^2 - 3.327C^2$. (2)

Table 2. Box-Behnken experimental design and response values.

Group	Ethanol concentration/%	Temperature/ ℃	Time/h	Color value
1	30	80	2.5	30.75
2	40	70	2.5	26.48
3	30	80	2.5	30.34
4	20	70	2.5	22.11
5	20	80	3	27.53
6	30	80	2.5	30.90
7	30	80	2.5	31.77
8	40	80	3	27.41
9	30	90	3	29.18
10	20	90	2.5	31.15
11	30	80	2.5	31.02
12	40	90	2.5	26.78
13	20	80	2	29.90
14	30	70	2	27.74
15	30	70	3	23.39
16	30	90	2	28.15
17	40	80	2	30.40

Table 3. ANOVA for response surface quadratic models.

Sources of variance	Summation	df	Mean square	F	ρ
Model	118.26	9	13.14	32.06	< 0.0001
A	0.019	1	0.019	0.05	0.8356
B	30.19	1	30.19	73.66	< 0.0001
C	9.44	1	9.44	23.04	0.0020
AB	19.10	1	19.10	46.60	0.0002
AC	0.093	1	0.093	0.23	0.6483
BC	7.24	1	7.24	17.66	0.0040
A^2	7.30	1	7.30	17.82	0.0039
B^2	38.13	1	38.13	93.05	< 0.0001
C^2	2.91	1	2.91	7.11	0.0322
Residual	2.87	7	0.41	-	-

Lack of fit	1.78	3	0.59	2.17	0.2342
Net Errors	1.09	4	0.27	-	-
SST	121.12	16	-	-	-

According to the variance analysis results in table 3, the ρ value of the regression model was less than 0.0001, at the same time the lack of fit (regression fitting failure) was equal to 0.2342, indicating that the regression model was fit well. Therefore, this regression model can be used to analyze and predict the optimal extraction conditions of chestnut shell pigment. The significances of first- degree terms affecting color value of chestnut shell pigments were A - not significant, B and C- significant. According to influence degree, the order was: temperature (B)> time (C)> ethanol concentration (A). The quadratic terms in the regression model, namely A^2, C^2 and B^2, were all significant. The interaction terms AB and BC were significant, while AC was not significant. It indicated that the interaction effects of ethanol concentration and temperature, and temperature and time on color value were significant, while the interaction effect of ethanol concentration and time was not significant.

3.2 Analysis of response surface

In this study, 3-dimensional response surface map and 2-dimensional contour map were plotted by Design-Expert software. Figure 1 showed the response surface and isogram of the effects of ethanol concentration, extraction temperature and time on color value of the chestnut shell pigment.

When the extraction time was 2.5h, the interaction effects of ethanol concentration and extraction temperature on the color value of chestnut shell pigments were shown in figure 1-a and d. From the diagram, when the ethanol concentration was in the range of 20 ~ 40% and extraction temperature was in the range of 70 ~ 90 ℃, the influence of temperature on color value played a leading role. With the extraction temperature increasing, the color value of the chestnut shell pigment increased firstly and then decreased. When the temperature was 80 ℃, the interaction influences of ethanol concentration and extraction time on the color value of the pigments were shown in figure 1-b and e. The response surface changed little in both axes of the independent variables, and the isogram was close to circular, indicating that the interaction between ethanol concentration and extraction time had no significant influence on the color value. When the ethanol concentration was 30%, the effects of extraction temperature and time on the color values were shown in figure 1-e and f. The contour curve was elliptic, indicating that the interaction between temperature and time had significant effects on the color value. When the extraction temperature was in the range of 70 ~ 90 ℃ and time was in the range of 2.0~3.0 h, the influence of temperature on color value still played a leading role. The color value of the chestnut shell pigment increased firstly and then decreased with the extraction temperature increasing.

Partial derivative method was used to determine the best point. The optimized extraction conditions were obtained with the color value as the index: ethanol concentration was 27.72%, temperature was 83.22°C and time was 2.31 h. Considering the convenience of actual production, the optimal extraction conditions were determined as follows: ethanol concentration was 28%, temperature was 83°C and time was 2.3 h. On the conditions, the theoretical color value can reach 31.47. The actual color value was measured by verification experiments. The color value of chestnut shell pigment was 31.25 ± 0.50 with the response surface optimization, which was more than double the color value (11.96 ± 0.58) of the pigment extracted by ammonium hydroxide [13].

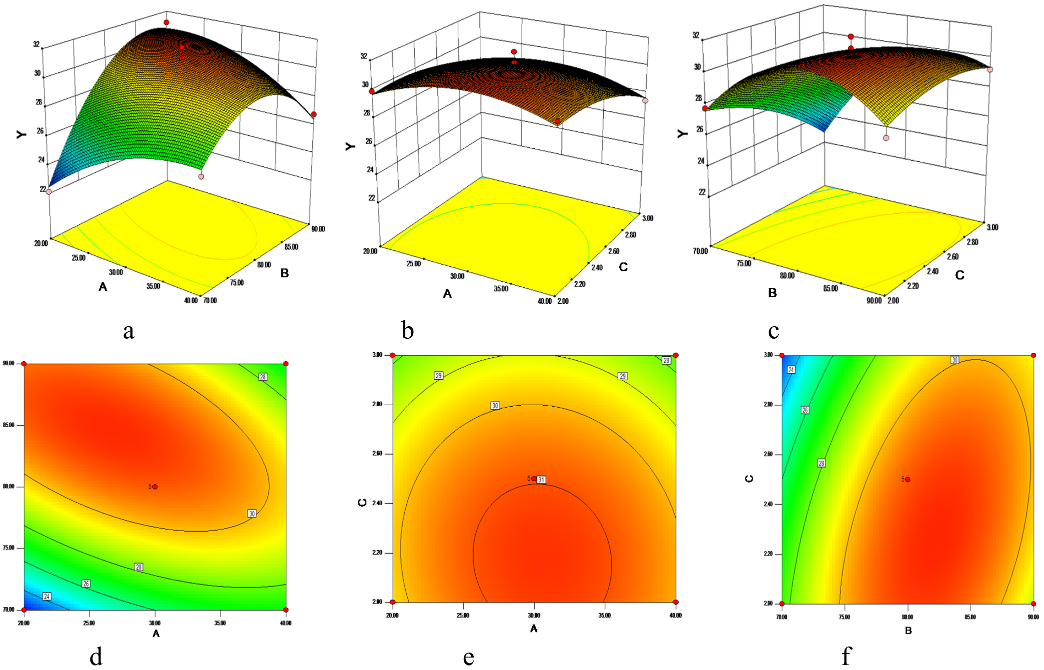

Figure 1. Response surface plots (a, b and c) and contour plots (d, e and f) showing the effect of ethanol concentration, reaction temperature and time on the color value.

4.Conclusions

Based on the single-factor experiment, the process conditions of brown pigment extraction from chestnut shell were optimized by response surface methodology. The color value was used as the index to optimize the extraction parameters. As a result, the optimal extraction conditions were obtained: the pH value of the extraction system was 13, ethanol concentration was 28%, temperature was 83 °C, and time was 2.3 h. The extraction temperature had the greatest influence on the color value of the pigment. The interaction between ethanol concentration and extraction temperature had significant effects on the color value. And the interaction between ethanol concentration and extraction time had no significant effects. The color value of chestnut shell pigment can reach 31.25 ± 0.50 with the response surface optimization. The study provided some practical guidance and theoretical basis for the utilization of discarded chestnut resources and the development of natural pigments.

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