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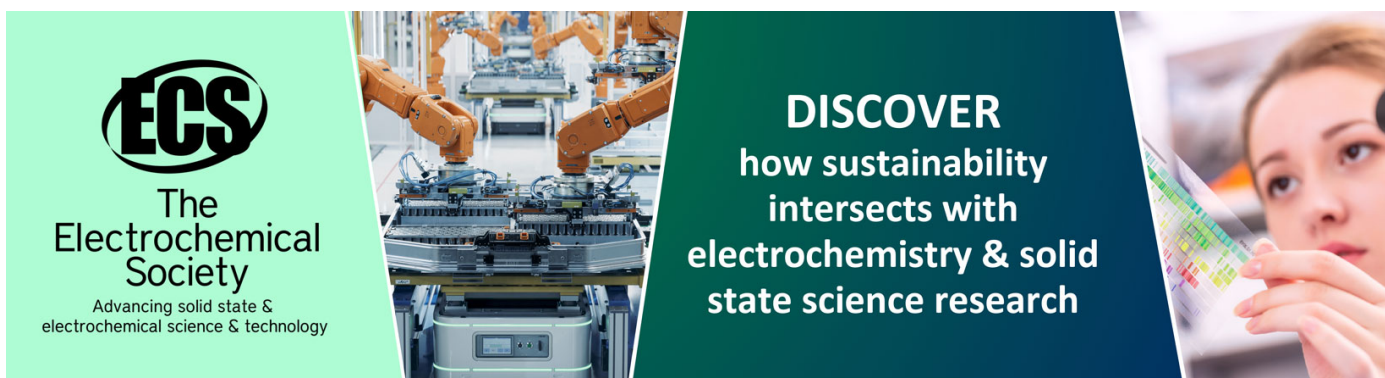
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Isolation of rotein from Residues of Macadamia Nuts After Oil Extraction

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Abstract. Macadamia nut produces plenty of residues after oil extraction which exhibit considerable quantities of protein and other nutrients. These residues, if not dealt with, become environmental pollutants and meanwhile lead to a great waste of resources. In this study, proteins were isolated from the residues of Macadamia nuts using the method of aqueous alkaline extraction at pH 9 and the identification of Amino acids was performed by cation-exchange chromatography. Box-Behnken Design (BBD) was used here, as an experimental method, to estimate and optimize the yield of protein. The results showed that 22.49% proteins were extracted under the optimized conditions. 18 Amino acids including 7 essential ones which took up 22.46% (Tryptophan was not detectable) could be found in it and was much higher than that in peanut meal, soybean meal and rapeseed meal, especially Glu, Arg, Asp and Gly whose content were up to 164.35 mg/g, 117.77 mg/g, 96.72 mg/g and 67.19 mg/g respectively.

1. Introduction

Macadamia nuts is a healthy and nutritious food and features high oil (65.4%), high protein (10.2%) and low cholesterol content^[1,2]. The residues of Macadamia nuts after oil extraction contain around 30% protein and deserve further processing. To date, there is no report relating the extraction and properties of these proteins. Aqueous alkaline is used widely in protein extraction from other protein sources like lychee and rice and the productivity is reported to be relatively high^[3,4]. Here aqueous alkaline is used to extract protein from residual macadamia nut and Box-Behnken Design (BBD)^[5,6] was used as an experiment design method to improve the yield of proteins.

2. Materials and methods

2.1. Materials

The residues of Macadamia nuts after extracting oil were obtained from our own laboratory (Key Laboratory of Tropical Agro-Products Processing, Ministry of Agriculture of China). All chemicals were analytical grade purchased from Guangdong Guanghua Chemical Factory Co., Ltd. (China).



2.2. *Single factor experiments*

The experiment involved four factors: materials to water ratios, pH, extraction time and temperature. The parameters of the effect of materials to water ratio on single-factor experiment of total protein extraction are as follows: samples of 1.0 g, soaked with ultra-pure water (varying materials to water ratios from 1:70 to 1:95 w/v, 55 °C, 2.5 h and pH 9). And the parameters of the effect of pH are as follows: samples of 1.0 g, soaked with ultra-pure water 90 ml (varying pH from 7 to 12, 55 °C and 2.5 h). Then the parameters of the effect of extraction time are as follows: samples of 1.0 g, soaked with ultra-pure water 90 ml (varying extraction time from 0.5 to 3 h, 55 °C and pH 9). The final parameters of the effect of extraction temperature are as follows: samples of 1.0 g, soaked with ultra-pure water 90 ml (varying extraction temperature from 40 to 65 °C, 2.5 h and pH 9). All samples were placed into the extraction vessel with 150 mL capacity separately.

2.3. *Protein quantification methods*

The determination of protein was described by Ohnishi and Barr method and slightly modified in the experiment. Five millilitres Bradford reagent was added to 100 µl sample and mixed. The absorbance at 595 nm was measured after an incubation period of 2 min at room temperature ^[7,8]. In the range of 100-1000 µg/mL, a good linear relationship was obtained, and the regression equation was $Y=0.0063X+0.0307$, with a correlation coefficients (R^2) of 0.9921, where Y is the absorbance of sample, X is the protein concentration (µg /mL).

2.4. *Response surface methodology*

The range of level values of each factors were determined through single-factor test, and Box-Behnken factorial design (BBD) was adopted to analyse this optimization study. Four factors (materials to water ratios, pH, extraction time and temperature) were selected as independent variables to optimize for the extraction of protein given in Table 1.

Table 1. Experimental design and responses of the dependent variables to the extraction parameters

Run	Coded and uncoded variable levels				Y/Extraction yield(%)	
	A/Extraction temperature (°C)	B/Extraction time (h)	C/Materials to water ratio	D/pH	Actual values	Predicted values
1	0(55)	-1(2)	-1(85)	0(9)	19.28	19.40
2	0(55)	+1(3)	-1(85)	0(9)	15.62	16.19
3	0(55)	-1(2)	+1(95)	0(9)	19.24	18.64
4	0(55)	+1(3)	+1(95)	0(9)	19.86	19.71
5	1(50)	0(2.5)	0(90)	-1(8)	18.34	17.95
6	1(50)	0(2.5)	0(90)	+1(10)	18.47	18.21
7	+1(60)	0(2.5)	0(90)	-1(8)	18.02	18.25
8	+1(60)	0(2.5)	0(90)	+1(10)	18.60	18.96
9	0(55)	0(2.5)	-1(85)	-1(8)	18.67	18.31
10	0(55)	0(2.5)	-1(85)	+1(10)	20.13	19.02
11	0(55)	0(2.5)	+1(95)	-1(8)	19.34	19.92
12	0(55)	0(2.5)	+1(95)	+1(10)	20.35	20.17
13	-1(50)	-1(2)	0(90)	0(9)	17.13	17.34
14	+1(60)	-1(2)	0(90)	0(9)	18.56	18.69
15	-1(50)	+1(3)	0(90)	0(9)	17.75	17.09
16	+1(60)	+1(3)	0(90)	0(9)	17.53	16.79
17	-1(50)	0(2.5)	-1(85)	0(9)	16.34	17.01
18	+1(60)	0(2.5)	-1(85)	0(9)	18.17	18.30
19	-1(50)	0(2.5)	+1(95)	0(9)	18.71	19.15
20	+1(60)	0(2.5)	+1(95)	0(9)	19.01	18.91
21	0(55)	-1(2)	0(90)	-1(8)	18.82	18.58
22	0(55)	-1(2)	0(90)	+1(10)	19.08	19.46
23	0(55)	+1(3)	0(90)	-1(8)	17.72	17.91
24	0(55)	+1(3)	0(90)	+1(10)	17.18	17.99
25	0(55)	0(2.5)	0(90)	0(9)	22.43	22.17
26	0(55)	0(2.5)	0(90)	0(9)	22.09	22.17
27	0(55)	0(2.5)	0(90)	0(9)	21.98	22.17

2.5. Statistical analysis

The data from triplicate measurements was analyzed by analysis of variance (ANOVA). Single-factor-test was estimated by Microsoft Excel software, and Design Expert software (Trial Version 8.0.6) and JMP 7.0.2 were used to estimate the response of each set of experimental design and optimized conditions. The behavior of the regression was explained by the following quadratic polynomial:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \beta_{00} + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (1)$$

where Y represents the measured response variables; β_0 , β_i , β_{ii} and β_{ij} are the regression coefficients for intercept, linearity, square and interaction, respectively and cross products of x_1 , x_2 , x_3 , ... on response.

3. Results and discussion

3.1. The analysis of single factor experiment

From Figure 1, effects of four factors (materials to water ratios, pH, extraction time and temperature) on extraction yield of protein were observed. The protein extraction yield was reached its peak at materials to water rate 1:90. There were previous papers involved that water played a key role in reaction process, however, overwhelming water decreased the concentration of substrate which

decreased the opportunity of collision at molecular level^[9]. The figure showed that the extraction yield was gradually increased with the increase of pH until 10 where the highest protein extraction yield was reached. The result was a little different from Sui XN et al^[3] described. The protein extraction yield was sharply increased with the increasing temperature (under 55 °C) and decreased with the increasing temperature (beyond 55 °C). The reason may be that the suitable temperature of extracting protein was below 55 °C and the higher temperature inactivated protein (beyond 55 °C). Additionally, better extraction yield was the extraction time under 2.5 h, and kept stable with the increasing of time. Therefore, the suitable extraction time was 2.5 h.

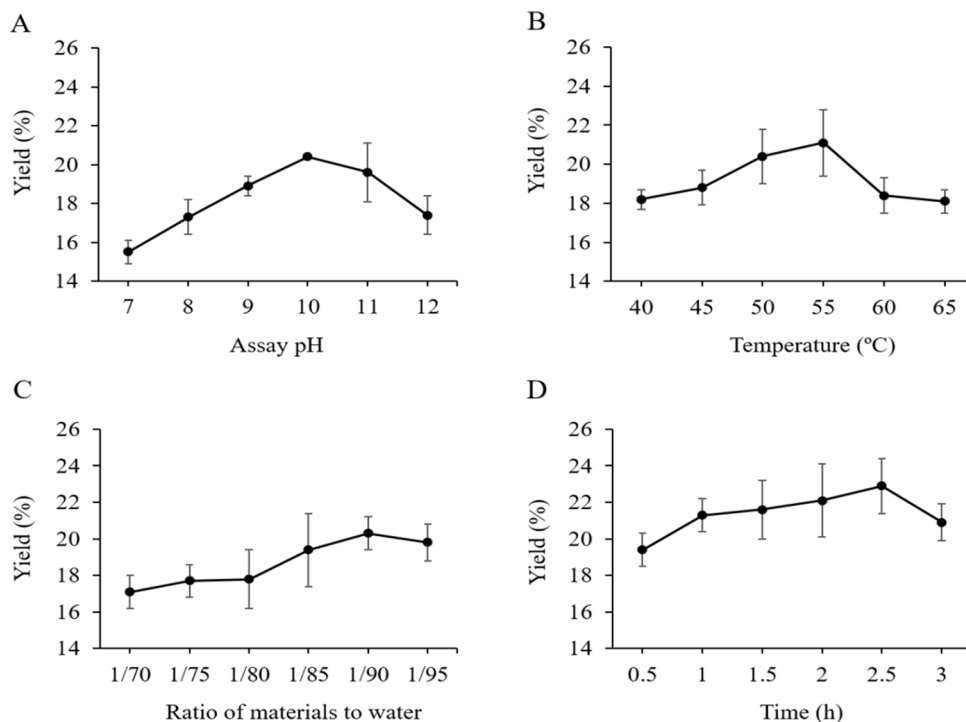


Figure 1. Effects of four factors (materials to water ratios, pH, extraction time and temperature) on extraction rate of protein

3.2. Optimization of the extraction parameters of protein

3.2.1. Statistical analysis and the model fitting

The experiments were designed according to BBD using a three-level-four-factor with three central points as listed in Table 2. And the response surface regression results obtained from BBD are F and P values along with the constant and coefficients (estimated using coded values) are given in Table 3. In general, larger value of F and smaller p-value would indicate a more significant effect as a tool to check the significance of each coefficient in the model^[10]. The analysis of variance (ANOVA) also shows that regression model is significant and lack of fit not significant, which means the variation in the response data can be very well explained by the model. According to multiple analysis on the experimental data, the behavior of the regression was explained by the following quadratic polynomial:

$$Y = 22.17 + 0.26A - 0.54B + 0.69C + 0.24D - 0.41AB - 0.38AC + 0.11AD + 1.07BC - 0.20BD - 0.11CD - 2.42A^2 - 2.28B^2 - 1.41C^2 - 1.41D^2 \quad (2)$$

Table 2. ANOVA for extraction yield of protein

Source	Sum of squares	df	Mean square	F value	P value
Model	61.56	14	4.40	9.47	0.0002
A	0.83	1	0.83	1.78	0.2068
B	3.47	1	3.47	7.47	0.0182
C	5.74	1	5.74	12.37	0.0042
D	0.70	1	0.70	1.51	0.2427
AB	0.68	1	0.68	1.47	0.2493
AC	0.59	1	0.59	1.26	0.2835
AD	0.051	1	0.051	0.11	0.7469
BC	4.58	1	4.58	9.78	0.0085
BD	0.16	1	0.16	0.34	0.5680
CD	0.051	1	0.051	0.11	0.7469
A ²	31.18	1	31.18	67.17	<0.0001
B ²	27.61	1	27.61	59.48	<0.0001
C ²	10.55	1	10.55	22.73	0.0005
D ²	10.55	1	10.55	22.73	0.0005
Residual	5.57	12	0.46		
Lack of fit	5.46	10	0.55	9.92	0.0950
Pure error	0.11	2	0.55		
Cor total	67.13	26			

3.2.2. Analysis of response surfaces

Response surfaces were plotted by using Design Expert software (Trial Version 8.0.6), and the effects of the four factors as well as their interactive effects on the extraction yield of protein are shown in Figure 2. The statistical analysis from Table 3 indicated the independent variables (B, C), the interaction between B and C and the quadratic terms (A², B², C² and D²) had significant effects on the yield of protein ($p < 0.05$).

Different shapes of the contour plots indicated different interactions between the variables, elliptical contour plots indicated the interactions between the variables were significant while circular contour plots means otherwise ^[11]. Combination response surfaces with Table 2, in the four variables, when two variables within the experimental range were depicted in three-dimensional surface plots, the third variable was kept constant at zero level. Although the interactions between materials to water ratios and extraction time were significant, the suitability of the model equation for predicting the optimum response values was tested by using the selected optimal conditions.

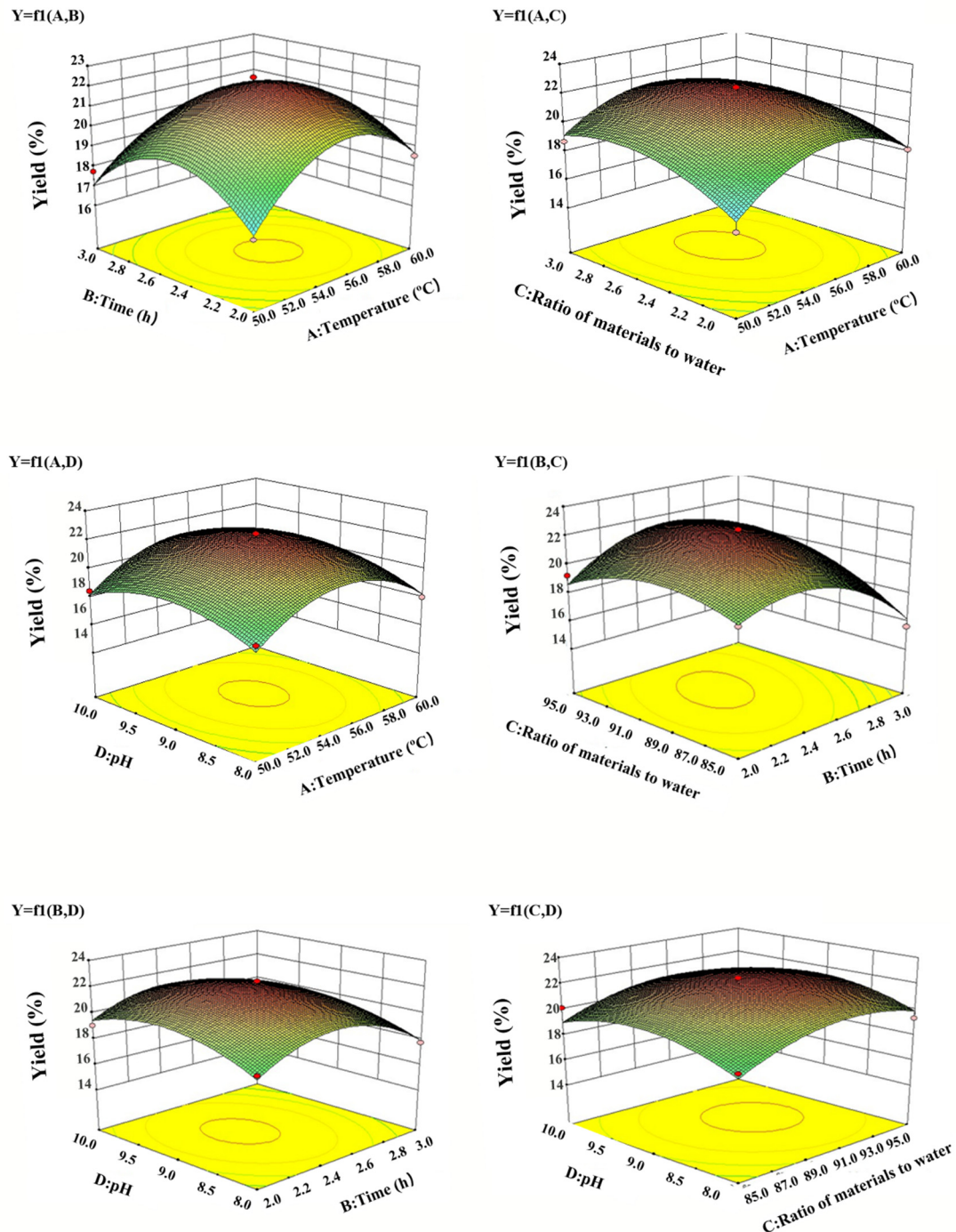


Figure 2. Response surface plot

3.3. Verification experiment

Three parallel experiments were taken under the best experimental conditions designed in the model (materials to water ratios 1:90, pH 9, extraction time 2.5 h and extraction temperature 55 °C), and the average result of extraction yield of protein was 22.17%. The optimal conditions were extracted from prediction profiler plotted by using JMP 7.0.2. From Figure 3, the results showed that the optimized conditions were materials to water ratios 1:91.03, extraction time 2.46 h, extraction temperature 55.23 °C and pH 9.09, and the extraction yield of maximize desirability was 22.28%. However, considering

the operability in actual production, the optimized conditions can be modified as follows: materials to water ratios 1:91, extraction time 2.5 h, extraction temperature 55 °C and pH 9.1. Then the extraction yield of protein was 22.49%, not only close to but also better than the predicted value. Therefore, the consequence of analysis demonstrated the validation of the RSM model and confirmed that the response model was adequate for reflecting the expected optimization.

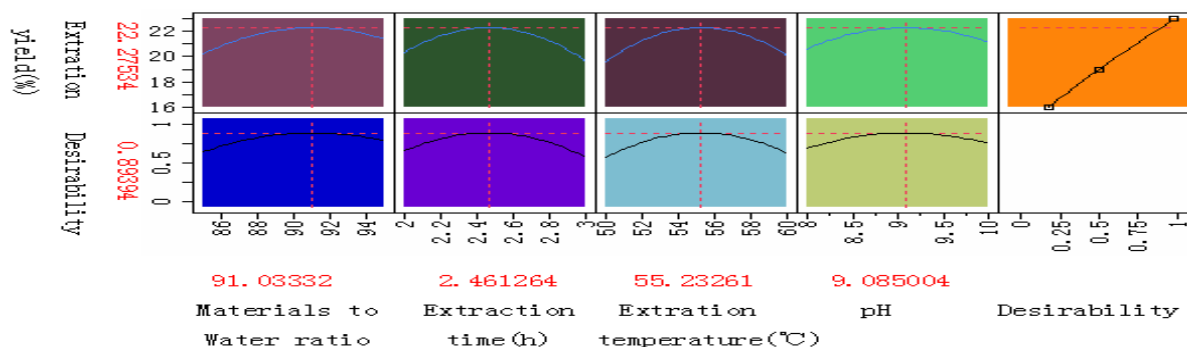


Figure 3. Dimension reduction analysis of effect of different parameters on extraction rate of protein

4. Conclusion

Aqueous alkaline extraction was performed for the extraction of protein from macadamia nuts residue after extracting oil. Based on the single-factor experiments, Box-Behnken Design (BBD) by using Design Expert software and JMP was used to estimate and optimize the experimental for improving the yield of protein. The optimum parameters are as follows: materials to water ratios 1:91, extraction time 2.5 h, extraction temperature 55 °C and pH 9.1. Under the optimized conditions, the extraction yield of protein was 22.49%, not only close to but also better than the predicted value.

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