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Procedia Engineering 15 (2011) 5250 – 5258

Procedia Engineering

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Advanced in Control Engineering and Information Science

Optimization of Extraction Process of Protein Isolate from Mung Bean

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Abstract

Protein was extracted from mung bean by water. Study the best parameter extraction. Based on single factor experiments, investigated liquid ratio, extraction temperature, extraction ph, settlement ph, and extraction time. Design by design expert to optimize the response of the face of extraction of technology. Optimal parameters: Liquid ratio of 10%, extraction temperature 31.74℃, extraction ph 8.97, settlement ph 4.4, extraction time 33.24min.

Key words：*A*lkali extraction and acid precipitation, Mung bean protein isolate, Response surface analysis

1. Introduction

Mung beans is leguminosae that is Cowpea. Originated in China, A long history of cultivation, widely distributed, abundant production. mung beans have Comprehensive nutrition. Protein content is 19.5%-33.1%, Effectiveness ratio is 1.87, Higher than wheat, rice, corn and other food crops. And the full range of amino acids, the ratio of equilibrium[1], in particular, the content of lysine is abundant close to the egg protein[2]. There is heat and detoxifying, refreshing thirst quencher, diuretic swelling, the effect of liver

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doi:10.1016/j.proeng.2011.08.973

detoxification, good food and medicinal value. Currently the use of mung bean focused on starch, while ignoring the development of mung bean protein, resulting in waste of resources, increasing the cost of downstream processing[3].

1977, Thompson LU [4] first extract of mung bean protein isolates. After, Adsuale RN gave a comprehensive overview on the nutritional quality of mung bean protein isolates. In recent years, Wang Jin-Shui et al [5] were preliminary study on the functional properties of mung bean protein isolates. Liang Liqin et al [6] studied the functional properties of mung bean protein isolates, the value of pH, temperature, ionic strength, protein content and other factors on the solubility of mung bean protein isolate, water absorption, oil absorption, foaming and foam stability. Emulsion, emulsion stability, viscosity systematically studied. Better for the mung bean protein isolates provide the theoretical basis for the food industry.

Currently, there are many ways of protein extraction. Such as the enzymatic extraction of protein, membrane, ion exchange. However, these advanced technologies is not yet mature. Separation of mung bean protein production is still alkaline extraction and acid precipitation method [7] based. In this study, this method further optimize the process parameters and get a mathematical regression equation, for later study and research provide a theoretical basis.

2. Methods

2.1. Materials

Mung bean flour(Homemade)，produced in Haerbin in China ,Other reagents were of analytical grade

2.2. Single factor experiments

(1) The parameters of the effect of materials to water ratio on Protein yield single-factor experiment are as follows: extraction temperature30℃, extraction pH 9, settlement pH 4.6, extraction time 30min. The chosen materials to water ratios are 8%、9%、10%、11% and 12%.

(2) The parameters of the effect of extraction temperature on Protein yield single-factor experiment are as follows: materials to water ratio 10%, extraction pH 9, settlement pH 4.6, extraction time 30min. The chosen extraction temperature are 20℃、25℃、30℃、35℃ and 40℃.

(3) The parameters of the effect of extraction pH on Protein yield single-factor experiment are as follows: materials to water ratio 10%, extraction temperature30℃,settlement pH 4.6, extraction time 30min. The chosen extraction pH are7、8、9、10 and 11.

(4) The parameters of the effect of settlement pH on Protein yield single-factor experiment are as follows: materials to water ratio 10%, extraction temperature30℃, extraction pH 9, extraction time 30min. The chosen settlement pH are4.2、4.4、4.6、4.8and5.0.

(5) The parameters of the effect of extraction time on Protein yield single-factor experiment are as follows: materials to water ratio 10%, extraction temperature30℃, extraction pH 9，settlement pH 4.6. The chosen extraction time is20 min、25 min、30 min、35 min and 40min.

2.3. Response surface method analysis

The range of level values of each factors were determined based on the single-factor experiments parameters. The response surface method was employed to analysis the effects of each factors on protein yield rate. Five factors (Liquid ratio, extraction temperature, extraction ph, settlement ph, extraction time) were selected as independent variable and protein yield was chosen as variable.

Determination of crude protein: according to GB 5009.5-2010

Weigh 30g mung beans grinding powder, and then mixed with distilled water according to a certain materials to water ratio. Then, transfer the mixture into water bath with continuous stirring and adjust the mixture pH using NaOH. Stiring and keeping the hydrolysis temperature during the extraction process. then, and absorb the mixture in upper layer. After then, adjust the mixture pH using HCl at Isoelectric poin absorb the mixture in lower layer and dry the residual materials. DesignExpert7.1.1 software was used to analysis the date of the experiment.

3. Results and discussion

3.1. The analysis of single factor experiment

3.1.1 The effect of materials to water rate on Protein yield rate

Fig.1 illustrates that with the mass fraction of mung bean flour gradually increased ,protein yield also will be gradually increased, when the growth to a certain extent, but have declined. May be due to excessive concentration of mung bean, powder and lye can not fully respond, thus affecting the protein solubility. Figure shows materials to water rate is 10% when the protein yield rate is best.

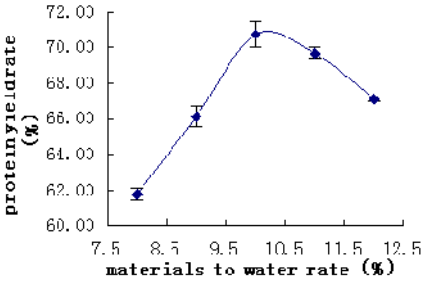


Fig.1 Effect of materials to water rate on Protein yield rate

3.1.2 The effect of hydrolysis temperature on oil extraction rate

Fig.2 illustrates that the Protein yield rate is increased with the increasing temperature (under 30 ºC) and decreased with the increasing temperature (beyond 30 ºC),and since High temperature can cause protein denaturation, alkaline solution can easily hydrolyzed protein molecular weight decreases, Figure shows extraction temperature is 30 ºC when the protein yield rate is best.

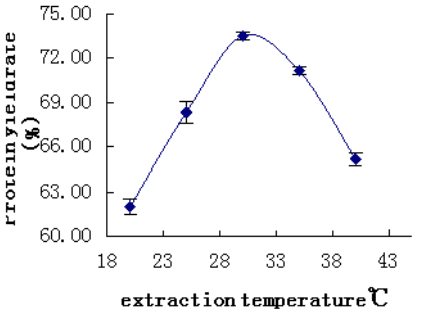


Fig.2 Effect of extraction temperature on Protein yield rate

3.1.3 The effect of hydrolysis time on oil extraction rate

Fig.3 illustrates that pH have significant impact to protein content, Protein yield rate is increased with the increasing extraction ph (under 9) and decreased with the increasing extraction ph (beyond 9) and then increases as the pH value of protein content started to decline, indicating that the protein should be extracted in an alkaline environment, especially under the conditions pH 9 extraction, results were better.

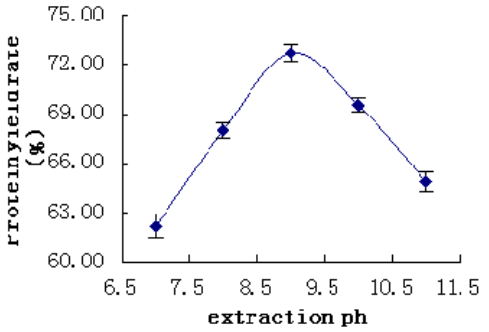


Fig.3 Effect of extraction ph on Protein yield rate

3.1.4 The effect of materials to water rate on oil extraction rate

Fig.4 illustrates that under different pH, protein deposition have different levels that is related to the mung bean protein isolates of isoelectric point when the pH to the isoelectric point of protein and water can be separated. Mung bean protein isolates obtained at pH 4.6 when the Protein yield rate was the highest.

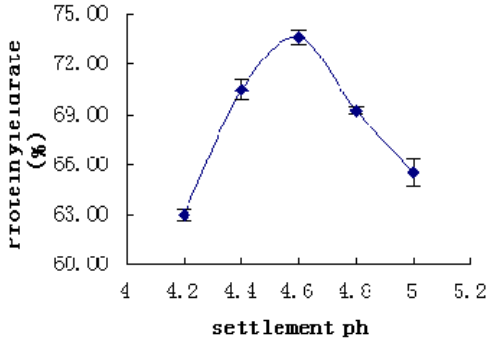


Fig.4 Effect of settlement ph on Protein yield rate

3.1.5 The effect of pH on oil extraction rate

Fig.5 illustrates that With the increase of extraction time, the protein yield rate is increased , when the growth reaches a certain level of slowing down time has decreased slightly, the reason is that after a certain time the green beans are in alkaline then protein has been fully raised, so the growth rate slowed,

so the growth rate slowed to Consider the efficiency of the experiment, the best extraction time is 30 minutes.

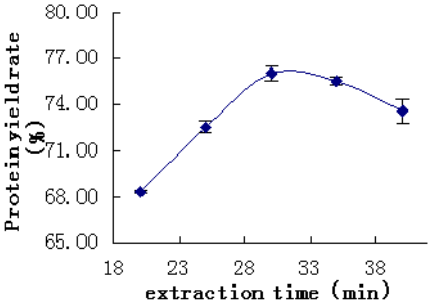


Fig.5 Effect of extraction time on Protein yield rate

3.2. Response surface analysis

The range of each factor's level is determined based on the data of single factor experiment. The response surface analysis method is employed to design and optimize the parameters in the experiment. The independent variables are as follows: materials to water rate (x1), extraction temperature (x2), extraction pH (x3), settlement pH (x4) and extraction time (x5). The response value is Protein yield rate. Tab.1 is the level coding and Tab.2 is the design and result of this experiment. Experimental No.1-40 is the factorial and 40-46 is center trial used to estimate the experimental error.

Table.1 Encode table of factors and levels

Code	factor				
	materials to water rate x_1（%）	extraction temperature x_2（℃）	extraction ph x_3	settlement pH x_4	extraction time x_5（min）
-1	9	25	8	4.4	25
0	10	30	9	4.6	30
1	11	35	10	4.8	35

Table.2 Design and results of response surface analysis

Run	**materials to water rate x_1（%）**	**extraction temperature x_2（℃）**	**extraction ph x_3**	**settlement ph x_4**	**extraction time x_5（min）**	**Protein yield rate y_1（%）**
1	-1	-1	0	0	0	69.34
2	1	-1	0	0	0	63.33
3	-1	1	0	0	0	67.98
4	1	1	0	0	0	71.95
5	0	0	-1	-1	0	70.34
6	0	0	1	-1	0	74.89
7	0	0	-1	1	0	72.29
8	0	0	1	1	0	62.9
9	0	-1	0	0	-1	68.99
10	0	1	0	0	-1	67.29

11	0	-1	0	0	1	63.89
12	0	1	0	0	1	73.33
13	-1	0	-1	0	0	72.82
14	1	0	-1	0	0	70.3
15	-1	0	1	0	0	67.91
16	1	0	1	0	0	62.91
17	0	0	0	-1	-1	66.27
18	0	0	0	1	-1	70.78
19	0	0	0	-1	1	75.01
20	0	0	0	1	1	63.8
21	0	-1	-1	0	0	67.88
22	0	1	-1	0	0	67.11
23	0	-	1	0	0	69.89
24	0	1	1	0	0	68.93
25	-1	0	0	-1	0	69.93
26	1	0	0	-1	0	73.89
27	-1	0	0	1	0	71.45
28	1	0	0	1	0	63.92
29	0	0	-1	0	-1	63.27
30	0	0	1	0	-1	70.38
31	0	0	-1	0	1	73.9
32	0	0	1	0	1	64.49
33	-1	0	0	0	-1	66.82
34	1	0	0	0	-1	67.88
35	-1	0	0	0	1	73.91
36	1	0	00	0	1	63.67
37	0	-1	0	-1	0	72.34
38	0	1	0	-1	0	74.81
39	0	-1	0	1	0	64.33
40	0	1	0	1	0	70.23
41	0	0	0	0	0	78.33
42	0	0	0	0	0	75.42
43	0	0	0	0	0	74.34
44	0	0	0	0	0	76.45
45	0	0	0	0	0	76.2
46	0	0	0	0	0	74.78

The response surface regression model Eq.2 is established by SAS 9.2.

$$Y=4028.57646+149.47896*x_1 3.37300*x_2+176.66562*x_3+901.72083*x_4+38.08450*x_5+0.49900*x_1*x_2-0.62000*x_1*x_3-14.36250*x_1*x_4-0.56500*x_1*x_5-9.50000E-003*x_2*x_3+0.85750*x_2*x_4+0.11140*x_2*x_5-17.42500*x_3*x_4-0.82600*x_3*x_5-3.93000*x_4*x_5-3.86229*x_1^2-0.14246*x_2^2-3.67896*x_3^2-56.61979*x_4^2-0.16892*x_5^2$$

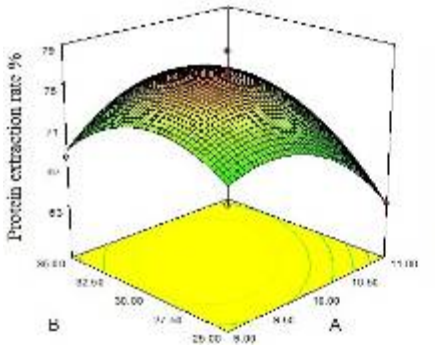
Tab.3 shows the results of regression and variance analysis and Fig.6 is the response surface analysis of significant effective interaction items.

Table.4 Results of regression and variance analysis

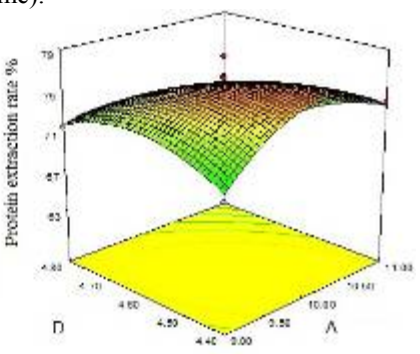
Various	**Degrees of freedom**	**Sum of squares**	**Mean square**	**F**	**Pr>F**
model	20	760.27	38.01	12.12	< 0.0001
x_1	1	31.11	31.11	9.92	0.0042
x_2	1	29.27	29.27	9.33	0.0053
x_3	1	15.23	15.23	4.86	0.0370
x_4	1	89.21	89.21	28.44	< 0.0001
x_5	1	6.66	6.66	2.12	0.1576
x_1x_2	1	24.90	24.90	7.94	0.0093
x_1x_3	1	1.54	1.54	0.49	0.4903
x_1x_4	1	33.01	33.01	10.52	0.0033

x_1x_5	1	31.92	31.92	10.18	0.0038
x_2x_3	1	9.025E-003	9.025E-003	2.877E-003	0.9576
x_2x_4	1	2.94	2.94	0.94	0.3421
x_2x_5	1	31.02	31.02	9.89	0.0043
x_3x_4	1	48.58	48.58	15.49	0.0006
x_3x_5	1	68.23	68.23	21.75	< 0.0001
x_4x_5	1	61.78	61.78	19.70	0.0002
x_1^2	1	130.19	130.19	41.51	< 0.0001
x_2^2	1	110.70	110.70	35.29	< 0.0001
x_3^2	1	118.12	118.12	37.66	< 0.0001
x_4^2	1	44.76	44.76	14.27	0.0009
x_5^2	1	155.65	155.65	49.62	< 0.0001
resdual	25	78.41	3.14	12.12	
lack of fit	20	68.20	3.41	1.67	0.2988
pure error	5	10.21	2.04		
cor total	45	838.69			

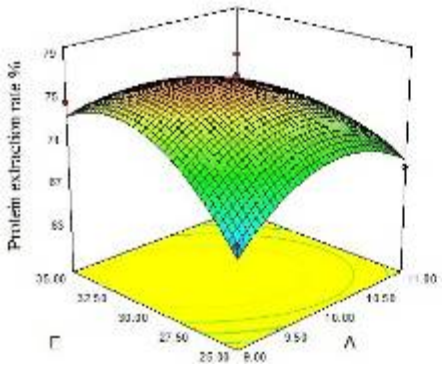
From Tab.4, the linear relationship between dependent variable and independent variables is significant, the regression model is significant ($p < 0.001$), lack of fit is insignificant, $R^2 = 90.65\%$ and $R^2_{Adj} = 83.17\%$, which indicate that the model fits well with the experimental data and the model can be used to analysis and predict the results of enzymatic extraction of pine kernel oil. Factor contribution rate obtained from the F-test are as follows: $x_4 > x_1 > x_2 > x_3 > x_5$, (settlement ph > materials to water rate > extraction temperature >extraction pH > extraction time).



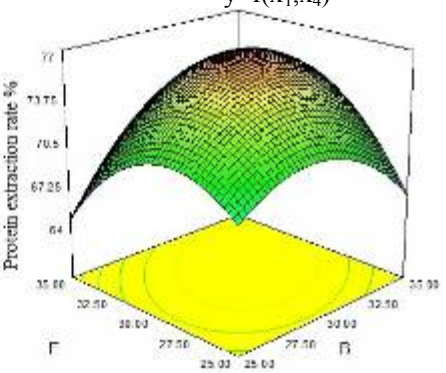
$y=f(x_1,x_2)$



$y=f(x_1,x_4)$



$y=f(x_1,x_5)$



$y=f(x_2,x_5)$

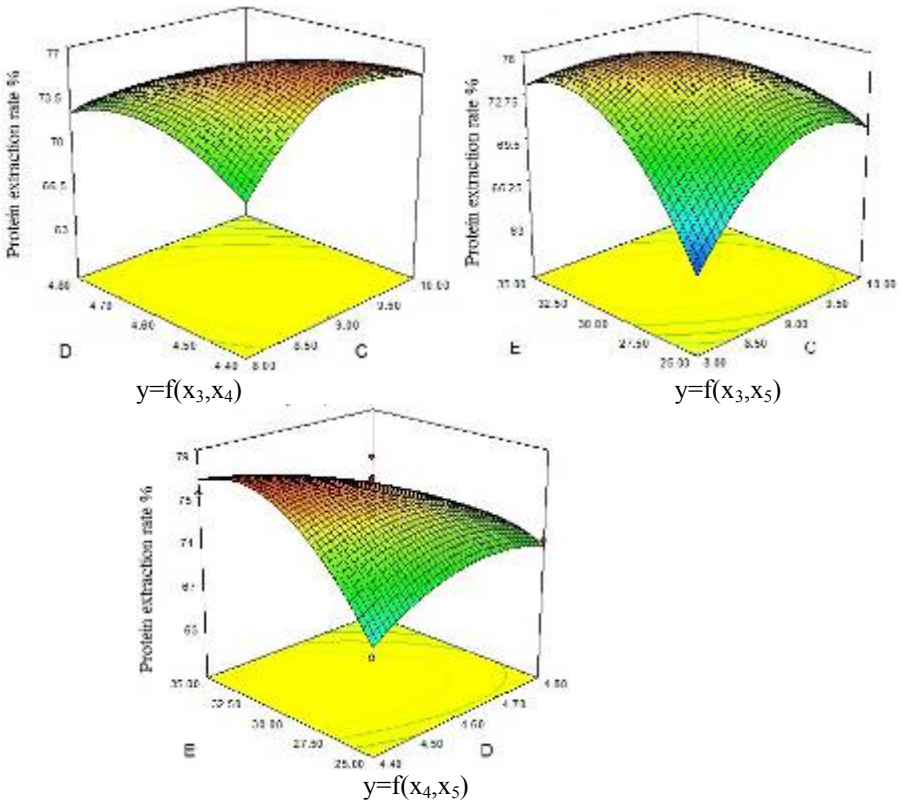


Fig.6 Response surface analysis of significant effective interaction items of different hydrolysis parameters on extraction rate of pine kernel oil

Fig.6 show that liquid ratio (x1) and extraction temperature (x2), solid to liquid ratio (x1) and settlement ph (x4), solid to liquid ratio (x1) and extraction time (x5), extraction temperature (x2) And extraction time (x5), extraction ph (x3) and extraction time (x5), extraction ph (x3) and extraction time (x5) and settlement ph (x4) and extraction time (x5) interaction effect degree of extraction. It can be seen in the extraction time is 25-35 range, the extraction time on the degree of extraction of factor contribution rate may be small, but the extraction time and the interaction of other factors influenced the degree of extraction significantly.

3.3. The optimal conditions for prediction and validation

Using design expert software optimization, optimization program has 9 groups, after the experiment: liquid ratio of 10%, 31.74 extraction temperature, extraction ph8.97, settlement ph4.4, extraction time 33.24min. Regression equation shows the optimized preparation of mung bean protein isolate for analysis and prediction of process parameters is very reliable.

4. Conclusion

In this study, we optimize and analysis the methons of alkali extraction and acid precipitation of mung bean protein isolates extracted to the process and get to a reliable regression equation.after experimental

verification, a good fit of this equation, the forecast and analysis of parameters can provide a good theoretical basis. Optimized analysis, the best experimental parameters: liquid ratio of 10%, 31.74 extraction temperature, extraction ph8.97, settlement ph4.4, extraction time 33.24min. The protein yield up to 77.60%.

Acknowledgements

The authors would like to thank the Heilongjiang Science and Technology Agency (research grant number: GA09B401-6), National Institute of Soybean Engineering Technology and Northeast Agricultural University for funding this work. We would also like to thank other students for their assistance.

References

[1] Fei Wang, Fan Mingtao. mung bean protein and amino acid composition characteristics of [J]. Jiangsu Polytechnic University (Natural Science), 2000,21 (3): 39 ~ 41.

[2]Adsuale R N.Chemistry and Technology of greengrain［J］.Critical Review Food Science and Nutrition,1986,25(1):73~105.

[3] Campbell K.A., C. E. Glatz CE, Johnson L. A., Jung S, de Moura J.M.N, Kapchie V, Murphy P. Advances in Aqueous Extraction Processing of Soybeans. Journal of Amercian Oil Chemists' Society. DOI 10.1007/s11746-010-1724-5

[4] Huang Y, Wu P. SAS analysis method and application. Bei Jing: Machinery Industry Press; 2006.

[5] Yi JH, Zhu ZB, Zhao F. Effects of enzyme on aqueous enzymatic extraction of walnut oil. China Oils and Fats,2007;32(2):27-29.

[7] Song YQ, Yu DY, Wang J, Li HL, Yang W. Study on Extraction Process of Hazelnut Oil by Aqueous Enzymatic Method [J].Food Science,2008;29(8):261-264.