Optimization of Dietary Fiber Extraction from Pineapple Pomace by Response Surface Methodology

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Abstract: Taking pineapple peel as raw material, the dietary fiber in pineapple pomace was extracted by enzymatic method combined with the chemical method. The effects of enzyme dosage, enzymolysis time, alkaline pH and alkali solution time on the yield of pineapple pomace dietary fiber were studied. And optimize the extraction process. The results showed that the optimal extraction process parameters of pineapple pomace were as follows: enzyme dosage 480u/g, enzyme hydrolysis time 43 minute, alkaline hydrolysis pH=12, alkaline hydrolysis time 60 minute, pineapple pomace dietary fiber under these conditions, the yield can reach 78.86%. The mathematical model is feasible for optimizing the extraction process of pineapple pomace dietary fiber.

Keywords: Pineapple pomace; Dietary fiber; Response surface analysis

1. Introduction

Dietary fiber refers to a general term for polysaccharides that are not easily digested and absorbed by the human body. It is a polymer composed of cellulose, pectin, hemicellulose, and glycoproteins[1]. According to the solubility, it is divided into two categories: soluble dietary fiber and insoluble dietary fiber. Among them, insoluble dietary fiber mainly acts as mechanical creep in the human body [2-3], and soluble dietary fiber has more physiological functions, which can prevent gallstones, eliminate harmful metal ions, prevent diabetes, lower blood fat and cholesterol, and prevent high blood pressure and other effects[4]. Dietary fiber is considered to be a "seventh nutrient" that can improve the body's nutritional status and regulate body functions after protein, fat, water, minerals, vitamins and carbohydrates[5]. Studies have shown that dietary fiber has many important physiological functions, such as relieving constipation, lowering blood sugar, lowering serum cholesterol levels, treating obesity, preventing hypertension, preventing oxidation, eliminating free radicals [6-8]。

Pineapple (*Ananas comosus).* it belongs to Ananas (*Bromeliaceae*), a perennial monocotyledonous evergreen tree, and is one of the tropical and subtropical typical fruits, its smell is fragrant, the flesh is sweet and crisp, rich in sugar, protein, multivitamins and minerals. Pineapple is not easy to store, except for a small part for fresh fruit sales, most of it is used for food processing such as processing juice or making canned pineapple products, almost 50% to 60% of pineapple peel and residue are not used during processing[9], while the pineapple pomace is the remainder of pineapple fresh food and processing, its nutrient composition is similar to pulp, rich in dietary fiber, direct discard will cause a lot of waste of resources and pollute the environment. This experiment explored the method of extracting dietary fiber from pineapple pomace by enzyme binding chemistry method. The extraction process was optimized by response surface method to provide the basis for further comprehensive utilization of pineapple pomace.

2. Materials and Methods

2.1 Materials

Pineapple pomace: Zhanjiang Siwei special company absolute ethanol, acetone, anhydrous ethanol, petroleum ether(boiling range of 30~60 °C), acetone, sodium hydroxide, glacial acetic acid, hydrochloric acid, all analytically pure; α-amylase, glucoamylase (activity greater than 100,000 U/g).

2.2 Pre-sample treatment

Pineapple peel was first washed with tap water to eliminate the bulk of sand and other inorganic materials. The peel was then immersed in 96% (v/v) boiling ethanol for 20 min and washed with 70% ethanol to remove low-molecular-weight sugars, organic acids, and inorganic salts and to inactivate enzymes. The residue was dried at 40 °C to generate an alcohol-insoluble residue (AIR). After drying, the pomace was ground and passed through a 50-mesh standard sieve.

2.3 Process flow

Pomace(3.0 g, dry basis) was suspended in distilled water with stirring (200 rpm) using a magnetic stirrer at room temperature (25 °C), Alpha-amylase: saccharification enzyme is added to the suspension in a 1:1 ratio，and the suspension was then transferred to a thermostatic water bath (60 °C)，then add a certain amount of sodium hydroxide and adjust the pH to a certain range, continue to extract for a certain time. The TDF-rich extract was precipitated by adding four volumes of ethanol at room temperature and allowing the mixture to stand for 60 min，the precipitates obtained after suction filtration is the TDF extract. TDF determination according to AOAC method.

2.4 Single-factor experiment

Accurately weigh 3 g of pretreated pineapple pomace, add distilled water according to the ratio of material to liquid 1:15 (g/ml), fix other factors, and check the amount of enzyme added (200u/g, 300u/g, 400u/g, 500u/g, 600u/g); enzymolysis time (20min, 30min, 40mim, 50min, 60min); alkaline concentration (pH=9, pH=10, pH=11, pH=12, pH=13); alkaline hydrolysis time (20 minutes, 40 minutes, 60 minutes, 80 minutes, 100 minutes) Effect on TDF yield. Dietary fiber yield as an indicator，it was calculated as：

$$DF\ (g/g)\ \frac{m_1 \times (1-r_1)}{m_2 \times (1-r_2)} \times 100\% \qquad (1)$$

Where m_1 and m_2 are extracted quality and raw material quality, $r_1$and r_2 are extract moisture and volatile content.

2.5 Response surface analysis

Response surface methodology comprises a group of empirical techniques for evaluating the relationships among a series of controlled experimental factors and measured responses according to one or more selected criteria[10]. Comprehensive single-factor experiments, using the Box-Behnken Design (BBD) response surface analysis design principle in Design-Expert 8.0 software, using the 4 factors and 3 levels of analysis methods to determine the optimal process of enzyme-chemical extraction of pineapple skin dregs dietary fiber condition. Related factors and level design are shown in Table 1.

Table 1 Related factors and level design

Level	Factor			
	X_1Enzyme dosage U/g	X_2 Enzymolysis time min	X_3Alk aline pH pH=	X_4Alkaline solution time min
-1	400	30	11	40
0	500	40	12	60
1	600	50	13	80

3. Results and discussion

3.1 Single-factor experiment

Taking the yield of dietary fiber from pineapple pomace as the final evaluation index, the effects of enzyme dosage, enzymolysis time, alkaline pH, and alkaline solution time on the yield of pomace were investigated. As shown in Figure 1.

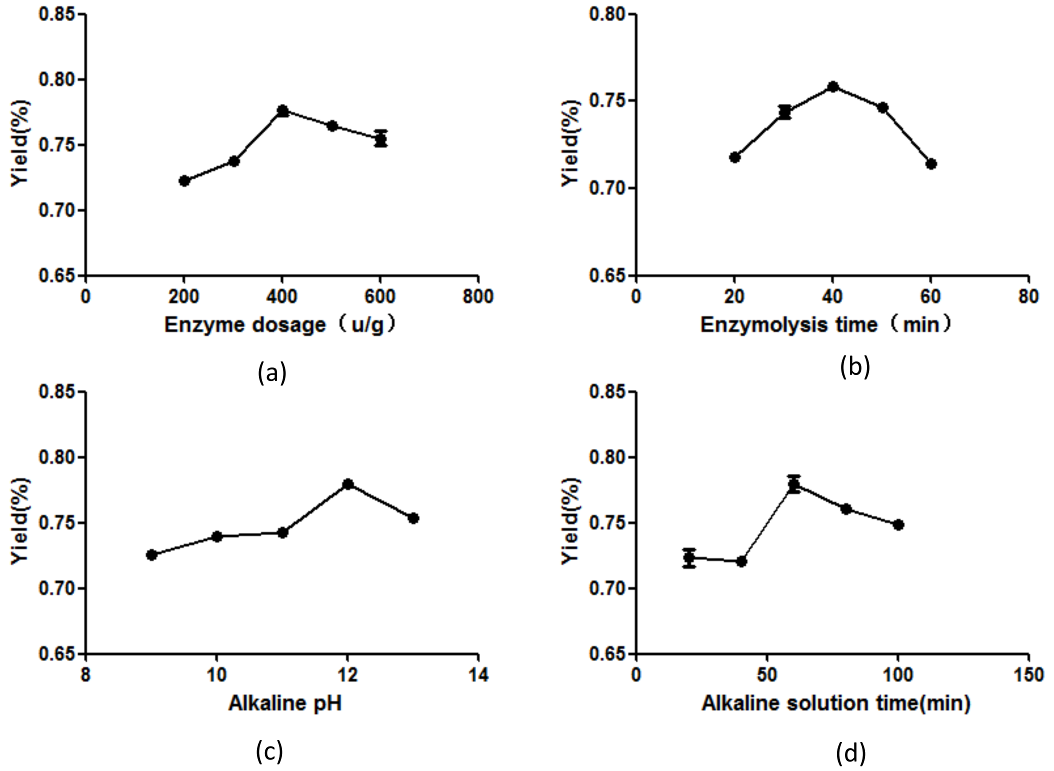

Fig. 1. Effect of different Enzyme dosage (a) Enzymolysis time, (b) Alkaline pH, (c) Alkaline solution time, (d)

The effect of the enzyme dosage on the DF yield was determined in the range of 400-600u/g. The DF yield first increased to its maximum level as the enzyme dosage was increased to 500u/g and then decreased with further increases in the enzyme dosage(Fig. 1a). The reason may be that the enzyme concentration is too low, and the starch covered by the surface of the pomace may not be completely hydrolyzed; if the enzyme concentration is too high, the hemicellulose and other physiologically active substances in the dietary fiber are easily eluted and the rate is reduced, Therefore[11], the optimum amount of mixed enzyme is 500u/g. The Enzymolysis time is also an important variable because Starch decomposition requires a certain time, but thermal degradation could occur if the extraction time is too long. In the present study, five extraction times (20, 30, 40, 50, and 60 min.) were tested to evaluate the impact of the extraction time on the DF yield. As shown in (Fig. 1b). An extraction time of 30-40 min is key for controlling the DF yield. The extraction time should not exceed 40 min because other extraction times will have adverse impacts on the extraction of DF. The yield of DF also first increases and then decreases with increases in the pH=12. As shown in (Fig. 1c), the highest yield was obtained with an suitable pH. Under appropriate pH conditions, the protein cell wall can be destroyed. When the alkali concentration is too high, slight hydrolysis of fibers and hemicellulose occurs[12], As a result, the yield of dietary fiber was reduced and the color of dietary fiber was increased. As can be seen from (Fig. 1d) the yield of dietary fiber increased first and then decreased with the prolongation of alkali hydrolysis time. When the alkali hydrolysis time was 60 min, the yield was the highest, and then it decreased rapidly. The reason may be that the alkali hydrolysis time is too short and the hydrolysis rate is incomplete, resulting in a low yield; and if the alkali hydrolysis time is too long, the dietary fiber will be softened, and even some parts will be slightly hydrolyzed, resulting in a decrease in yield[13], Therefore, alkali solution is suitable for 30min.

3.2 Enzymatic-chemical extraction of dietary fiber from pineapple pomace by response surface method

3.2.1 Model establishment and significance test

In this experiment, we used the BBD center combination experimental design principle and integrated single factor experimental results to select X_1, X_2, X_3 and X_4 as the response factors. The yield of pineapple skin dregs dietary fiber was the response value Y. The response surface experimental design scheme and results are shown in Table 2. There are 29 experimental points in the experimental design, of which 5 are repeated zero experiments to estimate the error. Statistical analysis of the results in Table 2 can establish the following multivariate quadratic regression equations: $Y = 78.82 - 0.28X_1 + 0.64X_2 + 0.19X_3 - 0.12X_4 - 0.20X_1X_2 + 0.85X_1X_3 - 0.050X_1X_4 + 0.050\ X_2X_3 + 0.48X_2X_4 - 0.83X_3X_4 - 1.33X_1{}^2 - 1.14X_2{}^2 - 1.14X_3{}^2 - 1.10X_4{}^2$, from table 2, we can see that the imaginary term is not significant (p-value is 0.3439, greater than 0.05). Variance and reliability of the quadratic regression equation are analyzed. The results are shown in tables 2 and 3. It can be seen from Table 3 that X_2, X_1X_3, X_3X_4, $X_1{}^2$, $X_2{}^2$, and $X_3{}^2$ in the first, second, and interaction terms of the quadratic regression equation all show significant levels, and the quadratic regression equation is an overall model. It is significant, and the missing quasi-terms are not significant. The regression model can be fitted well with the measured values.

Table 2. Scheme and experimental results for response surface analysis

NO.	X1	X2	X3	X4	Actual Value	Predicted Value
1	-1	-1	0	0	76.20	76.00
2	1	-1	0	0	75.30	75.43
3	-1	1	0	0	77.90	77.28
4	1	1	0	0	76.20	76.71
5	0	0	-1	-1	74.90	75.38
6	0	0	1	-1	78.40	77.41
7	0	0	-1	1	75.90	76.80
8	0	0	1	1	76.10	75.53
9	-1	0	0	-1	75.60	76.79
10	1	0	0	-1	75.90	76.23
11	-1	0	0	1	76.80	76.56
12	1	0	0	1	76.90	75.99
13	0	-1	-1	0	75.90	75.41
14	0	1	-1	0	76.80	76.69
15	0	-1	1	0	75.40	75.79
16	0	1	1	0	76.50	77.08
17	-1	0	0	-1	77.40	77.00
18	1	0	-1	0	75.10	74.73
19	-1	0	1	0	75.40	75.68
20	1	0	1	0	76.50	76.81
21	0	-1	0	-1	76.90	76.05
22	0	1	0	-1	77.50	77.34
23	0	-1	0	-1	74.80	75.82
24	0	1	0	1	77.30	77.10
25	0	0	0	0	79.60	78.82
26	0	0	0	0	79.10	78.82
27	0	0	0	0	78.40	78.82
28	0	0	0	0	78.90	78.82
29	0	0	0	0	78.10	78.82

Table 3 Analysis of variance of the regression equation for extraction efficiency of trypsin inhibitor

Source	Sum of Squares	df	Mean Square	F Value	P-Value Prob＞F
Model	38.66	10	3.87	7.05	0.0002
X1	0.96	1	0.96	1.76	0.2017
X2	4.94	1	4.94	9.01	0.0077
X3	0.44	1	0.44	0.80	0.3818
X4	0.16	1	0.16	0.30	0.5920
X1X2	0.16	1	0.160	0.25	0.6216
X1X4	0.98	1	0.98	0.016	0.9014
X2X4	0.90	1	0.90	1.44	0.2505
X2X3	0.98	1	0.98	0.016	0.9014
X1X3	2.89	1	2.89	5.27	0.0339
X3X4	2.72	1	2.72	4.96	0.0389
$X_1{}^2$	11.42	1	11.42	20.81	0.0002

$X_2{}^2$	8.42	1	8.42	15.35	0.0010
$X_3{}^2$	13.43	1	13.43	24.49	0.0001
$X_4{}^2$	7.87	1	7.87	14.35	0.0013
Residual	9.87	18	0.55		
Lack of Fit	8.49	14	0.61	1.75	0.3130
Pure Error	1.39	4	0.35		
Cor Total	48.53	28			

Note: $p < 0.05$, the difference is significant; $p < 0.01$, the difference is extremely significant

3.2.2 Response surface analysis

The degree of influence of the interaction of any two factors on the yield can be analyzed and evaluated by the response surface map and contour plot in Figure2, and the best factor level range can be obtained. The strength of the interaction between factors can be seen from the shape of the contours. The closer the shape is to the circle, the less significant the interaction is; the closer to the ellipse, the more significant the interaction between the factors, and the ellipse The degree of closeness may reflect the degree of influence of factors on the change in response value 14-15. From the response surface map above, the extreme conditions appear at the center of the contour. In the effect of the interaction item yield, the interaction between enzyme dosage (X1) and alkali addition (X3), and alkali hydrolysis time (X4) has a significant effect on dietary fiber yield.

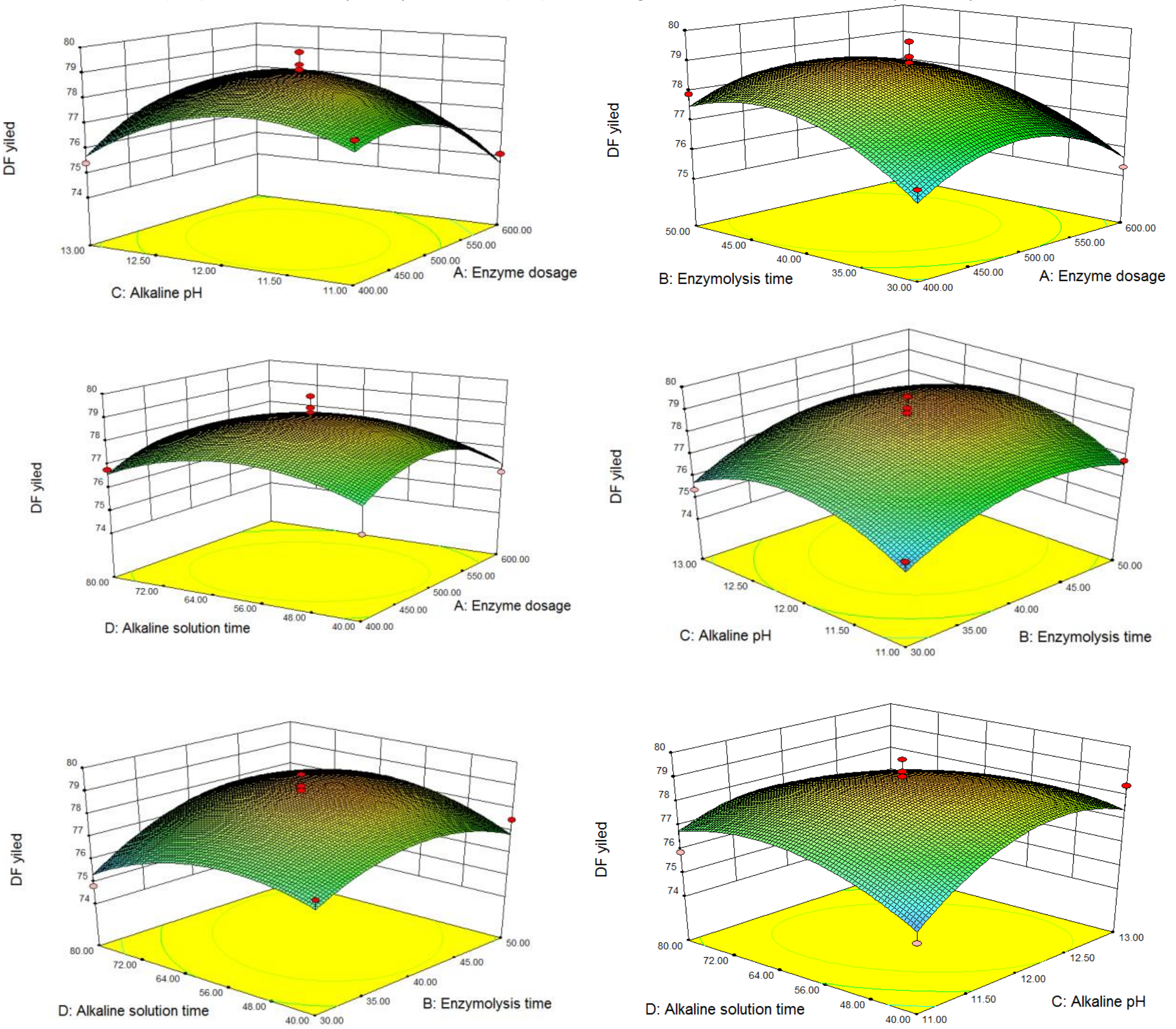

Fig.2 Response surface for the effects of pairwise interactions among various variables on extraction efficiency of DF

3.2.3 Extraction process optimization

The optimal extraction process parameters of pineapple pomace diet fiber were obtained through the analysis and optimization of Design-Expert 8.0 Trial software. The mixed enzyme dosage was 488.27u/g, the mixed enzymolysis time was 42.93 min, the alkaline hydrolysis pH was 12.04, and the alkaline hydrolysis time was 59.97Minute, the theoretical yield of wheat bran protein was 78.934% under the optimum process conditions. Considering the operability, the optimal extraction conditions were defined as: mixed enzyme addition amount 480u/g, mixed enzyme hydrolysis time 43 min, alkali The solution pH = 12, the alkaline hydrolysis time 60min. Under this condition for 3 parallel experiments, the relative error between the actual average yield value (78.86%) and the theoretical prediction value is small. Therefore, this mathematical model is feasible for optimizing the enzymatic-chemical extraction of pineapple pomace dietary fiber.

4. Conclusion

The enzyme-chemical method was used to extract pineapple pomace dietary fiber. Based on the single factor experiment, a response surface experiment was designed using the BBD center combination design method. A mathematical model was established to obtain a better extraction process. Combined with response surface analysis data and experimental verification, the best extraction conditions were: mixed enzyme dosage 480u/g, mixed enzymolysis time 43 minute, alkaline hydrolysis pH=12, alkaline hydrolysis time 60 minute, under this condition pineapple peel pomace dietary fiber yield can reach 78.86%. The quadratic equation model of dietary fiber yield and various factors variables was obtained. The regression model was extremely significant, and it fitted well to the experiment and had certain application value.

5. Acknowledgment

This work was supported by the Special Fund for Agro-scientific Research in the Public Interest (Grant Number 201503142), Hainan Provincial Natural Science Foundation of China (318QN260), and the Belt and Road Initiative Project for Joint Investigation and Exploitation Evaluation of Agricultural Resources in Tropical Countries (ZYLH2018010412).China Academy of Tropical Agricultural Sciences Fundamental Research Funds (1630122017013)

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