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Research Article

Cellulase-assisted extraction of total flavonoids from peanut hull

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Abstract: The total flavonoids extraction of peanut hull through using cellulase was investigated and the best process conditions were studied by one-factor experiment and orthogonal experiment. The result showed that the influential degree weakened as follows sequence: pH, enzyme quantity, time and temperature. The most suitable condition for extraction of total flavonoids from peanut hull was enzyme quantity 2.0%, pH 5.0, temperature 50˚C, and 130 minutes. Under the best condition, the total flavonoids yield of peanut hull was 2.99 mg/g.
Keywords: total flavonoids, cellulase, peanut hull, extraction.

INTRODUCTION

Peanut is a widely planted leguminous crop in China and the output is estimated to be approximately around 14.71 million tons in 2008. With an estimate of 30% waste biomass generation, the projected peanut hulls could reach 4.4 million tons, most of which were discarded as solid waste or burned off in stacks causing the resource dissipation and environmental pollution[1]. Research suggested that besides abundant carbohydrate and crude fiber, peanut hull contains many active substances such as flavonoids, polyphenols, and so on [2]. Flavonoids are natural compounds showing high physiological activities in therapies for inflammations, heart diseases and cancers [3,4].

Enzyme-assisted extraction of natural functional compounds from plants is widely investigated in recent years for its advantages in easy operation, high efficiency, and environment friendship. Most of the works in this field utilize cellulase, pectinase and β-glucosidase to hydrolyze and degrade plant cell wall constituents to improve the release of intracellular contents [5, 6]. In this work, the flavonoids of peanut hull were extracted using cellulase, and the effects of enzyme quantity, pH, temperature and time on flavonoids yield were investigated. Moreover, the extraction condition was optimized through orthogonal experiment. This work might explore an effective, simple method for total flavonoids extraction of peanut hull.

MATERIALS AND METHODS

Materials and Reagents

Peanut hull was originated of Shandong province, China; cellulase was purchased from ChengDu Kelong Chemical Co., Ltd. Absolute alcohol, sodium hydroxide, rutin, sodium nitrite, aluminum muriate and sodium carbonate (analytical grade) were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China).

Equipments and instruments

GZX-9246 MBE Digital blast drying box, Shanghai Boxun Industrial Co., Ltd. medical equipment factory, Shanghai, China; UV-1100 spectrophotometer, Shanghai Meipuda Instrument Co., Ltd., Shanghai, China; RJ-TDL-40C Centrifuge, Ruijiang Analysis Instrument Co., Ltd, Wuxi, China; SHA-C Water-bathing Constant Temperature Vibrator, Jintan Ronghua Instrument Manufacture CO., LTD, China; MJ-25BM04B Mill, Guangdong Midea premium appliances manufacturing Co., Ltd., Guangzhou, China.

Extraction of total flavonoids from peanut hull

Peanut hull was cleaned and drained. Afterward, it was dried for 10 h under 45˚C. After cooling, it was milled and sieved with 60 mesh. 6 g of peanut hull powder was placed into 250 ml-Erlenmeyer flask, and 120 ml of deionized water was added into the Erlenmeyer flask. The pH value of suspension was adjusted with citric acid or ammonia, and a quantity of cellulase was added into the suspension. And then the mixture was shaken with water-bathing constant temperature vibrator for extraction of total flavonoids from peanut hull. Subsequently, the samples were centrifuged and the total flavonoids of peanut hull were assayed.

Determination of total flavonoids

Total flavonoids content was measured according to a colorimetric assay [7]. A 1-mL aliquot of standard solution of rutin at different concentrations (0, 4, 10, 20,

40, 60 and 80 mg L-1) or appropriately diluted extracts of peanut hull was added to 10-mL volumetric flasks containing 4 mL water. At the onset of the experiment, 0.4 mL of 5% $NaNO_2$ was added to the flask. After 6 min, 0.4 mL of 10% $AlCl_3$ was added. At 6 min, 4 mL of 4% NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 mL with water and mixed thoroughly. The absorbance of the mixture was determined at 510 nm versus the prepared blanks. The standard curve of rutin was shown in Fig.1. Total flavonoids were expressed as mg rutin equivalents per g peanut hull.

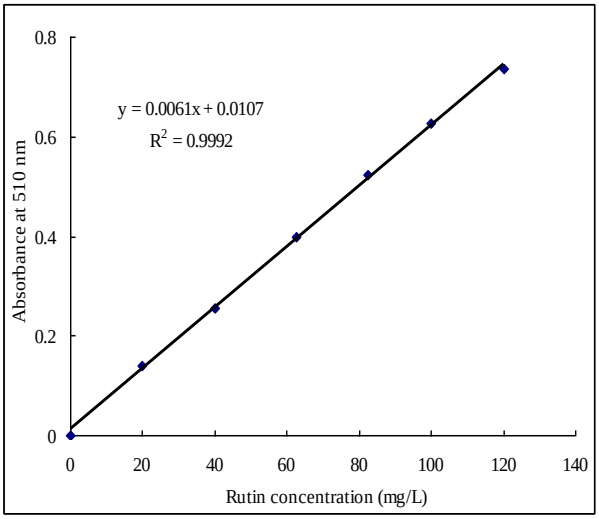

Fig-1: The standard curve of rutin

RESULT ANALYSIS AND DISCUSSION

Enzyme quantity

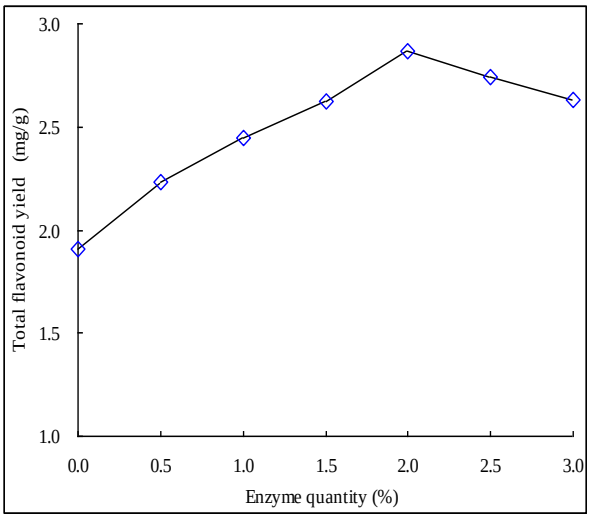

Fig-2: Effect of enzyme quantity on total flavonoids yield of peanut hull

In 120 ml deionized water containing 6 g of peanut powder, pH was adjusted to 5.0, and cellulase was added at 0, 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% of peanut powder, respectively. The suspension was shaken for 120 minutes at 50˚C. As shown in Fig.2, with cellulase quantity enhancement from 0 to 0.9%, the total flavonoids yield of peanut hull increased. When enzyme increased to 2.0%, the total flavonoids yield reached to the maximum of 2.87 mg/g, which was 50.3% higher than that of sample without adding cellulase. Afterward, with enzyme quantity further increase, the total flavonoids yield decreased instead. Cellulase could degrade the cell wall of peanut hull, so proper concentration of cellulase was beneficial to extraction of flavonoids yield. However, excessive cellulase might increase the viscosity of suspension. Flavonoids were water-soluble chemical, and higher viscosity might partly hinder their immigration from peanut hull to suspension [8]. As a result, with cellulase quantity enlargement from 2.0% to 3.0%, the total flavonoids yield decreased.

pH

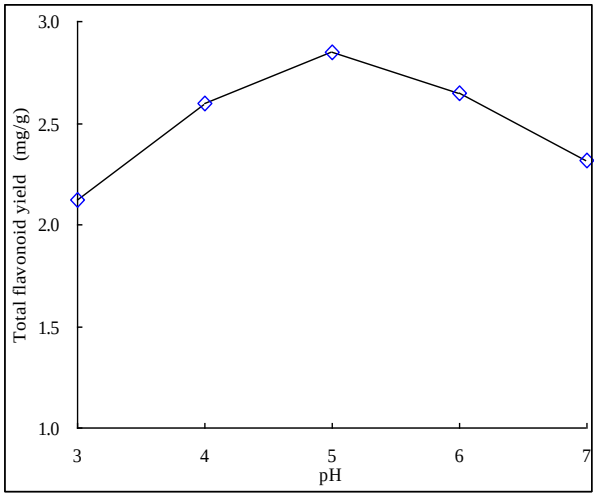

Fig-3: Effect of pH value on total flavonoids yield of peanut hull

In 120 ml deionized water containing 6 g of peanut powder, pH was adjusted to 3, 4, 5, 6 and 7, and cellulase was added at 2.0% of peanut powder, respectively. The suspension was shaken for 120 minutes at 50˚C. The influence of pH on the total flavonoids yield was summarized in Fig.3. The total flavonoids yield increased with the rise of pH value from 3.0 to 5.0. When the pH is 5.0, the total flavonoids yield achieved maximum for 2.85 mg/g. With the pH increasing further, the total flavonoids yield began to drop. As a biological catalyst, enzyme needs to maintain a certain space-conformation [9]. Maybe the catalytic activity of cellulase with the conformation at pH 5.0 was the largest, once the pH deviated 5.0, the conformation changed and catalytic activity decreased accordingly. Thus the total flavonoids yield also decreased.

Temperature

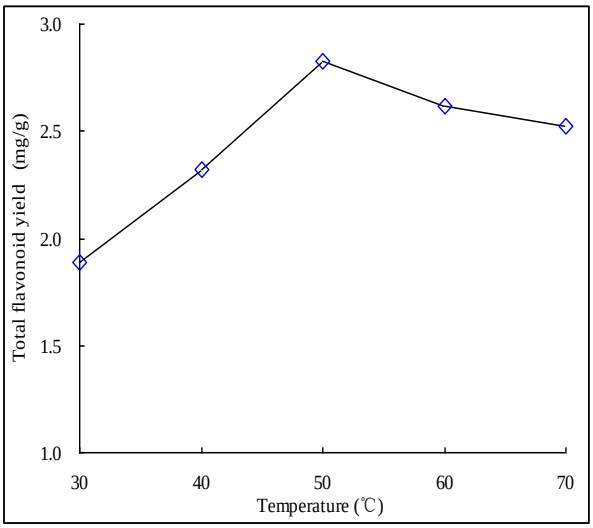

Fig-4: Effect of temperature on total flavonoids yield of peanut hull

In 120 ml deionized water containing 6 g of peanut powder, pH was adjusted to 5 and cellulase was added at 2.0% of peanut powder. The suspension was shaken for 120 minutes at 30, 40, 50, 60, and 70˚C, respectively. As shown in Fig.4, with temperature increasing from 30 ˚C to 50 ˚C, the total flavonoids yield increased accordingly. When the temperature rose to 50 ˚C, the total flavonoids yield achieved maximum for 2.83 mg/g. As the temperature continued to rise, the total flavonoids yield began to drop. When the temperature rose to 70 ˚C, the total flavonoids yield reduced to 2.52 mg/g, which was 11.0% lower than that of 50 ˚C. Enzyme was a kind of biological active protein, and each enzymatic reaction has an optimum temperature. At optimal temperature, enzyme activity reaches the peak, and catalytic efficiency is also the highest [10]. As the temperature further rose, enzymatic passivation occurred gradually, leading to the enzyme activity decline. Therefore, once the temperature was more than 50 ˚C, the total flavonoids yield gradually reduced as temperature rose.

Time

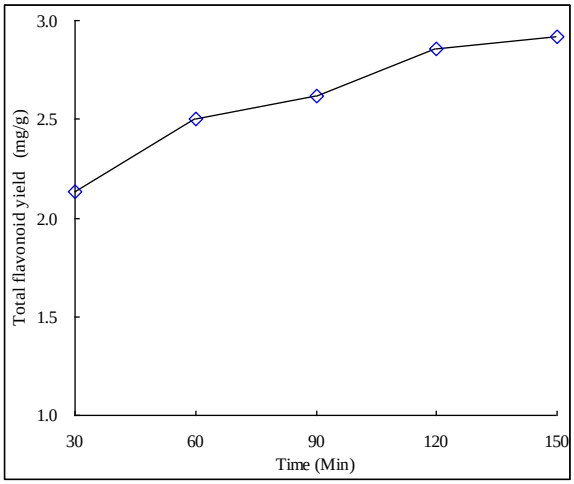

Fig-5: Effect of time on total flavonoids yield of peanut hull

In 120 ml deionized water containing 6 g of peanut powder, pH was adjusted to 5 and cellulase was added at 2.0% of peanut powder. The suspension was shaken for 30, 60, 90, 120 and 150 minutes at 50 ˚C, respectively. During extraction process, the total flavonoids yield of peanut hull increased with enzymolysis time extension (Fig.5). And total flavonoids yield at 150-minute was 33.3% higher that that of 30-minute. With time expansion, cellulase might adequately degrade the cell wall of peanut hull, so total flavonoids yield could effectively emigrate into suspension from cell [11].

Orthogonal experiment

Table-1: Result of orthogonal design $L_9(3^4)$

NO.	A enzyme %	B pH	C temperature ˚C	D time Min	Total flavonoids yield mg/g
1	1(1.8)	1(4.5)	1(45)	1(110)	2.82
2	1	2(5.0)	2(50)	2(120)	2.95
3	1	3(5.5)	3(55)	3(130)	2.85
4	2(2.00)	1	2	3	2.95
5	2	2	3	1	2.97
6	2	3	1	2	2.91
7	3(2.2)	1	3	2	2.85
8	3	2	1	3	2.92
9	3	3	2	1	2.82
k_1	2.87	2.87	2.88	2.87	
k_2	2.94	2.95	2.91	2.90	
k_3	2.86	2.86	2.89	2.91	
R	0.08	0.09	0.03	0.04	

According to orthogonal experiment (in table1), the extraction condition of $A_2B_2C_3D_1$ was the best, namely enzyme quantity 2.0%, pH 5.0, temperature 55˚C, and 110 minutes, and the total flavonoids yield was 2.97 mg/g. Analyzing k value ,the optimizing combination is $A_2B_2C_2D_3$, namely enzyme quantity 2.0%, pH 5.0, temperature 50˚C, and 130 minutes. By test, the total flavonoids yield was 2.99 mg/g. According to range R, in the process of extracting total flavonoids from peanut hull, the influential factors were B > A > D > C, namely pH > enzyme quantity > time> temperature.

CONCLUSION

The total flavonoids yield of peanut hull first increased and then decreased with enzyme quantity enhancement, pH enlargement and temperature rising, and they increased with time extension. The influential degree weakened as follows sequence: pH, enzyme quantity, time and temperature. The most suitable condition for extraction of total flavonoids from peanut hull was enzyme quantity 2.0%, pH 5.0, temperature 50˚C, and 130 minutes. Under the best condition, the total flavonoids yield of peanut hull was 2.99 mg/g.

Acknowledgments

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