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Ultrasonic-assisted Extraction of Total Flavonoid from Peanut Hull using Ionic Liquid Solution

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ABSTRACT----*The total flavonoid of peanut hull was extracted using ionic liquid (1-butyl-3- methylimidazolium bromide) combined with ultrasonic. The result showed that total flavonoid yield of peanut hull increased with the concentration enhancement of ionic liquid and reached to a higher value at 1.2 mol/L of ionic liquid. And total flavonoid yield was higher when the peanut hull was treated with lower frequency or larger power ultrasonic as well. It first increased and then decreased with ultrasonic treatment time extension or temperature increase. The most suitable condition for extraction of total flavonoid from peanut hull was 1.2 mol/L of ionic liquid, 300 W of Ultrasonic power, and 45 kHz of ultrasonic frequency, and the peanut hull was treated for 8 min at 60℃. Under the best condition, the total flavonoid yield of peanut hull was 3.967 mg/g.*

Keywords---- peanut hull, total flavonoid, ionic liquid, ultrasonic

1. INTRODUCTION

Peanut hull is the pod of peanut, having a lower utilization at present. Only a little peanut hull was served as animal feed, edible mushroom cultivation, chemical materials and pharmaceutical production, and most peanut hull was used as fuel or waste. Research suggested that besides abundant carbohydrate and crude fiber, peanut hull contains many active substances such as flavonoid, polyphenols, and so on [1,2]. Flavonoid has the physiological effects of lowering blood pressure, lowering blood lipid, or dilating coronary artery. In addition, it has many pharmacological activity including antioxidant, antitussive, antiasthmatic, anti-inflammatory, antibacterial, immune enhancement and anti-tumor. In the future, flavonoid probably exhibits great potentials in pharmaceutical, cosmetic, food and health care [3].

Ultrasonic may make cell rupture through cavitation effect. Thus, solvent was easy to penetrate into the inner of cell and accelerate the dissolution of target ingredient. Ultrasonic extraction method has the advantages of simple operation, low energy consumption. Moreover, it can shorten extraction time, increase extraction rate of effective components, and reduce the use amount of solvent [4, 5]. Ionic liquid is entirely composed of anion and cation, being a new media and soft functional material. Under the framework of green chemistry, it acquires fast development in recent years, possessing particular characteristics such as no volatilization, wide liquid range, strong solubility and conveniently design regulation [6, 7]. Extraction with ionic liquid is environmentally friendly and improves selectivity, integrating the advantages of efficient extraction and preconcentration.

At present, the extraction methods of flavonoid include solvent extraction, ultrasonic or microwave assisted extraction method [8-10]. In this experiment, based on traditional extraction method of peanut hull flavonoid, ultrasonic and ionic liquid (1- butyl -3- methylimidazolium bromide) were simultaneously applied to extract flavonoid from peanut hull. Our objective was to explore a simple and effective method for flavonoid extraction from peanut.

2. MATERIALS AND METHODS

2.1 Materials and reagents

Peanut hull (peanut with purple skin) was originated of Chengdu, China. 1-Butyl-3-methylimidazolium Bromide (purity≥97%) was purchased from Aladdin Industrial Corporation (Shanghai, China). Absolute alcohol, sodium hydroxide, rutin, sodium nitrite, aluminum muriate and sodium carbonate (analytical grade) were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China).

2.2 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; KQ300VDE ultrasound clean equipment, Kun Shan Ultrasonic Instruments Co., Ltd, Kunshan, China; MJ-25BM04B Mill, Guangdong Midea premium appliances manufacturing Co., Ltd., Guangzhou, China.

2.3 Extraction of total flavonoid from peanut hull

Peanut hull was cleaned and drained. Afterward, it was dried for 10 h under 45℃. After cooling, it was milled and sieved with 120 mesh. 3 g of peanut hull powder was placed into 150 ml-Erlenmeyer flask, and 30 ml of ionic liquid solution of 70% ethanol was added into the Erlenmeyer flask. And then the mixture was treated with ultrasonic at different temperature or time. Subsequently, the samples were centrifuged and the total flavonoid of peanut hull was assayed.

2.4 Determination of total flavonoid

Total flavonoid content was measured according to a colorimetric assay [11]. 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. Total flavonoid content was expressed as mg rutin equivalents per g peanut hull.

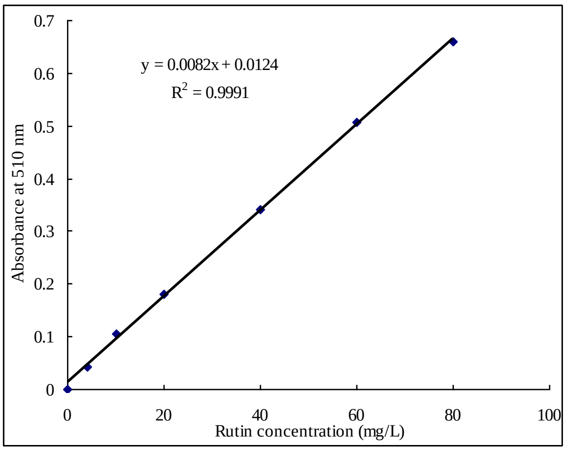

Fig.1 The standard curve of rutin

2.5 Data analysis.

Each treatment was repeated 3 times and the results were averaged. Excel 2003 was used to draw.

3. RESULT ANALYSIS AND DISCUSSION

3.1 Ionic liquid concentration

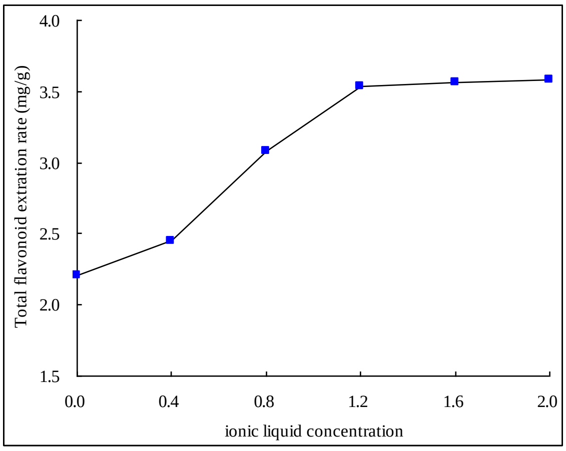

Fig.2 Effect of ionic liquid concentration on total flavonoid yield of peanut hull

3 g of peanut hull powder was placed into 150 ml-Erlenmeyer flask, and 30 ml of ionic liquid solution with the concentrations of 0, 0.4, 0.8, 1.2, 1.6 and 2.0 mol/L was respectively added into the Erlenmeyer flask. Afterward, the mixture was treated with ultrasonic at 60 ℃ through 180W of power and 100 KHz of frequency for 10 min.

As shown in Figure 2, total flavonoid yield of peanut hull generally increased with ionic liquid concentration enhancement. With the concentration enlargement of ionic liquid from 0 to 1.2 mol/L, total flavonoid yield increased by 60.9%, reaching to 3.557mg/g. however, when ionic liquid concentration was further enhanced, total flavonoid yield slightly increased. This suggested that flavonoid ingredients had been probably completely extracted from peanut hull with 1.2 mol/L of ionic liquid. In addition, solution viscosity increased with ionic liquid concentration enhancement and the diffusion capacity of ionic liquid decreased. Accordingly, to penetrate into the matrix sample for ionic liquid became more difficult [12]. Therefore, with the concentration increase of ionic liquid, total flavonoid yield of peanut hull enhanced a little.

3.2 Ultrasonic frequency

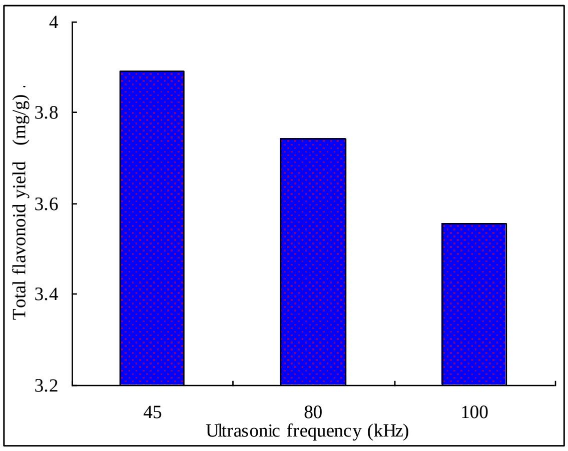

Fig.3 Effect of ultrasonic frequency on total flavonoid yield of peanut hull

3 g of peanut hull powder was placed into 150 ml-Erlenmeyer flask, and 30 ml of 1.2 mol/L ionic liquid solution was added into the Erlenmeyer flask. Afterward, the mixture was respectively treated with ultrasonic at 60 ℃ through 45, 80 and 100 kHz of frequency under 180W of power for 10 min.

The effect of ultrasonic frequency on total flavonoid yield was described in Figure 3. The total flavonoid yield of peanut hull treated at 45 kHz was the highest, the moderate at 80 kHz, and the lowest at 100 kHz. And the yield at 45 kHz was 9.5% higher than at 100KHz. Ultrasonic frequency is the sound vibration number per second, and the intension of ultrasonic cavitation is related to frequency. In high frequency, bubble originated from ultrasonic cavitation is small and its intension is lower. So the disruption degree to peanut hull cell was weaker and the flavonoid ingredients of peanut hull were not efficiently dissolved into solvent. As a result, low flavonoid yield was exhibited under high ultrasonic frequency [13]. Conversely, there was higher flavonoid yield when the peanut hull was treated at low ultrasonic frequency.

3.3 Ultrasonic power

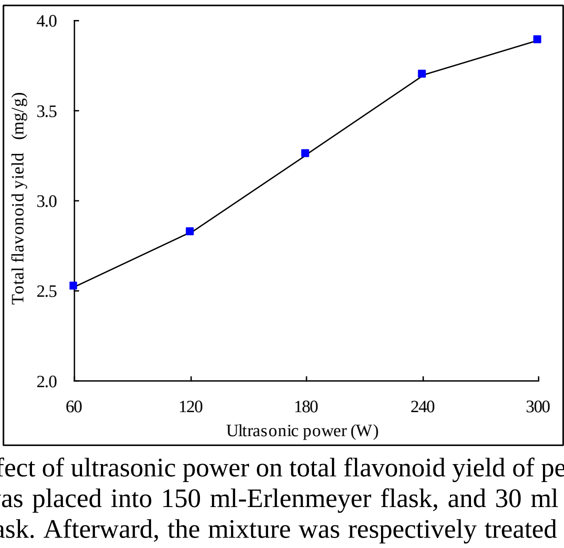

Fig.4 Effect of ultrasonic power on total flavonoid yield of peanut hull

3 g of peanut hull powder was placed into 150 ml-Erlenmeyer flask, and 30 ml of 1.2 mol/L ionic liquid solution was added into the Erlenmeyer flask. Afterward, the mixture was respectively treated with ultrasonic at 60℃ through 45 kHz of frequency under 60, 120, 180, 240 and 300 W of power for 10 min.

As shown in Fig. 4, the total flavonoid yield of peanut hull increased with ultrasonic power enhancement. Total flavonoid yield at 300 W was 3.893mg/g, which was 54.4% higher than that of 60 W. Ultrasonic mechanical effect intensifies with ultrasonic power enhancement. Thus, the moving speed of medium molecules increased. And the flavonoid ingredients of peanut hull were accelerated to dissolve into leaching solution and the extraction time might be shortened [14].

3.4 Ultrasonic treatment time

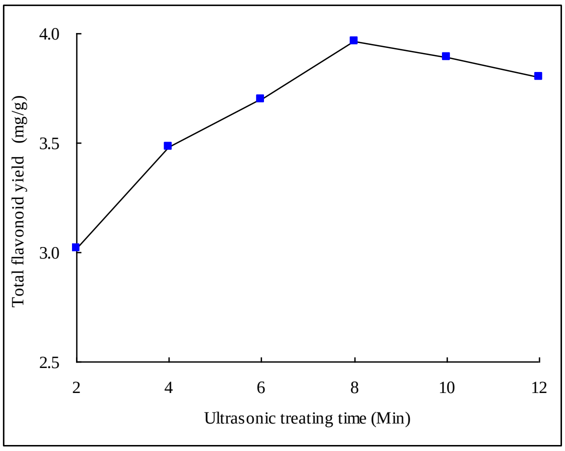

Fig.5 Effect of ultrasonic treatment time on total flavonoid yield of peanut hull

3 g of peanut hull powder was placed into 150 ml-Erlenmeyer flask, and 30 ml of 1.2 mol/L ionic liquid solution was added into the Erlenmeyer flask. Afterward, the mixture was respectively treated with ultrasonic at 60℃ through 300 W of power and 45 kHz of frequency for 2, 4, 6, 8, 10 min.

As shown in Figure 5, total flavonoid yield of peanut hull first increased and then decreased with ultrasonic treating time extension, reaching to the maximum value at 8 min. Before 8 min, it increased with time extension and the yield at 8 min was 31.2% higher than that of 2 min. And from 8 min to 12 min, total flavonoid yield slightly decreased. Properly treating peanut hull with ultrasonic might disrupt the cell of peanut hull owing to cavitation effect, and was beneficial to flavonoid migration from peanut. However, with the extra extension of ultrasonic time, drastic cavitation generated much heat due to local friction, thereby causing the degradation of flavonoid [15].

3.5 Ultrasonic treatment temperature

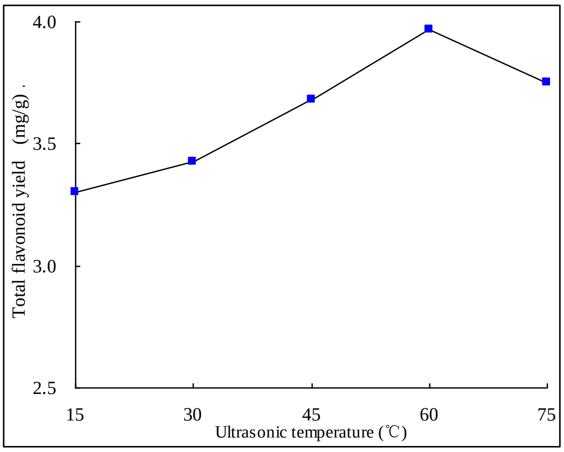

Fig.6 Effect of ultrasonic treatment temperature on total flavonoid yield of peanut hull

3 g of peanut hull powder was placed into 150 ml-Erlenmeyer flask, and 30 ml of 1.2 mol/L ionic liquid solution was added into the Erlenmeyer flask. Afterward, the mixture was respectively treated with ultrasonic at 15, 30, 45, 60 and 75℃ through 300 W of power and 45 kHz of frequency for 8 min.

The total flavonoid yield of peanut hull firstly increased and then decreased with ultrasonic treatment temperature enhancement (Figure 6). It reached to the maximum of 3.967 mg/g at 60℃. From 15 to 60℃, total flavonoid yield increased with temperature enlargement, and the yield at 60℃ was 20.2% higher than that of 15℃. With the further increase of treatment temperature, the yield began to decrease. Total flavonoid yield at 75 ℃ was 5.4% lower than that of 60℃. The reason was probably as followed. Proper high temperature could accelerate polar molecule motion and this was conducive to the dissolution of flavonoid ingredients from peanut hull. But too high temperature could cause flavonoid decomposition [16]. So total flavonoid yield was higher at 60℃ and lower at 75℃.

4. CONCLUSIONS

Total flavonoid yield of peanut hull increased with the concentration enhancement of ionic liquid and reached to a higher value at 1.2 mol/L of ionic liquid. It was higher when the peanut hull was treated with lower frequency or larger power ultrasonic. It first increased and then decreased with ultrasonic treatment time extension or temperature increase. The most suitable condition for extraction of total flavonoid from peanut hull was 1.2 mol/L of ionic liquid, 300 W of

Ultrasonic power, and 45 kHz of ultrasonic frequency, and the peanut hull was treated for 8 min at 60℃. Under the best condition, the total flavonoid yield of peanut hull was 3.967 mg/g.

5. ACKNOWLEDGMENTS

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