

## **Research Article**

# **Hydrophilic nano titanium dioxide assisted ethanol to extract total flavonoids from peanut shell**

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**Abstract:** In this experiment, the effect of hydrophilic nano titanium dioxide on the total flavonoids yield of peanut hull was investigated. Meanwhile, the extraction conditions were optimized. The result showed as followed. Adding hydrophilic nano titanium dioxide into solvent might increase the total flavonoids yield of peanut shell. The most suitable conditions for extraction of total flavonoids from peanut hull through adding 5 mg of hydrophilic nano titanium dioxide in 15 ml dissolvant, were sample to solvent ratio 1:15, ethanol concentration 70%, temperature 55°C, and 65 minutes. Under the best condition, the total flavonoids yield of peanut hull was 2.97 mg/g.

**Keywords:** total flavonoids, peanut hull, hydrophilic nano titanium dioxide, extraction.

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## **INTRODUCTION**

Peanut shell is a by-product, originated of peanut processing. It is usually burned as a waste or garbage and only a small amount served as feed additive or chemical raw material through simply initial processing. This situation wastes natural resources, decreasing the comprehensive utilization of peanut [1]. Peanut shell is rich in flavonoids that have important pharmacological effects such as enhancing immune function, anti-aging, lowering blood lipids and cholesterol, and anti-inflammatory [2, 3, 4]. Most researches suggested that peanut shell extract has excellent antioxidant and antibacterial activity. So extraction of flavonoids from peanut shell has important research value [5].

There were many reported methods to extract total flavonoids from plant material, such as microwave, ultrasonic or ionic liquid extraction method. In addition, enzymatic hydrolysis method for extraction of flavonoids had also been reported [6-9]. At present, some nano technologies had been reported or applied in food packaging, preservation, processing, testing, and so on [10-13]. In this work, hydrophilic nano titanium dioxide was used for extracting the total flavonoids of peanut shell. The effects of sample to solvent ratio, ethanol concentration, temperature and time on total flavonoids yield were investigated as well. This experiment might enrich the extraction methods of peanut shell total flavonoids.

## **MATERIALS AND METHODS**

### **Materials and Reagents**

Peanut hull was originated of Shanxi province, China. Absolute alcohol, sodium hydroxide, rutin,

sodium nitrite, and aluminum muriate (analytical grade) were purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China). Hydrophilic nano titanium dioxide (60nm, Anatase) was purchased from Shanghai crystal pure Industrial Co., Ltd.

### **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. 1 g of peanut hull powder was added into suitable amount of ethanol solution, and then a quantity of hydrophilic nano titanium dioxide was also added into the ethanol solution. Subsequently, the suspension was shaken for some time under different temperature with water-bathing constant temperature vibrator for extraction of total flavonoids from peanut hull. Finally, the samples were centrifuged and the total flavonoids content of peanut hull was assayed.

### Determination of total flavonoids content

Total flavonoids content was assayed according to a colorimetric assay [14]. A 1-mL aliquot of standard solution of rutin at different concentrations (0, 4, 10, 20, 40, 60 and 80 mg L<sup>-1</sup>) 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<sub>2</sub> was added to the flask. After 6 min, 0.4 mL of 10% AlCl<sub>3</sub> 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 deionized 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 content was expressed as mg rutin equivalents per g peanut hull.

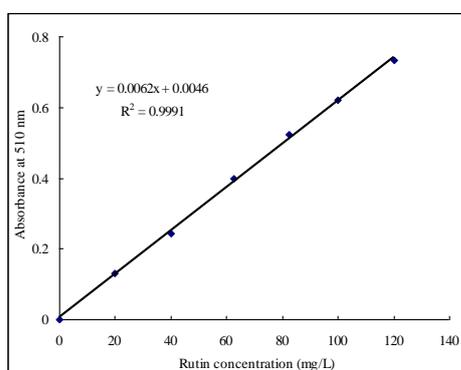


Fig-1: The standard curve of rutin

## RESULT ANALYSIS AND DISCUSSION

### Hydrophilic nano titanium dioxide quantity

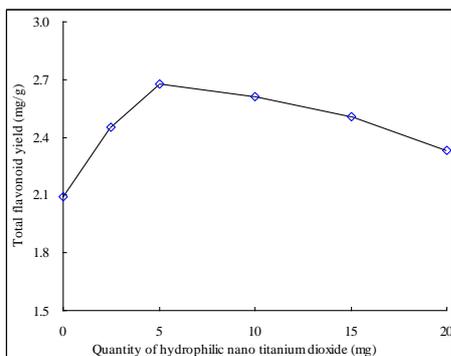


Fig-2: Effect of hydrophilic nano titanium dioxide quantity on total flavonoid yield of peanut shell

1 g of peanut powder was added into 15 ml of 75% ethanol, and then different quantities of hydrophilic nano titanium dioxide were also added into the ethanol. Subsequently, the suspension was shaken for 60 minutes at 55°C. As shown in figure 2, the total flavonoids yield of peanut shell first increased and then decreased with hydrophilic nano titanium dioxide enhancement. When the amount of hydrophilic nano titanium dioxide was 5 mg, total flavonoids yield reached to peak value of 2.68 mg/g, which was 28.2% higher than that of control sample. Afterward, it showed

down trends. The total flavonoids yield of adding 20 mg hydrophilic nano titanium dioxide was 13.1% lower than that of 5 mg.

### Sample to solvent ratio

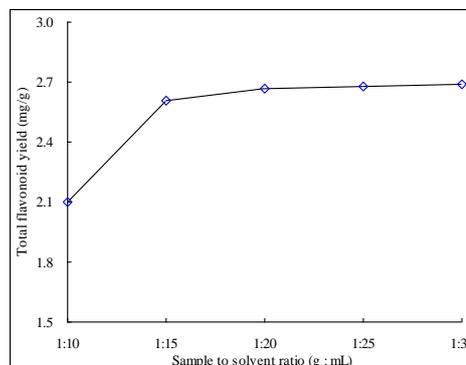


Fig-3: Effect of sample to solvent ratio on total flavonoid yield of peanut shell

1 g of peanut powder was added into 75% ethanol with certain volume, and then 5mg of hydrophilic nano titanium dioxide was also added into the ethanol. Subsequently, the suspension was shaken for 60 minutes at 55°C. As described in Figure 3, the total flavonoids yield of peanut shell gradually increased with solvent proportion expansion. When sample to solvent ratio was 1:15, total flavonoids yield reached to the maximum of 2.61 mg/g, which was 24.3% higher than that of 1:10. As solvent ratio further expanded, total flavonoids yield was almost no increase. Extraction is a solute migration processing from solid substance to liquid solvent. The appropriate increase of solvent ration might enhance concentration difference and promote more flavonoids to migrate into solvent [15].

### Ethanol concentration

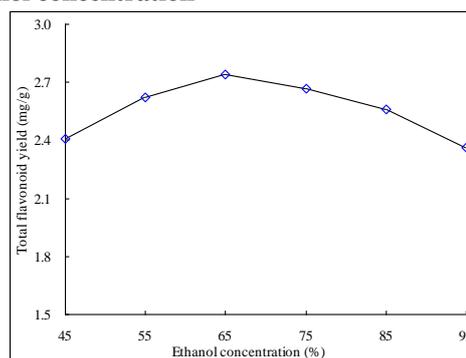
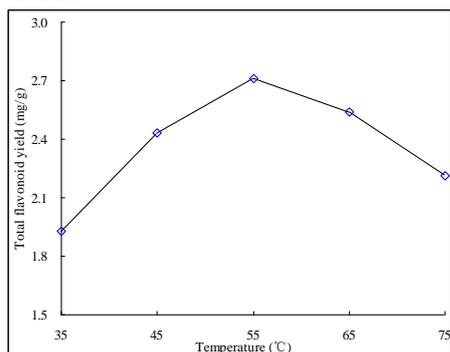


Fig-4: Effect of ethanol concentration on total flavonoid yield of peanut shell

1 g of peanut powder was added into 15 mL of ethanol with certain concentration, and then 5mg of hydrophilic nano titanium dioxide was also added into the ethanol. Subsequently, the suspension was shaken for 60 minutes at 55°C. As shown in Figure 4, the total flavonoids yield of peanut shell first increased and then decreased with ethanol concentration enhancement.

And at the ethanol concentration of 75%, it reached to the maximum of 2.74 mg/g, which was 16.1% higher than that of 75% ethanol. There are hydrophilic groups (hydroxyl) and lipophilic groups (phenyl) in the structure of flavonoids molecule, so certain polarity solvent was beneficial to extract flavonoids from peanut shell [16]. In too low ethanol concentration, solvent polarity was higher; in too high ethanol concentration, solvent polarity was lower. Only a suitable ethanol proportion was the most conducive to solute migration from solid peanut shell to solvent.

**Temperature**

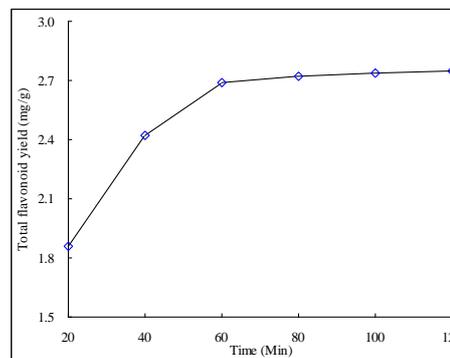


**Fig-5: Effect of temperature on total flavonoid yield of peanut shell**

1 g of peanut powder was added into 15 mL of 65% ethanol, and then 5mg of hydrophilic nano titanium dioxide was also added into the ethanol. Subsequently, the suspension was shaken for 60 minutes under deferent temperature. With temperature rising, the total flavonoids yield of peanut shell first increased and then decreased (Figure 5). At 55°C, it reached to the maximum of 2.71 mg/g. Afterward, total flavonoids yield began to decrease. And the total

flavonoids yield at 75 °C was 22.6% lower than that of 55 °C. Extraction is a solute migration process toward solvent. Higher temperature might accelerate flavonoids molecular motion, promoting flavonoids to migrate into solvent and improving total flavonoids yield [17]. However, excessively high temperature might cause the thermal degradation of flavonoids. Therefore, total flavonoids yield decreased from 55 to 75 °C.

**Time**



**Fig-6: Effect of time on total flavonoid yield of peanut shell**

1 g of peanut powder was added into 15 mL of 65% ethanol, and then 5mg of hydrophilic nano titanium dioxide was also added into the ethanol. Subsequently, the suspension was shaken for some time at 55 °C. As shown in Figure 6, the total flavonoids yield of peanut shell increased with time extension. It nearly reached to equilibrium at 60 minute, being 44.6% higher than that of 20 minute. As time further prolonged, total flavonoids yield slightly increased.

**Orthogonal experiment**

**Table-1: Result of orthogonal design L<sub>9</sub> (3<sup>4</sup>)**

NO.	A Sample to solvent ratio (g : mL)	B Ethanol concentration (%)	C temperature (°C)	D time (Min)	Flavonoid yield (mg/g)
1	1(1:13)	1(60)	1(50)	1(55)	2.76
2	1	2(65)	2(55)	2(60)	2.82
3	1	3(70)	3(60)	3(65)	2.87
4	2(1:15)	1	2	3	2.92
5	2	2	3	1	2.85
6	2	3	1	2	2.89
7	3(1:17)	1	3	2	2.83
8	3	2	1	3	2.92
9	3	3	2	1	2.93
k <sub>1</sub>	2.82	2.84	2.86	2.85	
k <sub>2</sub>	2.89	2.86	2.89	2.85	
k <sub>3</sub>	2.89	2.90	2.85	2.90	
R	0.08	0.09	0.03	0.04	

Based on orthogonal experiment via adding 5mg of hydrophilic nano titanium dioxide, the extraction conditions of  $A_3B_3C_2D_1$  were the best, namely sample to solvent ratio 1:17, ethanol concentration 70%, temperature 55°C, and 55 minutes, and the total flavonoids yield was 2.93 mg/g (in Table1). Analyzing k value, the optimizing combination is  $A_2B_3C_2D_3$ , namely sample to solvent ratio 1:15, ethanol concentration 70%, temperature 55°C, and 65 minutes. By test, the total flavonoids yield was 2.97 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 ethanol concentration > sample to solvent ratio > time > temperature.

## CONCLUSION

The total flavonoids yield of peanut hull first increased and then decreased with hydrophilic nano titanium dioxide quantity enhancement, ethanol concentration enlargement or temperature rising. It first increased and then reached to equilibrium with time extension or solvent ratio expansion. The influential degree strengthened as follows sequence: temperature, time, sample to solvent ratio and ethanol concentration. The most suitable conditions for extraction of total flavonoids from peanut hull through adding 5 mg of hydrophilic nano titanium dioxide in 15 ml dissolvent, were sample to solvent ratio 1:15, ethanol concentration 70%, temperature 55°C, and 65 minutes. Under the best condition, the total flavonoids yield of peanut hull was 2.97 mg/g.

## Acknowledgments

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