

Quantitative Analysis and Bacteriostasis of Polyphenols Extracts from Peanut Shells by Ultrahigh Pressure Extraction

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Keywords: Ultrahigh Pressure Extraction; Peanut Shells Polyphenols; Quantitative Analysis; Bacteriostasis.

Abstract. This study developed a method to extract polyphenols from peanut shells by ultrahigh pressure (PPSUP). The yield of PPSUP was up to 71.3 mg of gallic acid equivalents (GAE) /g under optimizing extraction parameters of ultrahigh pressure to 75% ethanol, 300 MPa, 4 min, 1:25 ratio of material to liquid. The results of antibacterial activities of PPSUP showed that it had bacteriostatic action against two food-related bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538), which indicates it can be used as an antiseptic aid in food.

Introduction

Peanut is cultivated on a large scale, with China, India being the biggest producers ^[1]. Peanut kernel has a high nutritional and commercial value because of its edible oil (43~55%). About 60% of the world's peanut kernels are used for extraction of their oil ^[2]. Peanut shells are abundant agricultural byproducts. Its contain many nutrients, including protein (4~7%), fat (1~2%), carbohydrates (10.6~21.2%), monosaccharide, oligosaccharides, hemicellulose ^[3]. Peanut shells also contain some medicinal ingredients, such as luteolin, β -sitosterol, β -carotene, saponins, and xylose ^[4], as well as antioxidant ingredients, such as catechol, pyrogallol, and pyrogallol acid ^[5]. Therefore, research has been done for development and utilization of peanut shells in the food, chemical, and medical industries as well as agriculture and other fields ^[6].

Hite pioneered research used ultrahigh hydrostatic pressure process to inactivate bacteria in milk and to denature egg albumin proteins in the late 1800s and early 1900s. Approximately 80 years later, ultrahigh pressure technique has been adapted to the specific requirements of food industry ^[7, 8]. It offers benefits such as inactivation of microorganisms and undesirable enzymes, thereby increasing shelf life and providing safer food. Fortunately, these pressure-treated products retain more of their natural colors and flavors for less detrimental effects on food than other methods such as heat treatment ^[9]. Studies showed that ultrahigh pressure technique could obtain higher yields, shorten processing time, and have less negative effects on activity of bioactive components ^[10, 11]. Recently, it had been used for extraction of lycopene from tomato paste waste, ginsenoside from Korean red ginseng, and catechins from green tea. Many efforts separated polyphenols from peanut shells using organic solvent extraction and water extraction successfully at this period. According to above discussion, this study develops a novel method extraction technique, ultrahigh pressure extraction, which can extract polyphenols from peanut shells. In addition, antibacterial activities of PPSUP against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 6538, which commonly cause food borne disease and food spoilage.

Materials and Methods

Peanut were kept in a sealed plastic bag and stored at 4 °C. Ethanol, gallic acid, Folin–Ciocalteu reagent, sodium carbonate were all purchased from the Beijing Chemical Agents Company (China). Luteolin, pyrogallol, catechol, phloroglucinol and quercetin were purchased from Sigma Company

(United States).

A 50 g sample of peanut shells were defatted with n-hexane (3400 mL) for 10 h at room temperature, and the remaining n-hexane in peanut shells was removed under reduced pressure in a vacuum oven. The defatted peanut shells were mixed with aqueous ethanol and placed into a sterile polyethylene bag. The bag was sealed after eliminating air from the inside and placed into a hydrostatic pressure vessel at room temperature under different ultrahigh pressure conditions. The experimental conditions were optimized by using orthogonal experiments. Standard curve was drawn first. Gallic acid standard solution (100 $\mu\text{g/mL}$) was dispensed at volumes of 0.05, 0.10, 0.15, 0.20, 0.25, and 0.30 (all in mL) into a 5 mL volumetric flask and combined with 1.95, 1.90, 1.85, 1.80, 1.75, and 1.70 mL distilled water, respectively. The solutions were mixed for 3-4 minutes after adding extra 1 mL Folin-Ciocalten reagent into each flask. One milliliter of 10 % Na_2CO_3 was then added and, after shaking, put into an electric-heated thermostatic water bath (25 $^\circ\text{C}$) for 2 h. The absorbance of the solution at 750 nm was then evaluated using glass cuvettes. Total phenolic content were determined with the Folin-Ciocalteu method.

Two pathogenic strains of bacteria were used in the antibacterial assay including gram-negative bacteria (*Escherichia coli*, ATCC 25922) and gram-positive bacteria (*Staphylococcus aureus*, ATCC 6538). Bacterial strains were activated by transferred from Luria broth (LB) agar medium into a test tube with sterile saline solution (0.9 % NaCl), and incubated at 36.5 ± 1.0 $^\circ\text{C}$ for 24 h, resulting in an inoculum with $(4-5)\times 10^6$ CFU/mL. All of these bacteria were obtained from the key laboratory of zoonosis research of Jilin University, China.

The antibacterial activity of PPSUP was measured by agar gel diffusion precipitation (AGP) method. Sterile filter paper discs (6 mm diameter) were impregnated with different PPSUP concentrations (100, 1000, 3000, 5000 $\mu\text{g/mL}$) for 24 h before testing. Then, the filter paper discs were placed on the surface of sterile agar plates which had been inoculated with *E. coli* and *S. aureus* in a biological safety hood. The plates were incubated at 36.5 ± 1.0 $^\circ\text{C}$ for 24 h, and the diameters of the clear inhibition zone were measured and reported with a verniercaliper.

MIC was determined by the two-fold dilution method. The *E. coli* was inoculated in the LB medium at 37 $^\circ\text{C}$ for 24h. PPSUP was dissolved in 5% dimethyl sulfoxide (DMSO) and further diluted with 5% DMSO, added to LB medium to achieve final concentrations of 0, 125, 250, 500, 750, 1000, 3000 and 5000 mg/mL. The different concentrations of PPSUP were added in the pre-grown bacterial cultures, incubating at 37 $^\circ\text{C}$ for 24 h. The MIC was defined as the minimum concentrations of PPSUP at which no visible growth was observed.

Results and Discussion

The extraction yields, were defined as the ratio of polyphenols weight to sample weight. The factors that can influence yields are different solvent, pressure level, pressure holding time, liquid/solid ratio, time to achieve treatment pressure, product initial temperature, solvent concentration, and so on.

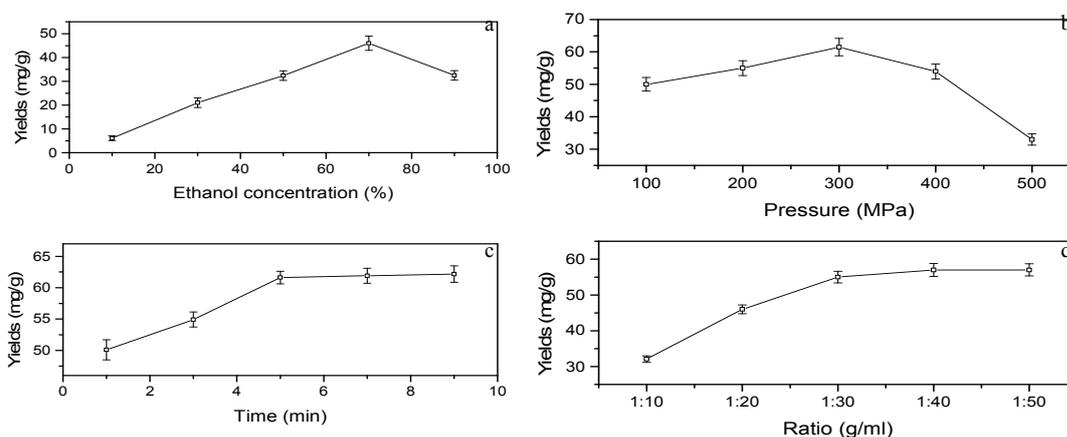


Fig.1 Effect of different factors on yields

Fig.1 (a) illustrated the yields variation trend of PPSUP in single-factor experiments of ethanol concentration. We found that the yields increased gradually as the ethanol concentration increased from 10% to 70%, with the highest yield reached when the ethanol concentration was 70%. Fig.1 (b) was the yields variation trend of PPSUP through single-factor test of ultrahigh pressure. The yields of PPSUP increased as pressure increased from 100 MPa to 300 MPa, and the highest yield were reached at 300 MPa. Fig.1 (c) illustrated the variance of PPSUP yields in single-factor test of pressure holding time. The yields increased as the time increased from 1 min to 5 min. The yields increased slightly with the time increase after 5 min. Five-minute was selected for further orthogonal experiment. Fig.1 (d) showed the yields of PPSUP varying through single-factor test of the ratio of solids to lipids. The yields increased as the ratio of solids to lipids increased from 1:10 to 1:30. The 1:40 was selected for further orthogonal experiments based on the single-factor trend shown in Fig. 1 (d).

The extraction parameters were optimized by the orthogonal experiment method. Table 1 listed the schedule of the orthogonal experiment including four key extraction parameters: (A) ethanol concentration, (B) pressure, (C) pressure holding time, and (D) ratio.

Table 1 Extraction parameters and levels for orthogonal experiment. (a) ethanol concentration, (b) pressure lever, (c) pressure holding time, and (d) ratio of material to liquid

Level	Extraction parameters			
	A (%)	B (MPa)	C (min)	D (g/ml)
1	65	250	4	1:25
2	70	300	5	1:30
3	75	350	6	1:35

Detailed extraction conditions and yields of PPSUP are listed in Table 2. From the mathematical processing of the data, the yield of PPSUP under 300 MPa pressure for 4 min were given the highest. Second, compared with ratio (D), the ethanol concentration (A), the pressure holding time (C), and pressure (B), showed much more significant effect on index point. So the ethanol concentration (A), the pressure holding time (C), and pressure lever (B) are the primary factors that may influence the yields of PPSUP.

Table 2 Experimental conditions and yield obtained during ultrahigh pressure extraction

Run number	A (%)	B (MPa)	C (min)	D (g/ml)	Yield (mg/g)
1	1	1	1	3	58.1±1.7
2	1	2	2	2	61.3±1.5
3	1	3	3	1	64.6±1.3
4	2	1	2	1	66.3±1.6
5	2	2	3	3	70.5±1.2
6	2	3	1	2	68.4±1.4
7	3	1	3	2	69.8±1.6
8	3	2	1	1	71.3±1.8
9	3	3	2	3	65.4±1.3

For further evaluating the performance of ultrahigh pressure extraction, we compared the yields of peanut shells polyphenols through two different extraction methods, ultrahigh pressure extraction and infuse extraction. It can be found in Table 3 that yields (71.3 mg/g) of polyphenols with ultrahigh pressure extraction (300 MPa) for 4 min at room temperature were higher than the yields (56.6 mg/g) of infuse extraction for 5 h at 75 °C.

Table 3 Comparison of process parameters and yield of polyphenols extracted from peanut shells by different methods

Extract method	Ethanol concentration (%)	Pressure level (MPa)	Temperature (°C)	Power (w)	Time	ratio of material to liquid (g/ml)	Yield (mg/g)
Infuse	65	-	75	-	5 h	1 : 30	56.6
Ultrahigh pressure	75	300	-	-	6 min	1 : 25	71.3

Results in this paper indicated that the yield of phenols was 71.3 mg of gallic acid equivalents (GAE) /g of shells under the optimized conditions (75% ethanol–water solution, 300 MPa, 4 min, 1:25 ration of solid to liquid). The maximum yield of total phenols was found to be 118 mg of gallic acid equivalents (GAE) /g of shells under the optimized conditions (30.8% ethanol–water solution, 30.9°C, 12 min).

PPSUP showed significant antibacterial activity against the two food-related bacteria (*E. coli* ATCC 25922, *S. aureus* ATCC 6538) from the data lined in Table 4. Growth inhibition zones of PPSUP with 5000 µg/ml resulted in 9.00, 9.50 mm for *E.coli*, *S.aureus*, respectively, while in infuse method sample, with 5000 µg/ml, these were 8.12, 8.45 mm. These results indicated that the PPSUP samples showed a higher anti-microbial activity than the sample obtained by infuse method.

Table 4 Diameter of inhibition zone and Minimum inhibitory concentrations (MIC) of polyphenols

Strains	Diameter of inhibition zone (mm)								MIC (µg/ml)	
	Infuse(µg/ml)				Ultrahigh pressure(µg/ml)				Infuse	Ultrahigh
	100	1000	3000	5000	100	1000	3000	5000	(µg/ml)	pressure (µg/ml)
<i>E. coli</i> (ATCC 25922)	6.12±0.15 ^a	6.95±0.13	7.96±0.21	8.12±0.23	6.69±0.31 ^a	7.75±0.15	8.65±0.20	9.00±0.1	200	100
<i>S. aureus</i> (ATCC 6538)	6.23±0.19	6.97±0.22	8.01±0.11	8.45±0.14	7.13±0.19	7.56±0.35	8.75±0.22	9.50±0.1	200	100

Conclusions

Ultrahigh pressure extraction is founded to extract polyphenols from peanut shells. In the investigated ranges of ethanol concentration, pressure, time, ratio, the optimized conditions of PPSUP are: 75 % ethanol solution as solvent, 300 MPa for pressure, 4-min pressure holding time, 1:25 of liquid to solid. Comparing with infuse extraction, ultrahigh pressure extraction has excellent advantages, such as shorter extraction time, higher yield, lower energy consumption, eco-friendly and so on. In antibacterial activities test, results showed that a low concentration of PPSUP could inhibit the growth of *E. coli* and *S. aureus*, indicating it could be used as an antiseptic aid in food.

Acknowledgements

The authors wish to thank the China Scholarship Council and the Science and Technology Department of Jilin Province (20140201018NY), China, for financial support of this research.

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