Phenolics Quantitative and their Chemical Fingerprint Analysis in Peanut Shells

Jiying Qiu, Xue Xin, Xiangyan Chen, Shuangzhi Zhao, Qingxin Zhou, Jinyu Yang, Xiaoyong Liu, Junhua Wang and Leilei Chen*

Institute of Agro-Food Science and Technology, China

Received: October 31, 2018; Published: November 13, 2018

*Corresponding author: Leilei Chen, Institute of Agro-Food Science and Technology, China

Abstract

To control the quality of peanut shells, a simple and reliable method by high-performance liquid chromatography (HPLC) was developed for phenolics quantitative determination and fingerprint analysis in 26 peanut cultivars. The ultrasonic extraction process of phenolics was firstly optimized by using 70% ethanol, a solid-liquid ratio of 1:15, and 3 extracted cycles of 50 min each. Three phenolics, including luteolin, eriodictyol, and 5,7-dihydroxychromone were quantified, with ranges of 0.988-4.688, 0.688-2.425, and 0.313-1.038 mg/g, respectively. Total of three phenolics in Shuang-ji 2, Xiang-hua 509-77 and Qing-lan 8 cultivars were 7.851, 7.638, and 7.514 mg/g, respectively, much higher than that in other cultivars. In the fingerprint analysis, a consensus fingerprint with fourteen common characteristic peaks was identified, and the chromatogram similarity values were all over 0.936. Based on these results, the quality of peanut shell used as herb or raw material of Chinese medicine can probably be controlled by a qualification test which was composed of simultaneous quantitative determination of phenolic compounds and fingerprint and similarity analysis.

Keywords: Peanut Shell; Phenolic Compounds; Quantification; Chromatographic Fingerprint

Abbreviations: HPLC: High Performance Liquid Chromatography; DPPH: 1,1-diphenyl-2-picrylhydrazyl; OAD: Orthogonal array design; SFDA: State Food and Drug Administration; RRT: Relative retention time; RPA: Relative peak area; OAD: Orthogonal Array Design

Introduction

Peanut is one of the top five oil crops in the world, is also the major oil and cash crop in China. There are more than three thousand peanut cultivars in China, hundreds of which are cultivated on a large-scale [1]. The peanut shell, a byproduct of oil production, was formerly used as animal feed in China. However, it was actually a traditional Chinese herb, and recently it has been used for drug production, as raw material of “Maishu Jiaonang”, a blood-fat decreasing Chinese medicine in clinical practice. Previous studies have reported that the physiological activities of the peanut shell are closely related to its phenolic compound content. Peanut shells containing higher quantities of phenolic compounds were shown to be more effective as Chinese herbs [2-4]. Therefore, it would be beneficial to develop a rapid method for the separation and quantification of the main phenolic compounds in peanut shells. Peanut shells are rich in phenolic compounds and are safe for use in clinical treatment [5]. The compounds luteolin, eriodictyol, and 5,7-dihydroxychromone have been screened and identified as the main components in peanut shells by the DPPH-HPLC-DAD-TOF/MS method [6]. Luteolin is the most representative bioactive compound in peanut shells. It has become popular as a primary component and is used extensively in synthetic drugs and health products [2].

Furthermore, the bioavailability of luteolin in peanut shell extract is significantly greater than that of pure luteolin [7], and its content varied wildly in different cultivars, as shown in an analysis of 42 peanut cultivars from Korea [8]. Therefore, it is important to screen for peanut shells with higher phenolic content for their application. There have been reports about the quality control of peanut shells, but most are limited to a qualitative analysis of the phenolic compounds or a quantitative analysis of luteolin rather than a chemical fingerprinting study of the peanut shell [9,10]. Chromatographic fingerprint analysis has been widely used for quality control of traditional Chinese herbal medicine, such as Chamomile, Gentiana crassicaulis, Mallotus apelta, and Dioscorea zingiberensis C. H. Wright [11-14]. It utilizes chromatographic techniques to construct specific recognition patterns for multiple...
compounds in herbal drugs [15]. This method has been accepted by the WHO, FDA (2000), and other authorities for herbal medicines quality assessment [15,16].

There is a great diversity of peanut cultivars in China, so it is meaningful to study phenolic compounds from peanut shells of different cultivars and also the chromatographic fingerprint of them. The current study aimed to adequately extract phenolic compounds from peanut shells and simultaneous determine the main phenolic compounds in the shells of 26 peanut cultivars in China and to develop a characteristic fingerprint, which combined with quantitative analysis, to control the quality of peanut shell used as herb or raw material of Chinese medicine.

Materials and Methods

Plant Materials: The shells of 26 peanut cultivars were provided by the Shandong Academy of Agricultural Sciences, including Bai-peanut (S1), Bai-sha 101 (S2), Feng-hua 1 (S3), Hai-hua 1 (S4), Hua-yu 6 (S5), Hei-peanut (S6), Hong-guan (S7), Hong-hua 1 (S8), Hua-guan wang (S9), Ji 9814 (S10), Lu-feng 2 (S11), Lu-hua 11 (S12), Lu-hua 14 (S13), Qing-lan 8 (S14), Shan-hua 9 (S15), Shuang-ji 2 (S16), Si-lihong (S17), Wu-cai peanut (S18), Xiang-hua 509-77 (S19), Xiu-hua 14 (S20), Yuan-hua 8 (S21), Yuan-za 9102 (S22), Zheng-nong 7 (S23), Zhong-hua 15 (S24), Zhong-nong 108 (S25), and Hua-yu 16 (S26). The samples were cleaned and dried at 45 °C for 4 hours. Then the dried shells were crushed into powder, passed through a 60-mesh sieve and stored in a desiccator for further analysis.

Chemicals and Reagents: 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Fresh DPPH stock solution (5 mM) was prepared by dissolving DPPH in methanol prior to the analysis. Reference compounds, including luteolin, eriodictyol, and 5,7-dihydroxychromone, were purchased from Shandong Engineering Technology Research Center (Jinan, China) with purities of over 98%. HPLC-grade methanol was purchased from Shandong Yuwang Group (Yucheng, China). Water for the HPLC analysis was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and solvents used were of analytical grade.

Optimization of Extraction Conditions of Phenolic Compounds from Peanut Shells: Three solvents (100% methanol, 70% ethanol and 60% acetone) were chosen for crude phenolic compound extraction. The extraction conditions were that approximately 1.0 g of peanut shell powder was immersed in 30 mL of solvent and ultrasonicated for 40 min at 40 kHz, 120 W, and 55 °C using X0-SM100 Ultrasonic microwave reaction system (Nanjing Atpio Instruments Manufacturer, Nanjing, China). After cooling to room temperature, made up the solvent to the original weight and centrifuged at 4000 rpm, 4 °C. The supernatants were collected for crude extracts. The respective extraction rates of luteolin, eriodictyol, and 5,7-dihydroxychromone and the DPPH scavenging activity of the crude extracts were taken as indices [6]. The ultrasonic extraction conditions were then optimized by the orthogonal array design (OAD) method. An L9 (34) orthogonal table was used to carry out an optimization experiment, and four important factors were set as follows:

a) Ethanol concentration (60%, 70%, and 80%)

b) Solid-liquid ratio (1:10, 1:15, and 1:20, w/v)

c) Extraction cycles (1, 2, and 3); D: extraction time (30, 40, and 50 min). Finally, the extracted solutions were centrifuged at 4000 rpm at 4 °C, and the supernatants were collected for further use. Total yield of luteolin, eriodictyol, and 5,7-dihydroxychromone in the supernatants was taken as an index.

Quantification of Phenolic Compounds from Peanut Shells by HPLC-DAD: Chromatographic analysis was performed on an Agilent 1200 series high performance liquid chromatography (HPLC) system (Agilent, Palo Alto, CA, USA) equipped with a diode array detector (190-400 nm), a quaternary solvent delivery system, a column temperature controller and an autosampler. The chromatographic data were processed with Agilent Chromatographic Work Station software. HPLC conditions and standard curves of luteolin, eriodictyol, and 5,7-dihydroxychromone were determined as described by Qiu et al. [6], carried out at 30 °C on an Agilent ZORBAS SB-C18 reserved-phase column (250 mmx4.6 mm i.d., 5 μm), with a flow rate of 1.0 mL/min, and a linear gradient elution of eluents A (water-acetic acid, 100:0.2, v/v) and B (methanol) was used for separation, the elution program was shown in Table 1 the fractions were monitored at 294 nm. A quantitative analysis of luteolin, eriodictyol, and 5,7-dihydroxychromone in the shells of 26 peanut cultivars was carried out by an external standard method. Five different concentrations of standard mixtures according to levels estimated in the samples were injected for the calibration curve establishment, and calibration curves were constructed from peak areas versus concentration. The concentrations of luteolin, eriodictyol, and 5,7-dihydroxychromone in the extracts were calculated by corresponding calibration curves and finally converted into content values for the raw peanut shell powders. Each sample was analyzed in triplicate to determine the mean content (mg/g).

Table 1: Elution program of HPLC for quantification of phenolic compounds.

| Time (min) | Elution program A (%) | Elution program B (%) |
|------------|----------------------|----------------------|
| 0          | 95                   | 5                    |
| 3          | 95                   | 5                    |
| 5          | 70                   | 30                   |
| 30         | 50                   | 50                   |
| 60         | 20                   | 80                   |
| 75         | 20                   | 80                   |

HPLC Fingerprint Analysis and Similarity Calculations

Data analysis was performed by a software of the Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A), which was recommended by the State Food and Drug Administration in China (SFDA). The software was employed for synchronization and quantitative comparison between different samples. The correlation coefficients of the...
complete chromatographic profiles of the samples were calculated, the simulative median chromatogram was also calculated and generated, and the similarities of different chromatographic patterns were compared with the median chromatogram. In addition, the relative retention time (RRT) and relative peak area (RPA) of each characteristic peak relative to the reference peak were also calculated for quantitative expression of the chemical properties shown in the chromatographic pattern of the peanut shells.

Results and Discussion

Organic solvents combined with ultrasonic methods have been proven to be efficient for phenolic extraction [17-19] and were both optimized here for phenolic extraction from the peanut shell. The extraction efficiency of different organic solvents, including methanol, ethanol and acetone were firstly compared. The respective extraction rates of luteolin, eriodictyol, and 5,7-dihydroxychromone from peanut shells and the DPPH scavenging activity of the crude extracts were determined (Figure 1). It was shown that the extraction rates of phenolic compounds and DPPH scavenging activities in 70% ethanol and 60% acetone were significantly higher than that in 100% methanol. Finally, ethanol was selected as the extraction solvent because there was no significant difference between 70% ethanol and 60% acetone (P>0.05), and ethanol is more cost effective and less toxic than acetone.

Table 2: Results of the orthogonal design of L9 (34) and range analysis.

| Test No. | A (%) | B (g/ml) | C | D (min) | Results (mg/g) |
|---------|-------|----------|---|---------|----------------|
| 1       | 60    | 1:10     | 1 | 40      | 5.510          |
| 2       | 60    | 1:15     | 2 | 50      | 6.744          |
| 3       | 60    | 1:20     | 3 | 60      | 7.553          |
| 4       | 70    | 1:10     | 2 | 60      | 7.314          |
| 5       | 70    | 1:15     | 3 | 40      | 7.089          |
| 6       | 70    | 1:20     | 1 | 50      | 6.107          |
| 7       | 80    | 1:10     | 3 | 50      | 7.049          |
| 8       | 80    | 1:15     | 1 | 60      | 6.044          |
| 9       | 80    | 1:20     | 2 | 40      | 6.187          |
| k1      | 6.601 | 6.623    | 5.886|        | 6.261          |
| k2      | 6.837 | 6.625    | 6.748|        | 6.633          |
| k3      | 6.427 | 6.616    | 7.230|        | 6.970          |
| R       | 0.410 | 0.009    | 1.344|        | 0.709          |

Figure 1: Effects of solvents on the extraction rates of phenolic compounds and DPPH scavenging of the peanut shell. *Significant at 0.05 level; **Significant at 0.01 level.
Ultrasonic extraction conditions were optimized by the orthogonal array design (OAD) method, which has been commonly used in phenolic extraction [20,21]. Based on the results of single factor tests [22], a further optimization process using OAD method was carried out (Table 2). According to the range analysis, the effects of each factor on the extraction of luteolin, eriodictyol, and 5,7-dihydroxychromone in order were as follows: extraction cycles (C)>extraction time (D)>ethanol concentration (A)>sample-solvent ratio (B). According to variance analysis, the effect of factor C was significant (P<0.1), while the effects of factors D, A and B were not significant (P>0.1). Based on this analysis, the optimum extraction conditions were determined to be an ethanol concentration of 70%, a solid-liquid ratio of 1:15 (g/mL), 3 extraction cycles, and an extraction time of 50 min. And the amount of extracted total phenolics reached as high as 7.553 mg/g. The whole phenolic extraction process is simple, economical and feasible for industrial production.

Table 3: The mean contents (mg/g) of three phenolic compounds in the shells of 26 peanut cultivars.

| Samples | 5,7-dihydroxychromone | Eriodictyol  | Luteolin  |
|---------|------------------------|-------------|-----------|
| S1      | 0.575                  | 1.100       | 3.163     |
| S2      | 0.550                  | 1.075       | 2.300     |
| S3      | 0.839                  | 1.575       | 3.588     |
| S4      | 0.738                  | 2.425       | 2.338     |
| S5      | 0.825                  | 1.550       | 3.463     |
| S6      | 0.575                  | 2.225       | 2.563     |
| S7      | 0.575                  | 0.688       | 2.488     |
| S8      | 0.400                  | 0.763       | 1.400     |
| S9      | 0.875                  | 1.025       | 2.375     |
| S10     | 0.313                  | 1.138       | 0.988     |
| S11     | 0.788                  | 1.988       | 4.075     |
| S12     | 0.563                  | 1.300       | 2.100     |

Note: (A) ethanol concentration; (B) sample-solvent ratio; (C) extraction cycles; (D) extraction time; (ki) The average value of the experimental results corresponding to the horizontal number (i) of any column; (R) The maximum of ki value minus the minimum of ki value under each factor. The peanut shells of 26 peanut cultivars (S1-S26) were extracted by the optimized ultrasonic method described above, and the contents of three main phenolic compounds (luteolin, eriodictyol and 5,7-dihydroxychromone) were simultaneously determined by HPLC. As shown in Table 3, luteolin, eriodictyol, and 5,7-dihydroxychromone were present in the shells of all 26 peanut cultivars, with content ranges of 0.988-4.688, 0.688-2.425, and 0.313-1.038 mg/g, respectively. The total quantity of the three main phenolic compounds in S16, S19 and S14 were, respectively, 7.851, 7.638, and 7.514 mg/g, much higher than in the other cultivars. To obtain a consensus chromatographic fingerprint, the HPLC fingerprints of 26 peanut shell samples were generated at the UV absorption of 294 nm (Figure 2).
The median chromatogram was regarded as the consensus fingerprint of peanut shells. The peaks present in all chromatograms of the samples were assigned as the "common peaks". Fourteen common characteristic peaks in the 26 chromatograms were selected (Figure 3). The retention times of these peaks were 10.37, 12.12, 12.79, 14.22, 19.50, 21.76, 26.41, 28.06, 31.15, 32.61, 34.51, 37.29, 40.42, and 50.27 min, respectively. Peak 11 (retention time=34.51 min, luteolin) was chosen as the internal reference peak to calculate the relative retention time (RRT) and relative peak area (RPA). The RRT and RPA of common peaks in the 26 samples are shown in Table 4. A similarity analysis of all of the peanut shell samples was conducted by comparison to the consensus fingerprint, and the results are shown in Table 5. The closer the cosine values are to 1, the more similar two chromatograms are. The similarity values of the 26 samples were all over 0.936, suggesting that the peanut shells had relatively similar chemical compositions.

### Table 4: The relative retention time (RRT±SD) and the relative peak area (RPA±SD) of fourteen characteristic peaks in chromatograms of 26 samples.

| Peak No. | RRT±SD | RPA±SD  |
|----------|--------|---------|
| 1        | 0.3029±0.0021 | 0.0476±0.0131 |
| 2        | 0.3532±0.0016 | 0.0398±0.0196 |
| 3        | 0.3729±0.0018 | 0.0355±0.0121 |
| 4        | 0.4138±0.0018 | 0.0915±0.0402 |
| 5        | 0.5665±0.0012 | 0.3160±0.0712 |
The results showed that the optimized ultrasonic extraction method is simple, economical and feasible. The contents of luteolin, eriodictyol, and 5,7-dihydroxychromone in the shells of 26 peanut cultivars in China were significantly different (P<0.05) between cultivars. Among them, total of three phenolics in cultivars S16, S19 and S14 were much higher than in the other cultivars, with values of 7.851, 7.638, and 7.514 mg/g, respectively. The result of fingerprint and similarity analysis suggested that the peanut shells had relatively similar chemical compositions. Based on above results, the quality of peanut shell used as herb or raw material of Chinese medicine can probably be controlled by a qualification test which was composed of simultaneous quantitative determination of phenolic compounds and fingerprint and similarity analysis by the lowest possible phenolic content and similarity value recommendation.

**Acknowledgement**

This work was supported by grants from the National Key Research and Development Program of China (No. 2017YFC1601404); the Shandong Provincial Natural Science Foundation of China (No. ZR2016YL022); the Special Funds for the Shandong Foreign Experts Double 100 Plan.

**References**

1. Wan SB (2003) Chinese peanut cultivation, Shanghai scientific and Technical Publishers.
2. Bi J, Yang QL, Yu LN, Sun J, Zhang CS (2011) Evaluation of antioxidant activity of alcoholic extract from peanut hulls. Advanced Materials Research 233: 2849-2854.
3. Win MM, Abdul Hamid A, Baharin BS, Anwar F, Sabu MC, et al. (2011) Phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour. Pakistan Journal of Botany 43(3): 1635-1642.
4. De Camargo AC, Regitano-d Arce MAB, Shahidi F (2017) Phenolic profile of peanut by-products: Antioxidant potential and inhibition of
alpha-glucosidase and lipase activities. Journal of the American Oil Chemists Society 94(7): 959-971.

5. Gao F, Ye HQ, Yu YL, Zhang TH, Deng XM (2011) Lack of toxicological effect through mutagenicity test of polyphenol extracts from peanutshells. Food Chemistry 129(3): 920-926.

6. Qiu JY, Chen LL, Zhu QJ, Wang DJ, Wang RL, et al. (2010) Screening natural antioxidants in peanut shell using DPPH-HPLC-DAD-TOF/MS methods. Food Chemistry 135(4): 2366-2371.

7. Zhou P, Li LP, Luo SQ, Jiang HD, Zeng S (2008) Intestinal absorption of luteolin from peanut hull extract is more efficient than that from individual pure luteolin. Journal of Agricultural and Food Chemistry 56(1): 296-300.

8. Radhakrishnan R, Pae SB, Lee BK, Baek JY (2013) Evaluation of luteolin from shells of Korean peanut cultivars for industrial utilization. African Journal of Biotechnology 12(28): 4477-4480.

9. Zhao DM, Zhang XH, Feng LJ, Qi Q, Wang SF (2011) Sensitive electrochemical determination of luteolin in peanut hulls using multi-walled carbon nanotubes modified electrode. Food Chemistry 127(2): 694-698.

10. Sheng SJ, Zhang LY, Chen G (2014) Determination of 5,7-dihydroxychromone and luteolin in peanut hulls by capillary electrophoresis with a multiwall carbon nanotube / poly (ethylene terephthalate) composite electrode. Food Chemistry 145: 555-561.

11. Zhang XX, Liang JR, Liu JL, Zhao Y, Gao J, et al. (2014) Quality control and identification of steroid saponins from Dioscorea zingiberensis CH Wright by fingerprint with HPLC-ELSD and HPLC-ESI-Quadrupole/Time-of-flight tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 91: 46-59.

12. Tu VA, Cuong NX, Thanh NV, Dimitrov V, Nguyen NH (2016) Development of a chromatographic fingerprint for the quality control of Mallotus apelta by using HPLC-DAD-FLD-ELSD with Malloapelta B as marker compound. Natural Products Chemistry & Research 4: 234.

13. Vapiana A, Struck Lewicka V, Konieczynski P, Wesolowski M, Kaliszan R (2016) An approach based on HPLC-fingerprint and chemometrics to quality consistency evaluation of Matricaria chamomilla L. commercial samples. Frontiers in Plant Science 7: 1561.

14. Song J, Chen F, Liu J, Zou Y, Luo Y, et al. (2017) Combintative method using multi-components quantitation and HPLC fingerprint for comprehensive evaluation of Gentiana criminalis. Pharmacognosy Magazine 13(49): 180-187.

15. Liang YZ, Xie PS, Chen K (2004) Quality control of herbal medicines. Journal of Chromatography B 812: 53-70.

16. Wei H, Sun LN, Tai ZG, Gao SH, Xu W, et al. (2010) A simple and sensitive HPLC method for the simultaneous determination of eight bioactive components and fingerprint analysis of Schisandra sphenanthera. Analytica Chimica Acta 662(1): 97-104.

17. Conde Hernández LA, Guerrero Beltrán JA (2014) Total phenolics and antioxidant activity of Piper auritum and Porophyllum ruderale. Food Chemistry 142: 455-460.

18. Reboredo Rodríguez P, Rey Salgueiro I, Regueiro J, González Barreiro C, Cancho-Grande B, et al. (2014) Ultrasound-assisted emulification-microextraction for the determination of phenolic compounds in olive oils. Food Chemistry 150: 128-136.

19. Rezaie M, Farhoosh R, Iranshahi M, Sharif A, Golmohamadzadeh S (2015) Ultrasonic-assisted extraction of antioxidative compounds from Bene (Pistacia atlantica subsp. mutica) hull using various solvents of different physiochemical properties. Food Chemistry 173: 577-583.

20. Wu T, Yan J, Liu RH, Marcone MF, Aisa HA, et al. (2012) Optimization of microwave-assisted extraction of phenolics from potato and its downstream waste using orthogonal array design. Food Chemistry 133(4): 1292-1298.

21. Zhang B, Han SY, Hu YS, Pu LM, Wang WY (2012) Optimization of Preparation Conditions of Crosslinked Potato Starch Microsphere through Orthogonal Experimental Design. Advanced Materials Research 524: 2188-2198.

22. Qiu JY, Du FL, Chen LL, Liu XY, Sun X, et al. (2011) Optimization of extract technique of total flavonoids from peanut hulls with assistance of ultrasonic. Food and Nutrition in China 17: 34-36.