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Screening natural antioxidants in peanut shell using DPPH–HPLC–DAD–TOF/MS methods

Jiying Qiu [a,1], Leilei Chen [a,1], Qingjun Zhu [a], Daijie Wang [b], Wenliang Wang [a], Xin Sun [a], Xiaoyong Liu [a], Fangling Du [a,*]

[a] Institute of Agro-Food Science and Technology, Shandong Academy of Agricultural Sciences, Jinan 250100, China
[b] Shandong Analysis and Test Center, Shandong Academy of Sciences, Jinan 250014, China

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ABSTRACT

Peanut shell, a byproduct in oil production, is rich in natural antioxidants. Here, a rapid and efficient method using DPPH–HPLC–DAD–TOF/MS was used for the first time to screen antioxidants in peanut shell. The method is based on the hypothesis that upon reaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH), the peak areas of compounds with potential antioxidant activities in the HPLC chromatogram will be significantly reduced or disappeared, and the identity confirmation could be achieved by HPLC–DAD–TOF/MS technique. With this method, three compounds possessing potential antioxidant activities were found abundantly in the methanolic extract of peanut shell. They were identified as 5,7-dihydroxychromone, eriodictyol, and luteolin. The contents of these compounds were 0.59, 0.92, and 2.36 mg/g, respectively, and luteolin possessed the strongest radical scavenging capacity. DPPH–HPLC–DAD–TOF/MS assay facilitated rapid identification and determination of natural antioxidants in peanut shell, which may be helpful for value-added utilization of peanut processing byproducts.

1. Introduction

Peanut (*Arachis hypogaea* L.) is the pod or legume belonging to the family Leguminosae. On a worldwide scale, peanut is grown primarily for its seed oil, and also an important protein food resource in developing and developed countries (Bertiolia et al., 2011; Emekli-Alturfan, Kasikci, & Yarat, 2008). Peanut shells are abundant and inexpensive byproducts of peanut processing operations. Every year, the yield of peanut shells reaches as high as 5 million tonnes in China alone (Liu & Sun, 2010). Most of this agricultural byproduct is set on fire or arbitrarily discarded, except for the small quantity that is manufactured into medium-density fibreboard, agglutinant, plastic stuffing, etc. (Akgül & Tozluoğlu, 2008). However, peanut shells contain many functional components which have been demonstrated to be safe to human (Gao, Ye, Yu, Zhang, & Deng, 2011). The functional compositions in peanut shells still remain underutilized. Therefore, it is of great economic importance to explore the high-value utilization of peanut shells.

Oxidative stress enhances pathological processes contributing to cancer, cardiovascular disease, and neurodegenerative diseases, and dietary antioxidants could counteract these deleterious processes (Mariani, Polidori, Cherubini, & Mecocci, 2005). Many natural antioxidants have been identified from hazelnut, almond, cashew, Brazil nuts, walnuts, and so on (Andrade et al., 2011; John & Shahidi, 2010; Li, Tsao, Yang, Kramer, & Hernandez, 2007; Mourea, Pazos, Medinab, Domíngueza, & Parajó, 2007; Shahidi, Alasalvar, & Liyana-Pathirana, 2007). Peanut shell is also a good and readily available source of natural antioxidants, which has attracted much attention from nutritional and chemical researchers. Peanut shell was demonstrated to be rich in antioxidants and other potential health promoting compounds, which could be extracted for use in food (Duncan, Gorbet, & Talcott, 2006; Negi, Chauhan, Sadia, Rohinishree, & Ramteke, 2005). Many physical and chemical techniques have been used to extract antioxidants from peanuts (Ballard, Mallikarjunan, Zhou, & O'Keefe, 2009; Chukwumah, Walker, Verghese, & Ogutu, 2009; Monagas et al., 2009; Potrebko & Resurreccion, 2009; Yu, Ahmedna, & Goktepe, 2005). Duh, Yeh, and Yen (1992) isolated eighteen fractions from peanut shells by thin-layer chromatography (TLC). Recently, four compounds with antioxidant activities have been isolated from the methanolic extract of peanut shells and identified as 5,7-dihydroxychromone, eriodictyol, 3′,4′,7-trihydroxyflavanone, and luteolin by electron impact-mass spectrometry (EI-MS) and nuclear magnetic resonance (NMR) analysis (Wee et al., 2007).

Despite the employment of many methods in the extraction of antioxidants from peanut shells, the strategy used now is time-consuming, cumbersome, and less efficient for screening

* Corresponding author. Address: Institute of Agro-Food Science and Technology, Shandong Academy of Agricultural Sciences, 202# Gongye North Road, Jinan 250100, China. Tel.: +86 531 83179292.
E-mail addresses: chenleilei@yahoo.cn, dufl@saas.ac.cn (F. Du).
[1] These authors contributed equally to this work.

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antioxidants from peanut shells, thus could not be used in a processing plant scale. Additionally, the activities of some natural antioxidants are often decreased during the isolation and purification processes due to their decomposition. DPPH–HPLC–DAD–TOF/MS, which was developed by Tang, Li, Chen, Guo, and Guo (2008), had been successfully used in the identification and quantitative analysis of antioxidants in Chinese medicinal herbs. The hypothesis was that upon reaction with 1,1-diphenyl-2-picrylhydrazyl (DPPH), the peak areas (PAs) of compounds with potential antioxidant effects in the HPLC chromatogram would be significantly reduced or disappeared, and the identity confirmation could be achieved by HPLC–DAD–TOF/MS technique (Tang et al., 2008). Here, HPLC–DAD–TOF/MS technique was used for the first time to screen antioxidants from methanolic extract of peanut shells.

2. Materials and methods

2.1. Materials

Peanut shell of variety "Huayu 16" was provided by Shandong Academy of Agricultural Sciences. The outer shell accounts for about 33% of the whole peanut weight of variety "Huayu 16". 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Fresh DPPH stock solution (5, 10, 15 mM) was prepared by dissolving proper amount of DPPH in methanol before analysis. Reference compounds, including 5,7-dihydroxychromone (1), eriodictyol (2), and luteolin (3) (Fig. 1), were purchased from Shandong Engineering Technology Research Center (Jinan, China) with purities of over 98%. L-Ascorbic acid was purchased from Sigma (St. Louis, MO, USA), and methanol of HPLC grade from Shandong Yuwang Group (Yucheng, China). All other chemicals and solvents used were of analytical grade.

2.2. Preparation of Peanut Shell extract

Peanut shells were cleaned and dried at 45 °C for about 3–4 h. The dried peanut shells were then crushed into powder of 60 mesh. Dried powder (approximately 1.0 g) was immersed in 30 mL methanol, and ultrasonicated for 40 min at 40 kHz, 120 W, and 55 °C using BK-120F Ultrasonic cleaners (Jinan, China). After cooling to room temperature, the methanolic solution was centrifuged at 4000 rpm, 4 °C. The supernatant was collected, dried by vacuum evaporation, and then redissolved in a volumetric flask containing 10 mL methanol. The prepared methanol extract was immediately used or kept at 4 °C for further analysis.

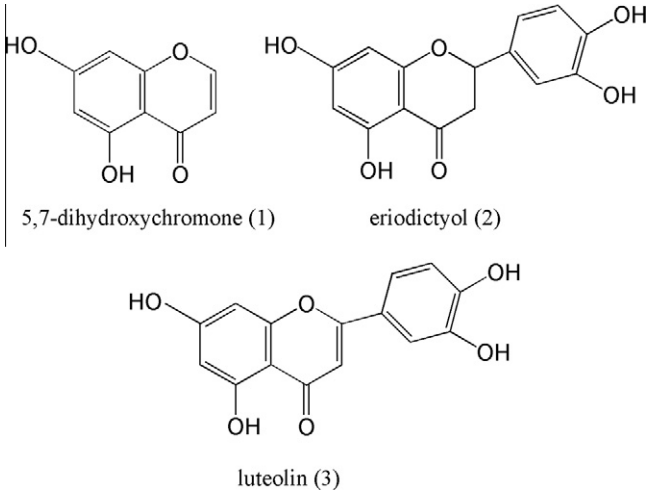

Fig. 1. Chemical structure of the three reference compounds.

2.3. DPPH–HPLC analysis for screening of main antioxidants in peanut shell extract

The methanolic extract of peanut shells was mixed with DPPH of different concentrations (5, 10, 15 mM) at the ratio of 1:1 (v/v), and then the mixture was incubated at room temperature for 30 min. After that, the mixture was filtered through a 0.45 μm filter paper, and then applied onto the HPLC column. The control sample was prepared by adding methanol instead of DPPH to the methanolic peanut shell extract.

The mixture and control samples were analyzed using a LC-1200 high performance liquid chromatograph (HPLC) (Agilent, Palo Alto, CA, USA) equipped with a ZORBAX Eclipse SB-C18 column (Agilent) and a photodiode array detector (DAD). HPLC conditions including detection wavelength and the elution gradient of mobile phase were optimized. With a flow rate of 1.0 mL/min at 30 °C, the sample injection volume was set at 10 μL, and acetic acid (0.2%) and methanol were used as the mobile phases A and B, respectively. The solvent gradient was used as follows: 5% B at 0–3 min, 5–30% B at 3–5 min, 30–50% B at 5–30 min, 50–80% B at 30–60 min, 80% B at 60–75 min. Fractions was monitored at 294 nm. By comparing the chromatographic profiles of DPPH-reacted samples and control samples, the main antioxidants in peanut shell extract could be screened and collected.

2.4. Identification of main natural antioxidants in peanut shell extract by Q-TOF LC/MS and HPLC

In order to identify the screened antioxidants, Q-TOF LC/MS was used to determine their molecular weights. It was performed on a Q-TOF LC/MS equipped with an electrospray interface (Agilent 6520). TOF/MS analysis was performed in a positive mode using full scan mode and the mass range of 100–3000 Da. The conditions of the ESI source were used as follows: drying gas (N_2) flow rate, 10.0 L/min; drying gas temperature, 350 °C; Nebulizer, 30 psi; capillary voltage, 3500 V; fragmentor 175 V; skimmer voltage, 65 V; and octopole radio frequency, 750 V. Reference masses consisted of unknown fluorinated compounds which were provided by the manufacturer (Agilent) with empirical formulas.

To further identify the main antioxidants screened by HPLC, three reference compounds having the same molecular weights with the screened antioxidants, including 5,7-dihydroxychromone, eriodictyol, and luteolin, were used. The peanut shell extract and reference compounds were, respectively, applied onto the C-18 HPLC column, performed at the same HPLC conditions, and the consistency of their retention time was compared.

2.5. Quantitative analysis of main natural antioxidants in peanut shell extract

The quantitative analysis was performed using an external standard method with brief modifications (Zhang et al., 2007). Mixed standard methanol solutions containing 5,7-dihydroxychromone (10.87–108.7 μg/mL), eriodictyol (9.47–94.7 μg/mL) and luteolin (15.2–152 μg/mL) were prepared, and then respectively injected onto HPLC column. The HPLC conditions were used as mentioned above. Standard curves of three reference compounds were made based on the linear relationship between concentrations and peak areas (PAs), and the main antioxidants in the extract of peanut shell powder were calculated.