ความสามารถในการต้านออกซิเดชันและปริมาณสารฟีโนลิครวมของเยื่อหุ้มเมล็ดถั่วลิสง

Antioxidant capacity and total phenolics of peanut testae

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บทคัดย่อ: เยื่อหุ้มเมล็ดถั่วลิสงมักจะถูกแยกทิ้งไปในการแปรรูปถั่วลิสง ซึ่งเยื่อหุ้มเมล็ดถั่วลิสงนี้ประกอบไปด้วยสารพฤกษเคมีที่มีประโยชน์ ซึ่งสามารถต้านการเกิดออกซิเดชันทั้งในหลอดทดลองและในอาหาร การศึกษานี้มีวัตถุประสงค์เพื่อวิเคราะห์ความสามารถในการต้านการเกิดออกซิเดชันของเยื่อหุ้มเมล็ดถั่วลิสง 9 สายพันธุ์ ผลการวิเคราะห์แสดงให้เห็นว่า เยื่อหุ้มเมล็ดถั่วลิสงมีน้ำหนักโดยเฉลี่ยเท่ากับ 3.62 ± 0.80% ของน้ำหนักถั่วทั้งเมล็ด และมีความชื้นโดยเฉลี่ยเท่ากับ 11.00 ± 0.90 % สารสกัดด้วยเมทานอลจากเยื่อหุ้มเมล็ดถั่วลิสงสามารถยับยั้งอนุมูล DPPH และอนุมูลไฮดรอกซิลได้ในหลอดทดลอง ปริมาณสารฟีโนลิครวมของเยื่อหุ้มเมล็ดถั่วลิสง 9 สายพันธุ์มีค่าในช่วง 6,267.70 – 10,292.08 mg GAE/100 g โดยมีค่าเฉลี่ยเท่ากับ 8,554.97 ± 1,264.80 mg GAE/100 g ปริมาณสารฟีโนลิครวมนี้มีความสัมพันธ์แบบผกผันกับค่า IC_{50} ของสารสกัดจากเยื่อหุ้มเมล็ดถั่วลิสงทั้งจากวิธีที่ใช้ DPPH และ deoxyribose โดยพบว่ามีค่าสัมประสิทธิ์สหสัมพันธ์เท่ากับ -0.69 และ –0.64 ตามลำดับ ซึ่งความสัมพันธ์ดังกล่าวหมายถึง ปริมาณสารฟีโนลิครวมมีความสัมพันธ์โดยตรงกับความสามารถในการต้านการเกิดออกซิเดชันของเยื่อหุ้มเมล็ดถั่วลิสง

คำสำคัญ: เยื่อหุ้มเมล็ดถั่วลิสง, ความสามารถในการต้านออกซิเดชัน, ปริมาณสารฟีโนลิครวม

ABSTRACT: Peanut testae or peanut skins are normally discarded as a by-product of peanut processing. Peanut testae contain some beneficial phytochemicals that can show the antioxidant effect *in vitro* and in food. Therefore, the aim of this study was to determine the antioxidant capacity of the peanut testae of 9 peanut varieties. The average amount of peanut testa was 3.62 ± 0.80 % of whole seed. The average moisture content of peanut testae was 11.00 ± 0.90 %. The methanol extracts of peanut testae exhibited the ability to scavenge both DPPH radical and hydroxyl radical *in vitro*. The total phenolic contents of peanut testae were 6,267.70 – 10,292.08 mg GAE/100 g, with an average of 8,554.97 ± 1,264.80 mg GAE/100 g. The total phenolics of peanut testae exhibited a negative correlation with IC_{50} values of peanut testa methanol extracts obtained from both DPPH assay and deoxyribose assay, by giving the correlation coefficient (r) values of -0.69 and -0.64, respectively. This implied the positive relationship between total phenolics and antioxidant capacity of peanut testae.

Keywords: peanut testa, antioxidant capacity, total phenolics

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Introduction

Peanuts (*Arachis hypogaea* L.) are grown primarily for their seeds, which are used as foods such as roasted peanut, peanut confection and peanut butter. Mature seeds are normally roasted before use or consumption. The seed coats or skins or testae are normally removed and discarded as a by-product of peanut production processing. According to Sobolev and Cole (2003), based on world in-shell peanut production of 29.1 million tons in 1999/2000 and an average skin content of 2.6 %, world production of peanut skin could be estimated at over 750,000 tons per year. The commercial value of peanut skins is extremely low and they are used as a minor component in cattle feed. Peanut skins were investigated and found that they consisted of some oils, proteins and phenolic compounds. The composition of peanut skin, especially the phenolic content has been interested by many researchers. Hundred of studies showed that phenolic compounds obtained from vegetables and fruits could inhibit or scavenge free radicals *in vitro*. Scientists in many countries have already investigated peanut skin in order to search for the active compounds, which could be used in foods, drugs and cosmetics for specific purposes. In Thailand, peanuts are produced about 60,000 tons annually, and peanut testae are used for producing the valueless compost. It would be more valuable for peanut testae, if they could be used for a production of bioactive compounds in the future. The aim of this study was to determine antioxidant capacity and total phenolic content in the peanut testae of peanut lines selected from Khon Kaen University.

Materials and methods

Nine genotypes of peanut were obtained from Faculty of Agriculture, Khon Kaen University. They were TN 9, KK 4, KK 6, KK 60-3, KS 2, KKU 1, KKU 40, KKU 60 and KKU 72-1, which were stored at 4°C prior to analysis. Peanut testae were manually removed from seeds of each genotypes. The weight percentage of peanut testae was determined. Moisture content of peanut testae was determined by weight difference after drying at 70°C for at least 5 hrs in an oven.

Extraction of peanut testa

One gram of peanut testae was ground with liquid nitrogen in a mortar. Fifty milliliters of methanol was added to ground peanut testae and stirred for 2 hrs at room temperature in a beaker and filtered through Whatman no. 4 paper. Then methanol was evaporated at 60°C by rotary evaporator. The methanol extract was collected and blown with nitrogen gas until 2 mL was obtained. The methanol extract of peanut testae was kept at 4°C until used.

Total phenolic content

Total phenolic content of the methanol extract of peanut testae was determined by the Folin-Ciocalteu assay (Torres et al., 1987). Fifty microliters of the methanol extract was added to the mixture of 3.0 mL of distilled water, 0.25 mL of Folin-Ciocalteu reagent and 0.75 mL of 20% Na_2CO_3. The final volume of 5 mL was made up by adding distilled water. The mixture was vortexed and incubated at 50°C for 2 hrs. Then the absorbance was read at 765 nm. The phenolic content was expressed as gallic acid equivalent (mg GAE/100 g of peanut testae).

Antioxidant capacity

Two assays for evaluation of antioxidant capacity were DPPH assay (Kang and Salveit, 2002) and Deoxyribose assay (Chung et al., 1997). For DPPH assay, 10 μL of the appropriate concentrations of methanol extract was added to 3 mL of 0.1 mM DPPH (in methanol) and mixed well. The absorbance was read at 515 nm within 2 min. The antioxidant capacity was calculated as 50 % Inhibitory Concentration (IC_{50}). For Deoxyribose assay, 20 μL of the appropriate concentrations of methanol extract was added to a mixture of 0.1 mL of 10 mM $FeS0_4.7H_2O$, 0.1 mL of 10 mM EDTA and 0.2 mL of 10 mM 2-deoxyribose. Phosphate buffer (0.1 M) pH 7.4 was added to make a volume of 1.8 mL. Twenty microliters of 10 mM H_2O_2 was added to the mixture, which was then incubated at 37°C for 1 hr. One milliliters of 2.8 % trichloroacetic acid solution and 1 mL of 1 % thiobarbituric acid solution were added into the mixture and kept in boiling water for 10 min. The mixture was cooled immediately and the absorbance was recorded at 532 nm. The antioxidant capacity was calculated as IC_{50}.

Statistical analysis

The experimental results were expressed as mean ± standard deviation. The correlation coefficient (r) values between total phenolics and IC_{50} values were determined. Analysis of variance was performed by ANOVA procedure. Differences at $p < 0.05$ were considered statistically significant.

Results and discussion

The results of this study were summarized in **table 1. Table 1** shows testae content of peanut seed, moisture content, antioxidant capacity and total phenolic content of each peanut lines in this study. The average weight percentage of peanut testae was 3.62 ± 0.80. The moisture contents of peanut testae of all peanut lines were not significantly different. The average moisture content was 11.0 ± 0.90 %.

Table 1 Testa content of peanut seed, moisture content, antioxidant capacity and total phenolics in peanut testa

Peanut genotypes	Testa (%)	Moisture (%)	IC_{50} (%, v/v) (DPPH assay)	IC_{50} (%, v/v) (Deoxyribose assay)	Total Phenolics (mg GAE/100 g)
KKU 1	3.95±0.04b	9.98±0.00a	0.0088±0.0006a	0.047±0.0021a	7,518.66±345.01c
KKU 72-1	4.51±0.47ab	11.30±2.80a	0.0077±0.0001b	0.050±0.0006a	9,001.82±543.68b
KKU 60	2.98±0.03c	10.90±1.05a	0.0065±0.0001c	0.043±0.0006b	10,292.08±854.90a
TN 9	2.47±0.04d	11.82±0.28a	0.0078±0.0002b	0.050±0.0017a	8,631.14±362.28b
KKU 60-3	3.41±0.02c	10.55±0.01a	0.0080±0.0005b	0.043±0.0010b	8,607.14±307.15b
KK 4	3.30±0.33c	11.42±0.43a	0.0085±0.0002a	0.048±0.0020a	7,534.41±822.52c
KS 2	5.13±0.01a	10.56±0.47a	0.0076±0.0001b	0.051±0.0026a	6,267.70±388.02c
KKU 40	3.41±0.18c	12.64±2.57a	0.0065±0.0002c	0.042±0.0006b	9,863.31±536.07a
KK 6	3.43±0.64c	9.84±0.19a	0.0063±0.0064c	0.037±0.0025c	9,270.39±397.59a
Average	3.62±0.80	11.00±0.90	0.0075±0.0009	0.046±0.0046	8,554.97±1,264.80

Values are means ± SD. Different letters within the same column indicate significant different at $p < 0.05$.

The total phenolic contents of methanol extract of peanut testae were in the range of 6,267.70 - 10,292.08 mg GAE/100 g with the average of 8,554.97 ± 1,264.80 mg GAE/100 g. This result was comparable to total phenolic contents of 9,010 mg GAE/100 g dry skin reported by Yu et al. (2005) and 9,700 mg GAE/100 g of defatted peanut skin reported by Wang et al. (2007). Yu et al. (2005) identified that phenolic compounds in peanut skin were phenolic acids, flavonoids and stilbenes (resveratrol). Francisco and Resurreccion (2009) could quantify the amount of each phenolic compound in peanut skin. They found that flavanols (particularly epigallocatechin, catechin and epicatechin) were the most abundant phenolics in peanut skin.

The antioxidant capacities of methanol extracts of peanut testae of all peanut lines determined by DPPH assay and Deoxyribose assay were also shown in **Figure 1 and Figure 2**, respectively. The methanol extracts of peanut testae showed the ability to scavenge DPPH radical and hydroxyl radical from DPPH assay and Deoxyribose assay, respectively. The IC_{50} values (DPPH assay) were very low compared to those of methanol extracts of whole seeds (data not shown). This implied that higher content of antioxidants were found in peanut testa. Yu et al. (2005) found that antioxidant activity of peanut skin extracts (both water and ethanol extracts) exhibited approximately 2 times higher than those of green tea extracts. O'Keefe and Wang (2006) revealed that phenolic compounds of peanut skin (200-400 ppm) could significantly reduce the oxidation of meat products and extend their storage stability without negative effects on color and aroma. The negative correlation coefficient (r) values were demonstrated between total phenolics and IC_{50} values of both assays (r = -0.69 for DPPH assay and r = -0.64 for Deoxyribose assay). These values implied that peanut testae with higher phenolic content exhibited higher antioxidant capacity (lower IC_{50} value). This research is a preliminary study of the peanut testae from peanut lines grown in Thailand. We will extend our study in the near future to identify and quantify phenolic compounds in peanut testae and show how they could be a valuable natural compound to be used as food ingredient or food additive for specific purposes.

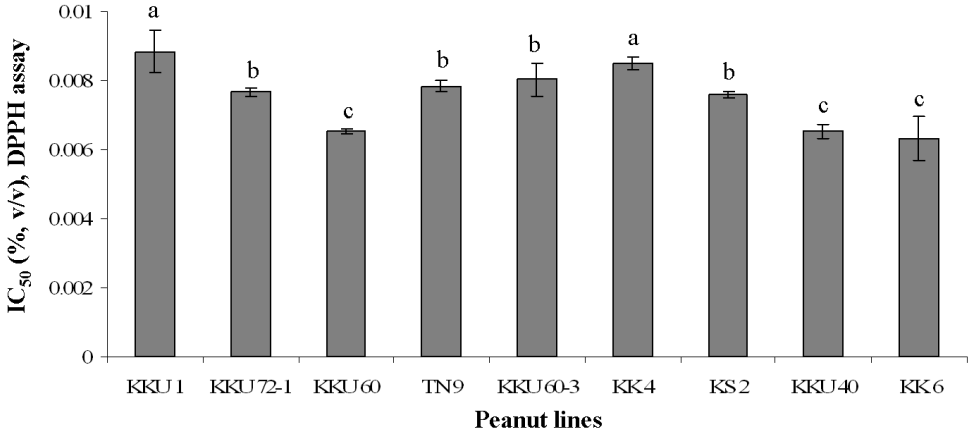

Figure 1 IC_{50} values of peanut testae determined by DPPH assay. Different letters indicate significant different at $p < 0.05$

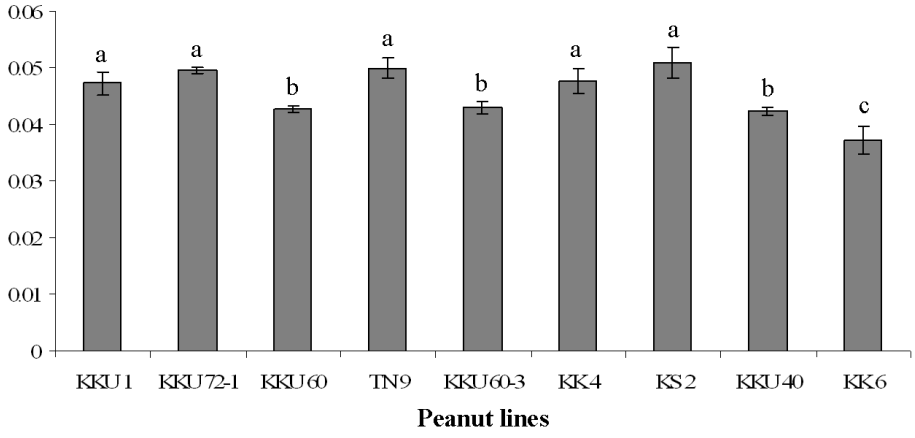

Figure 2 IC_{50} values of peanut testae determined by Deoxyribose assay. Different letters indicate significant different at $p < 0.05$

Conclusion

The average of weight percentages for peanut testae from whole seed was 3.62 $\pm$ 0.80 %. The moisture contents of peanut testae from 9 peanut lines tested were not significantly different. The average of total phenolics contents of peanut testae was 8,554.97 $\pm$ 1,264.80 mg GAE/100 g. The methanol extracts exhibited the ability to scavenge both DPPH radical and hydroxyl radical *in vitro*. The antioxidant capacity of peanut testae appeared to have a correspondence with the quantity of total phenolic content present in the peanut testae.

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