Antioxidant activity of Peanut (*Arachis Hypogaea* L.) Skin Extract: Application in Soybean and Mustard Oil

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Abstract: Total phenolics of peanut skin obtained by different methods were estimated; the effect of different solvents on extractability of total phenolic compounds has also been evaluated. The effect of peanut skin extract, possessing highest phenolics and DPPH radical scavenging activity, was evaluated by schaal oven test in the soybean oil, while oxidative stability of mustard oil was evaluated by Rancimat method. Total phenolics were obtained highest (76.0 ± 2.12 to 101.7 ± 5.54 mg/g GAE dw) in the roasted samples, followed by the dry samples (54.7 ± 1.78 to 89.1 ± 3.78 mg/g GAE dw), and lowest (14.5 ± 0.95 to 21.6 ± 1.02 mg/g GAE dw) in the blanched samples; 80% methanol extraction provided better extractability of phenolic compounds than the aqueous and 80% ethanol extraction method. The peroxide value and induction period of different oils was also evaluated in the present study, which clearly showed that peanut skin extract offered significantly ($P < 0.05$) better or at least similar protection against oxidation in the oils; than the synthetic antioxidant (BHT). Thus, peanut skin may be a good source of natural antioxidants for stabilization of various vegetable oils, during harsh processing and unavoidable storage conditions.

Keywords: Antioxidant, DPPH, Induction time, Peanut skin, Peroxide value, Vegetable oils.

INTRODUCTION

The shelf-life of the raw or processed food is decided mainly by two parameters: oxidation and microbial contamination [1]. Oxidation reactions mainly occur in the unsaturated fraction of lipid, which result in development of off-flavor [2, 3]. Thus, lipid oxidation reduces the consumer acceptability of food products. Vegetable oils are the main source of fat in our diets and their use in culinary processes is a constant. However, intense heating during frying and microwave cooking, improper storage conditions and presence of prooxidants are known to induce oxidative deterioration of vegetable oils; therefore, it is necessary to find suitable strategies to maintain the oxidative stability of vegetable oils.

Various antioxidants are used in food products to prevent lipid oxidation. The most popular antioxidants are *BHA* (butylated hydroxyanisole), *BHT* (butylated hydroxytoluene), *PG* (propyl gallate) *etc*. However, these synthetic antioxidants have been proved for their negative health implications like cancer, endocrine malfunction, affect organ function, reproductive disorders; thus, the food processing industry now prefers natural antioxidants, free of any synthetic chemicals, and provide similar protection on lipid oxidation in different food products [1, 4]. Many natural antioxidants from a variety of sources have been identified and evaluated for their antioxidative potential in various food products; for example, curry berry, thuza cone and peach seed extracts in meat products [2, 4]; chokecherry in sunflower oil [5]; Canadian rowanberry and crabapple in rapeseed oil [6]. However, identification and evaluation of natural antioxidants from the by-products of existing processing industry is a recent trend; from the utilization of by-products, as well as from the economics point of view.

Peanut (*Arachis hypogaea* L.) is an important food, as well as an oilseed crop of the tropical and sub-tropical world. Peanut processing for a variety of edible products (peanut butter, milk, roasted snack peanuts, peanut oil and other value added products) is being done in various developed, as well as developing countries. Peanut skins are low value by-products of peanut blanching and roasting operations; and is currently used as an ingredient of animal ration up to a certain limit [7, 8]. Research has indicated that some natural phenolic compounds can be extracted from peanut skins and hulls, like proanthocyanidins [9]; caffeic acid, chlorogenic acid, ferulic acid, coumaric acid, flavonoids (catechins and procyanidins), and stilbene (resveratrol) [10]; and ethyl protocatechuate [11]. Thus, peanut skin may be utilized as a source of natural antioxidants that may have commercial application in a variety of food products. Therefore, the objectives of this research were: (i) to study the antioxidant potential of peanut skin extract obtained by various methods, (ii) to identify the effect of various solvents on extractability of total phenolic compounds from the peanut skin and (iii) to evaluate the effect of peanut skin extracts on oxidative stability of soybean and mustard oils.

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MATERIALS & METHODS

Materials

The peanuts (*variety: M 542*) were obtained from Punjab Agriculture University, Ludhiana, India; and stored at 4 °C until use. Folin-Ciocaltieu (*FC*) reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma–Aldrich Japan (Tokyo, Japan). All other chemicals were obtained from SD Fine Chem. Ltd.

Methods

Preparation of Peanut Skin Extract

Peanut skins were removed by three different methods (i) direct method, the skin was directly peeled; (ii) the moist method, peanuts were blanched in boiling water for 3 min, after which water was drained and boiled kernels were cooled to room temperature, their skin was hand peeled and dried; (iii) the roasting method consisted of roasting peanuts at 175 °C for 6 min, cooling to room temperature, and skin was removed by rubbing with hand. Dry skins obtained from each method were separately blended into powder and stored in plastic bottles in the refrigerator 4 ˚C until used.

Three solvents, water, 80% methanol, and 80% ethanol, were used to prepare peanut skin extract. About 30 g of peanut skin powder was mixed with 300 ml of solvent in a flask, wrapped by aluminium foil, and shaken overnight at room temperature. Mixture was filtered (Whatman, 1) to obtain crude extract. The crude extract was centrifuged at 7000 rpm for 30 min, and the supernatant was collected and filtered (Whatman, 42). Each solvent extraction was carried out in triplicate.

Total Phenolics

The amount of total phenolics in the extracts was determined with the Folin-Ciocalteu (*FC*) method [12], with slight modifications. Each extract (0.1 ml) was mixed with 0.9 ml of deionized water in a test tube; 5 ml of 0.2 N *FC* reagent was added to each tube; 4 ml of 7.5% Na_2CO_3 was added in each tube. All tubes were incubated at 45 ˚C for 15 min. The absorbance of all samples was measured at 765 nm (UV-1800 PharmaSpec, SHIMADZU, Japan). Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dry weight).

DPPH Radical-Scavenging Activity

The method of yogesh and ali [4] was employed to assess the ability of extracts to scavenge 1, 1-diphenyl 1-2- picrylhydrazyl (DPPH) radicals; with slight modification. The methanolic peanut skin extract (500 µl) was diluted with 500 µl, 50 mM Tris-HCl buffer (pH 7.4), and mixed with 1 ml of DPPH (0.1 mM) with vigorous shaking. BHT was used as positive control, water as negative control and water with 80% methanol was used as blank. The tubes were stored in the dark at room temperature for 20 min and the absorbance was measured at 517 nm.

Addition of Extracts to Oil

The methanolic peanut skin extract (0.5%) was added to soybean oil in a flask, the solvent was evaporated under vacuum at 50 °C using a rotary evaporator.

Accelerated Storage – Schaal Oven Test

The ability of the extract to inhibit oxidative deterioration of oil during storage was determined using the Schaal oven test [6]. Soybean oil (1.0 g), fortified with extracts at 0.5%, were introduced in the vials (2 ml). The vials (uncapped) were stored in darkness at 65 °C. Samples were examined at 24 h intervals by collecting individual vials at the particular period. The oxidative stability of the samples was evaluated by peroxide value (PV). The effectiveness of the extract was compared with BHT, a synthetic antioxidant as a positive control. Experiments were set up in triplicate, and samples from each replication were analyzed in duplicate.

Peroxide Value (PV)

PV was assessed according to procedure described by Szterk *et al.* [13] with slight modifications. Briefly, 250 mg of oil was dissolved in 5 ml of hexane. 250 µl of the solution was mixed with 5 ml of methanol/-chloroform/HCl solution (1:1:0.012; v/v). Thereafter, 100 µl of $FeCl_2$ (0.4 g/100 ml water) and 100 µl of NH_4SCN (30 g/100 ml water) were added. The reaction was kept at room temperature for 5 min, and the absorbance was measured at 480 nm using all reagents for the blank sample.

Thermal Oxidation by Rancimat

Mustard oil fortified with extracts (0.2%, 0.3% and control) was submitted to thermo-oxidation under Rancimat conditions using a 743 Rancimat (Metrohm, Filderstadt, Germany) [6]. In brief, 3.6 g oil was weighed into the reaction vessel, which was placed into the heating block kept at 120 °C. The air flow was set at 20 L/h for all determinations. Volatile compounds released during the degradation process were collected

in a receiving flask filled with 60 ml distilled water. The conductivity of this solution was measured and recorded. The software of the Rancimat automatically evaluated the resulting curves. BHT was used as the reference antioxidant, and experiments were set up in triplicate.

Statistical Analysis

Data are presented as means ± standard deviation. Data were analysed by single factor analyses of variance (ANOVA) using SPSS package (version 13.0). Statistically significant differences between means were determined by Duncan's multiple range tests for $P < 0.05$.

RESULTS AND DISCUSSION

Total Phenolics

Total phenolic content in the peanut skin was ranged from 14.5 ± 0.95 to 101.7 ± 5.54 mg GAE/g dry skin (Table **1**). In this study, the peanut skin was obtained by three different methods, and extraction was carried out using three solvents, both of these variable had a significant effect ($P < 0.05$) on total phenolic content of the final extract. The aqueous extraction is a good choice, because of the absence of harmful organic solvents (organic residues). However, aqueous extraction is advisable only when the water soluble components are more. In this study, the total phenolic content was observed lowest in the aqueous extracted samples, while organic solvents were shown to extract more phenolic components. It was also observed that there was much differences in the phenolic content of aqueous extracted and organic extracted (80% C_2H_5OH and 80% CH_3OH); however, not a much difference was observed between two organic solvent extracted samples (Table **1**). This proves that, peanut skin contains mainly hydrophobic components, which may also be lipophilic in nature. This finding is important because there is a need of antioxidative compounds with lipophilic nature, having good solubility in oil and oil products. Similar results have been reported on total phenolic contents when ethanol was used as extraction solvent [14].

Different removal methods employed in this study, also had a significant effect on total phenolic content of peanut skin (Table **1**). Total phenolic content was observed lowest in the blanched treated samples while roasting was shown to improve the total phenolic content. It is important to notice here that the dry removal of peanut skin has been resulted in more phenolic content than the blanching treatment, which shows that peanut skin also contains water soluble phenolic compounds that might be washed away during blanching operation. Moreover, in the roasted samples, products formed due to the Maillard reaction might contribute to the increase of total phenolics or phenolics like complexes that contributed to higher absorbance of the samples [10, 15]. Similar results on phenolic content of peanut by-products have been reported earlier [10, 16].

DPPH Radical-Scavenging Activity

The data on radical scavenging activity of 80% methanolic extract of peanut skin (dry removal) and BHT is shown in Figure **1**. The scavenging activity was similar to that of BHT samples ($P < 0.05$) when compared at lower concentration and somewhat lesser when compared at higher concentrations. The negative health implications of synthetic antioxidants (*BHT*) are well established; thus, natural antioxidants are now preferred by the food industry; moreover, consumers are now also well aware of health benefits of natural ingredients; thus, they favour natural ingredients over synthetic one. In the present study, the scavenging activity of peanut skin is similar to that of BHT, which has been used widely in the food industry as an antioxidant for a variety of food products. Thus, peanut

Table 1: Total Phenolics (TP) (mg GAE/g Dry Weight) in the Peanut Skin Obtained by Various Methods

	TP (mg GAE/g Dry Weight)		
Methods	**Water Extraction**	**80% Ethanol Extraction**	**80% Methanol Extraction**
Dry	54.7 ± 1.78^{cC}	81.9 ± 3.05^{bB}	89.1 ± 3.78^{bA}
Blanching	14.5 ± 0.95^{bC}	18.2 ± 0.56^{cB}	21.6 ± 1.02^{cA}
After roasting	76.0 ± 2.12^{aB}	103 ± 2.85^{aA}	101.7 ± 5.54^{aA}

*Mean ± SD; experiments were done in triplicate; GAE-gallic acid equivalent.
*Values bearing different small superscript letters in a column are differed significantly ($P < 0.05$).
*Values bearing different capital superscript letters in a row are differed significantly ($P < 0.05$).

skin may be a good alternative as a source of natural antioxidants. Moreover, this peanut skin is a low-value by-product obtained during the processing of peanut; thus, the efficient use of this component may further improve the economics of peanut processing. In some previous studies, the correlation between total phenolic content and antioxidant activity (DPPH, ORAC, TAA) has been evaluated; and similar to our findings, authors have reported higher total phenolic content in the peanut skin [14, 15, 17].

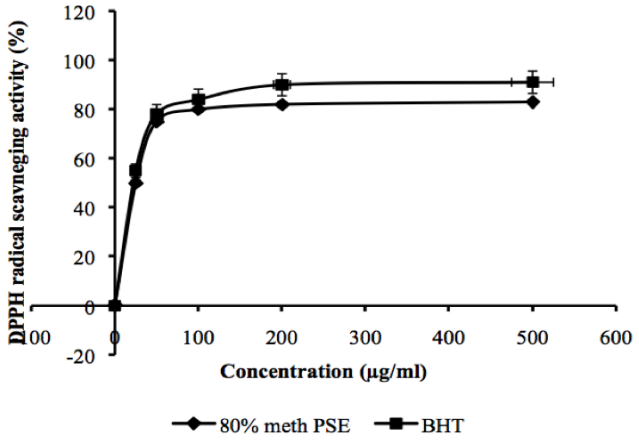

Figure 1: DPPH radical scavenging activity of methanolic extract of PSE and BHT at different concentrations (0–500 µg/ml). Data represent the means ± SD of triplicate experiments. No significant difference was observed at $P < 0.05$.

Peroxide Value (PV)

Total phenolic content provides only quantitative information, and the DPPH radical scavenging activity can provide information on the potential of an antioxidative compound, only *in vitro*. Thus, assessment of new antioxidants in real food system is mandatory; for this reason, ability of the peanut skin extract to protect fats/oils during storage was evaluated in refined oil (soybean) during accelerated storage at 65 °C for 7 days. Formation of lipid hydroperoxides, the primary products of oxidative deterioration was monitored by peroxide value (PV). The increase in the PV in the oil substrate over the storage period is depicted in Figure **2**. The peanut skin extract (80% methanolic; dry removal only) as well as BHT (standard; positive control) showed significant ($P < 0.01$) protection against oxidative deterioration of the oil, in comparison to the control samples. Formation of hydroperoxides in the oil was inhibited by more than 50% in the presence of peanut skin extract. Thus, the action of the peanut skin on the prevention of oxidative damage to the oil was comparable to that of BHT. The use of olive leaves and tea extracts as antioxidants against the oxidation of soybean oil under microwave heating was carried out [18] during earlier studies. These authors concluded that the olive leaves extracts increased the vitamin E availability in the samples, and were able to reduce the formation of oxidation compounds, and exhibited a higher protection against polyunsaturated fatty acids loss; white tea extracts provided higher antioxidant activity and oxidative resistance to the soybean oils. Likewise, some researchers [19] evaluated the effect of natural antioxidants extracted from plant and animal resources on the oxidative stability of soybean oil and concluded that olive leaf extract had a suitable antioxidant activity in soybean oil.

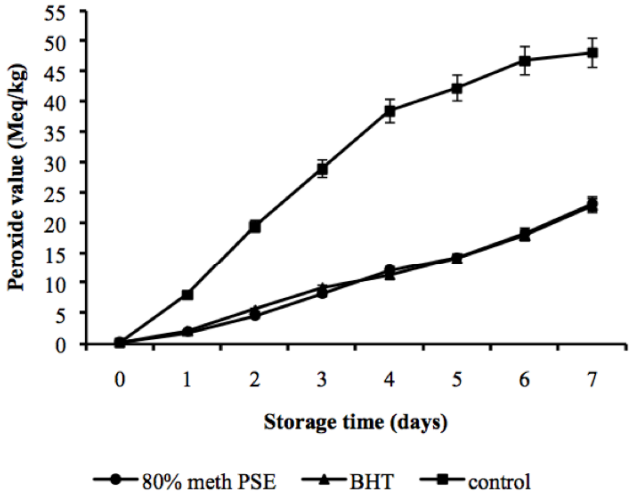

Figure 2: Changes in peroxide values during accelerated storage of soybean oil fortified with methanolic extract of peanut skin. BHT – butylated hydroxytoluene; control – refined soybean oil without extract. Significant difference was observed between 80% meth PSE vs control and BHT vs control ($P < 0.01$).

Thermal Oxidation by Rancimat

The peroxide value provides a static measure for the assessment of oxidation in fats and oils; while, the determination of the oxidative stability by means of Rancimat method is a dynamic measurement. Thus, the induction period (IP) measured by Rancimat method can be a useful screening method for measuring the oxidative stability of frying oils. The IP for mustard oil is presented in Figure **3**; the peanut skin extract was used at two concentration (0.2% and 0.3%), with BHT as an positive control. The extracts exhibited a concentration dependent increase in activity, with efficiency markedly better ($P < 0.05$) than BHT at the higher concentration. The poor efficiency of

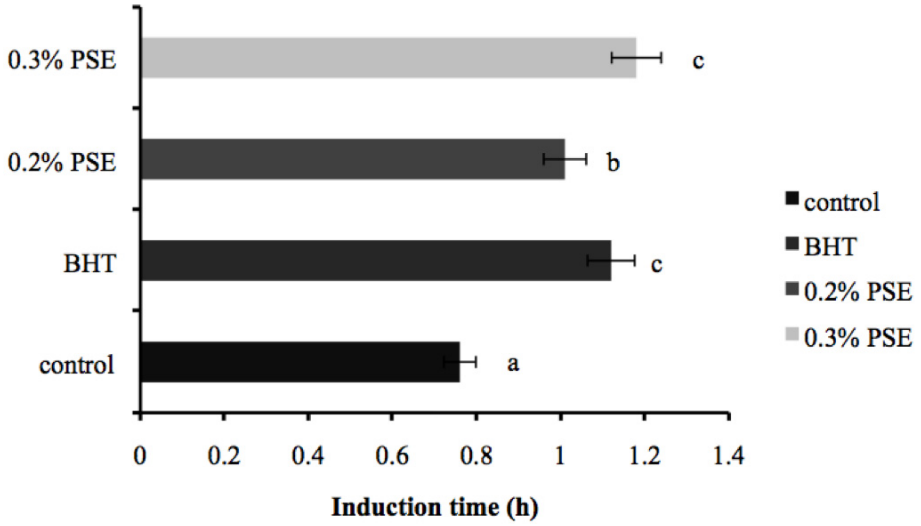

Figure 3: Oxidative stability of mustard oil fortified with peanut skin extract (PSE) as measured by Rancimat induction period. BHT – butylated hydroxytoluene; Control –oil without extract/BHT. Bars bearing the same letter are not statistically significant ($P < 0.05$).

the fractions at 0.2% is presumably related to the thermal degradation of the phenolic compounds under the harsher Rancimat condition. Some authors [5, 6] reported antioxidant potential of plant extract in the rapeseed and sunflower oil, with similar findings. Similarly, antioxidative potential of ethanolic extract of *Polygonum cuspidatum* was evaluated in peanut oil [20].

CONCLUSIONS

Total phenolics of peanut skin obtained by three different methods were estimated; the effect of three different solvents on extractability of total phenolic compounds has also been evaluated. The effect of peanut skin extract, possessing highest phenolics and DPPH radical scavenging activity, was evaluated by schaal oven test in the soybean oil, while oxidative stability of mustard oil was evaluated by Rancimat method. The results from the present study indicated that the peanut skin possess higher amount of phenolic compounds, which showed similar DPPH radical scavenging activity to that of BHT. The extractability of phenolic compounds was observed maximum with 80% methanol, while aqueous extraction showed lowest extractability. The PV and IP of different oils evaluated in the present study, clearly showed that peanut skin extract offered significantly ($P < 0.05$) better or similar protection against oxidation in the oils; than BHT, the synthetic antioxidant.

REFERENCES

[1] Hygreeva D, Pandey MC, Radhakrishna K. Potential applications of plant based derivatives as fat replacers, antioxidants and antimicrobials in fresh and processed meat products. Meat Sci 2014; 98: 47-57.

[2] Yogesh K, Jha SN, Yadav DN. Antioxidant activities of murraya koenigii (l.) spreng berry extract: Application in refrigerated (4 ± 1 °c) stored meat homogenates. Agric Res. 2012; 1: 183-9.

[3] Falowo AB, Fayemi PO, Muchenje V. Natural antioxidants against lipid-protein oxidative deterioration in meat and meat products: A review. Food Res Int. 2014; 64: 171-81.

[4] Yogesh K, Ali J. Antioxidant potential of thuja (*thuja occidentalis*) cones and peach (*prunus persia*) seeds in raw chicken ground meat during refrigerated (4 ± 1 °c) storage. J Food Sci Technol 20121-7.

[5] Aladedunye F, Przybylski R, Niehaus K, Bednarz H, Matthaus B. Phenolic extracts from *crataegus* x *mordenensis* and *prunus virginiana*: Composition, antioxidant activity and performance in sunflower oil. LWT - Food Sci Technol 2014; 59: 308-19.

[6] Aladedunye F, Matthaus B. Phenolic extracts from *sorbus aucuparia* (l.) and *malus baccata* (l.) berries: Antioxidant activity and performance in rapeseed oil during frying and storage. Food Chem 2014; 159: 273-81.

[7] Lee, J. Usda press release. 2002.

[8] Sobolev VS, Cole RJ. Note on utilisation of peanut seed testa. J Sci Food Agric 2004; 84: 105-11.

[9] Lou H, Yuan H, Ma B, Ren D, Ji M, Oka S. Polyphenols from peanut skins and their free radical-scavenging effects. Phytochemistry 2004; 65: 2391-9.

[10] Yu J, Ahmedna M, Goktepe I. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chem 2005; 90: 199-206.

[11] Huang SC, Yen G-C, Chang L-W, Yen W-J, Duh P-D. Identification of an antioxidant, ethyl protocatechuate, in peanut seed testa. J Agric Food Chem 2003; 51: 2380-3.

[12] panos GA, Wrolstad RE. Influence of processing and storage on the phenolic composition of thompson seedless grape juice. J Agric Food Chem 1990; 38: 1565-71.

[13] Szterk A, Roszko M, Sosinska E, Derewiaka D, Lewicki PP. Chemical composition and oxidative stability of selected plant oils. J Am Oil Chem Soc 2010; 87: 637-45.

[14] Ballard TS, Mallikarjunan P, Zhou K, O'Keefe S. Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. Food Chem 2010; 120: 1185-92.

[15] Talcott ST, Passeretti S, Duncan CE, Gorbet DW. Polyphenolic content and sensory properties of normal and high oleic acid peanuts. Food Chem 2005; 90: 379-88.

[16] Valeria N, Nelson RG, Guzman CA. Extraction of antioxidant components from peanut skins Grasas Aceites. 2002; 53: 391-5.

[17] Ma Y, Kosinska-Cagnazzo A, Kerr WL, Amarowicz R, Swanson RB, Pegg RB. Separation and characterization of phenolic compounds from dry-blanched peanut skins by liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr A 2014.

[18] Malheiro R, Rodrigues N, Manzke G, Bento A, Pereira JA, Casal S. The use of olive leaves and tea extracts as effective antioxidants against the oxidation of soybean oil under microwave heating. Ind Crop Prod 2013; 44: 37-43.

[19] Taghvaei M, Jafari SM, Mahoonak AS, Nikoo AM, Rahmanian N, Hajitabar J, Meshginfar N. The effect of natural antioxidants extracted from plant and animal resources on the oxidative stability of soybean oil. LWT - Food Sci Technol 2014; 56: 124-30.

[20] Pan Y, Zhang X, Wang H, Liang Y, Zhu J, Li H, Zhang Z, Wu Q. Antioxidant potential of ethanolic extract of polygonum cuspidatum and application in peanut oil. Food Chem 2007; 105: 1518-24.

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