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ORIGINAL PAPER

Effects of roasting on phenolics composition and antioxidant activity of peanut (*Arachis hypogaea* L.) kernel flour

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Abstract The effects of roasting on the phenolics composition and antioxidant activity of peanut (*Arachis hypogaea* L.) kernel flour were appraised. Peanut kernel flour, with and without skin, were roasted at 160 °C for 10, 20, 30, 40 and 50 min. The resultant changes in the antioxidant activity of roasted peanut kernel flour were assessed by the determinations of total phenolics, 1,1-diphenyl-2-picryl-hydrazyl free radical-scavenging capacity, percent inhibition of linoleic acid oxidation and thiobarbituric acid test and compared with those of unroasted kernel flour. It was observed that roasting significantly ($p < 0.05$) increased the antioxidant activity of the peanut kernel flour. HPLC analysis revealed the detection of three phenolic acids (*p*-hydroxybenzoic, chlorogenic, *p*-coumaric), two flavonols (quercetin, kaempferol), and a stilbene (resveratrol) both in the roasted and unroasted samples. In peanut kernel flour without skin, the contents of the phenolics increased in the initial roasting phase, however, decreased gradually in the later phase (>20 min of roasting time). In contrast, over the course of heating, the amounts of phenolics were noted to be slightly increased in the peanut kernel flour with skin; the most significant ($p < 0.05$) increase occurred in the concentration of *p*-coumaric acid and quercetin at 30, 40, and 50 min of roasting. The results of this study reveal that optimum roasting time should be sought to enhancing the antioxidant capacity and phenolics concentration in peanut kernel flour.

Keywords Peanut kernel · Roasting time · TPC · Colorimetric antioxidant assays · Phenolics acids · Flavonols

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Introduction

Peanut (*Arachis hypogaea* L.) is one of the major oilseed crops cultivated in many regions across the world. It is valued as an important food protein source in the developing and developed countries. Peanuts are not only used as a source of edible oil but are also consumed directly or incorporated into different foods such as snacks and confectionary products for nutrition purposes. Epidemiological studies suggest that consumption of peanut and peanut based-products can protect against the incidence of coronary heart diseases (CHD) by decreasing low-density lipoprotein (LDL) cholesterol and reducing the risk of development of type II diabetes as well as controlling weight gain [1, 2]. Nearly, 80% of the fat in peanut is in the form of monounsaturates (oleic acid) that may help to lowering LDL cholesterol level in blood. Oleic acid may also play a key role in food-derived hormonal interaction in the intestine, which boosts satiety between meals by

prolonging the feeling of fullness, therefore, contributing to weight management [3, 4].

Peanut kernels are typically considered as a good source of antioxidant components and phytosterols and contain about 50% lipid, 25% protein, and 16% carbohydrate making them a nutritious alternative to meat products. A number of phenolics such as hydroxybenzoic acid, ferulic acid, coumaric acid, resveratrol, flavonoids (catechin and procyanidins), and flavonols (quercetin and kaempferol) have been identified in peanuts kernels along with considerable amount of total tocopherols (80–140 mg/kg). In addition to its desirable fatty acids profile, the purported health benefits associated with consumption of peanut kernel are mainly attributed to these bioactive compounds [5, 6]. A dry powder (peanut flour), obtained after partial extraction of oil, from the roasted peanut kernel, is commercially used as additive to increase the protein content of various food commodities including baked goods, sauces, dressing, etc. Some recent studies also report the rheological, foaming, emulsifying, and water holding properties of peanut flour [7, 8]. Peanut skin (testa or seed coat), although a potential source of plophenols, especially proanthocyanidins and condensed tannins, is sometime consumed along with the peanut-derived foods; however, it is mostly discarded as an agro-waste during peanut processing [9, 10].

Roasting is an important step in peanut processing industry as it is used to enhance the flavor, color, texture, and overall palatability of the end-user products. Mature peanut kernels are commonly dry roasted at 160 °C for 20–30 min to prepare roasted, salted peanuts [11]. However, actual roasting intensities may depend upon the required characteristic, flavor, and applications. During the process of roasting, some chemical changes may occur in which sugars can condense with free amino acids, peptides, or proteins leading to the formation of brown Maillard reaction products with potential antioxidant activity [12]. In addition to free forms, plants also contain considerable amounts of bound form antioxidant phenolics, it is therefore perceived that some processing methods might be employed to break these covalently bounded polymeric compounds to liberate into free forms so as to enhancing their antioxidant capacity [13]. For instance, previously, it has been reported that heat treatment liberated the low-molecular weight compounds into their free forms and hence increased the antioxidant capacity of peanut skin, hulls, and kernels [10, 14, 15].

As far as we know, there have been no earlier reports yet available studying the influence of roasting times on the phenolics composition and antioxidant activity of peanut kernel flour, with and without skin. Such investigations are important to devise an optimum roasting time offering peanut products with better antioxidant attributes. The main objective of this research, therefore, was to elucidate the effect of different roasting times on the phenolics composition and antioxidant attributes of two different forms of peanut kernel flour (with and without skin) leading to exploring their potential uses for functional foods.

Materials and methods

Reagents

Trifluoroacetic acid (TFA), standards of phenolic acids (*p*-hydroxybenzoic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, and gallic acid), flavonoids (epicatechin, dihydroquercetin, luteolin, and kaempferol) stilbene (*trans*-resveratrol), butylated hydroxyanisole (BHA), and α-Tocopherol, 2,2-Diphenyl-1-picryhydrzyl radical (DPPH) were from Sigma Chemical Company (St. Louis, MO, USA) and ferrous chloride, 2,4,6-tripyridyl-*s*-triazine, linoleic acid, thiobarbituric acid, ferric chloride, and tween 20 were obtained from Fisher Scientific (Ottawa, ON, Canada). All other reagents used were of analytical grade from Fisher Scientific.

Sample preparation and roasting

Raw, peanut pods (Virginia spreading type variety) were obtained from an Agricultural Farm in Nay-Pyi-Taw Township, Myanmar. Three different peanut raw samples, harvested from different agriculture plots, were assayed. The selection of the present peanut variety was based upon its high yield and productivity and popularity among local consumers. The pods (3 kg) were manually dehulled and the kernels recovered roasted in an electric oven (Memmert, UL40, Germany) equipped with an air circulation system. The roasting temperature was set at 160 °C following the method of Damame et al. [11] and maintained for 2 h to reach equilibrium before used. The roasting times were increased gradually from 10 to 20, 30, 40, and 50 min. After cooling to room temperature, the skin was removed from an appropriate amount/batch of the kernels, producing kernels with and without skin. Triplicate treatments were applied for roasting process. The roasted peanut kernels with and without skin were ground separately using a commercial grinder (Pensonic, Malaysia) and then sieved to get fine power (peanut flour). Peanut flour (10 g) was defatted with *n*-hexane (100 mL) using a water bath shaker for 8 h at 45 °C. After that, the defatted samples were ambient dried, packed in amber bottles, and preserved at −20 °C until used for antioxidants extraction and HPLC analysis. Control samples (unroasted samples) of peanut flour with and without skins were also defatted and kept at the same conditions.

Extraction of antioxidant components

Extraction of antioxidant components was carried out according to method of Chukwumah et al. [16] with slight modifications. Each of the defatted ground kernel sample (10 g) was extracted with pure methanol (100 mL) using a water bath shaker for 2 h at room temperature (28 °C). The suspension was filtered through a Whatman No. 1 filter paper, and the residue obtained was re-extracted twice, with additional 100 mL of methanol. The filtrates were pooled and the solvent distilled off under reduced pressure using a rotary evaporator at 40 °C. The crude concentrated extracts were used for the following antioxidant activity-related experiments.

Total phenolic contents

The amounts of total phenolics in the roasted and unroasted peanut kernel flour with and without skin were determined using Folin–Ciocalteau procedure as described by [17]. Methanol extract (0.4 mL) of each sample (1 mg/mL concentration) was mixed with 2 mL of the Folin–Ciocalteau reagent. After 5 min, 1.6 mL of sodium carbonate solution (75%) was added to maintain basic condition for the reaction to occur between phenolic components and Foiln reagent. The reaction mixture was mixed well using vortex machine and then incubated for 30 min at room temperature (28 ± 1 °C) in the darkness. The absorbance of the reaction mixture was then noted at 750 nm using a UV–visible spectrophotometer (Shimadzu, Japan). The standard calibration curve was prepared using gallic acid standard solutions of known concentrations (0.02, 0.04, 0.06, 0.08, 0.10, and 0.20 mg/mL), and the amounts of total phenolics were calculated as gallic acid equivalent mg/g of dry sample (mg GAE/g dry sample).

Free radical-scavenging capacity (DPPH)

Antioxidant activity of methanol extracts from roasted and unroasted peanut kernel flour samples was measured using 2,2-diphenyl-1-picrylhdrazyl (DPPH) according to the method as described by Brand-Willian et al. [18], with minor modifications. The extracts (0.5 mL) of varying concentration were mixed with 2.5 mL of freshly prepared DPPH solution (25 mg/L). The solution was then incubated in the darkness at room temperature for 30 min, and the decrease in absorbance was noted at 515 nm using a UV–visible Spectrophotometer (Shimadzu, Japan). For blank, pure methanol was used in place of the sample. For positive controls, 0.1 mg/mL of each α-tocopherol and BHA (reference antioxidants) were employed. The percent DPPH free radical scavenged by each sample extract was calculated by the following equation:

$$\%\,\text{DPPH} = \frac{A_0 - A_1}{A_0} \times 100$$

A_0 absorbance of DPPH solution without sample/standard solution, and A_1 absorbance of the sample/standard solution at 30 min reaction.

Inhibition of linoleic acid peroxidation

Methanol extracts of roasted and unroasted peanut kernel flour were tested for their antioxidant activity by measuring the inhibition of linoleic acid peroxidation following a previously reported method of Yen and Hsieh [19]. Test sample 0.5 mL (1 mg/mL in absolute ethanol) was mixed with 2.5 mL of linoleic acid emulsion (0.02 M, pH 7.0) in phosphate buffer. This emulsion was prepared by mixing and homogenizing 0.280 g of linoleic acid, 0.280 g of tween 20 as emulsifier, and 50 mL of phosphate buffer solution. The resulting reaction mixture was subjected to incubation at 37 °C for 96 h. The magnitude of linoleic acid oxidation was determined by the peroxide value following a colorimetric method as described by Yen et al. [20]. Briefly, to 0.1 mL sample solution, 4.7 mL of ethanol (75%), 0.1 mL of ammonium thiocyanate (30%), and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl) were added sequentially. After 3 min of reaction, the absorbance of the resulting reaction mixture was read at 500 nm using a spectrophotometer (Shimadzu, Japan). The degree of oxidation was measured after every 24 h until a day after the absorbance of the control reached its maximum. A control contained all reagents without the sample extract was prepared simultaneously. Besides, BHA, α-tocopherol, and quercetin were used as positive controls (1 mg/mL each compound). The percent inhibition of linoleic acid oxidation was calculated as the following equation:

$$\text{Inhibition of linoleic acid oxidation } (\%) = \left(1 - \frac{\text{Absorbance at 500 nm in the presence of sample 96 h}}{\text{Absorbance at 500 nm in the absence of sample 96 h}}\right) \times 100$$

Thiobarbituric acid method

The antioxidant activity of the extracts from roasted and unroasted peanut kernel flour was also measured using thiobarbituric acid (TBA) method as described by Ottolenghi [21] and Kikuzaki and Nakatani [22]. The same reaction mixture as employed for measurement of percent inhibition of linoleic acid oxidation test at the final day of the experiment was used for TBA test. Briefly, to 1 mL of assay mixture, 2 mL of 20% trichloroacetic acid (TCA) solution and 2 mL of TBA solution were added. The reaction mixture was then incubated in a boiling water bath for 10 min. After cooling to room

temperature, the solution was centrifuged at 900×*g* for 20 min, and the absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Shimadzu, Japan).

HPLC analysis of phenolic compounds

The analysis of selected phenolic acids (*p*-hydroxybenzoic, caffeic, chlorogenic, *p*-coumaric, and ferulic), flavonoids (epicatechin, quercetin, kaempferol, and luteolin), and stilbene (*trans*-resveratrol) was based on the fact they are commonly found in peanuts. The analysis was performed by HPLC on the acid-hydrolyzed samples according to the method as described by Wang et al. [23], with slight modifications. Briefly, roasted and unroasted kernel flour (0.5 g) was mixed with 10 mL of 80% methanol and 1 mL of 1.2 M hydrochloric acid and hydrolyzed by incubation in a water bath at 80 °C for 2 h. After hydrolysis, it was cooled at room temperature and centrifuged at 1,200×*g* for 5 min. The supernatant was filtered using a 0.45 μm nylon membrane filter, prior to an HPLC analysis.

A Waters HPLC system equipped with Waters 2487 Dual Wavelength Absorbance Detector, Waters 600 Pump and controlled by Waters Empower2 software (Waters, Milford, MA) was used. The separation of the phenolics was carried out on Waters reverse-phase (RP) Symmetry C_{18} column (150 × 3.9 mm, 5 μm) operated at room temperature. The mobile phase consisted of TFA in deionized water (pH 2.5) as solvent A and absolute methanol (99.99%) as solvent B. The gradient conditions used were as follows: 100–50% solvent A (0–20 min), 50–40% solvent A (20–30 min), and 40–100% solvent A (30–40 min). The mobile phase flow rate was set at 1.0 mL/min, and a 20 μL sample volume was injected. The detection of the phenolic was monitored at 280 nm because this wavelength is near the maximum absorbance wavelength for hydroxybenzoic acids (270–280 nm), hydroxycinnamic acids (290–300 nm), and some flavonoids (250–270 nm, 330–350 nm) [24]. The phenolic standard solutions were prepared by dissolving respective pure compounds in absolute methanol at various concentrations (0.005, 0.01, 0.02, 0.05, 0.1, and 0.2 mg/mL) and injected into the HPLC system to construct the calibration curves for each standard compound. Identification of the unknown phenolics was based on matching their retention times with those of pure standards of phenolics. Peak areas, based on external standard calibration curves, were used for quantification purposes. The amounts were expressed as μg/g defatted sample.

Statistical analysis

Three different peanut samples were analyzed individually in triplicate. All experiments were performed in triplicates. The data were expressed as means ± standard deviations of triplicate determinations ($n = 3 \times 3$). One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were carried out to assess the significance of the differences between means ($p < 0.05$) using SAS System for Windows Version 7 (SAS Institute Inc., Cary, NC, USA).

Results and discussions

Total phenolic contents

It is well known that there is a positive association between consumption of plant foods rich in phenolic antioxidants and health. Thermal processing is reported to significantly alter the phytochemical and functional composition in legumes including peanuts [16, 25]. Chukwumah et al. [16] investigated the effects of different processing techniques such as boiling, oil-, and dry-roasting on the phenolics composition and antioxidant activity of peanuts. In the present study, we investigated the effects of different roasting times on the total phenolic contents (TPC) of peanut kernel flour with and without skin. Results of the study showed that TPC of peanut kernel flour with and without skin increased significantly ($p < 0.05$) as function of roasting times (Fig. 1). The amount of TP in the roasted peanut kernel without skin significantly ($p < 0.05$) increased from 0.94 mg GAE/g in unroasted control to 1.61, 1.83, 2.16, and 2.04 mg GAE/g in the samples roasted for 20, 30, 40, and 50 min at 160 °C, respectively. However, TPC of roasted sample without skin were almost unaffected during the initial stage (10 min) of roasting. In agreement with our present finding, some previous studies also revealed that initial stage of heating or roasting

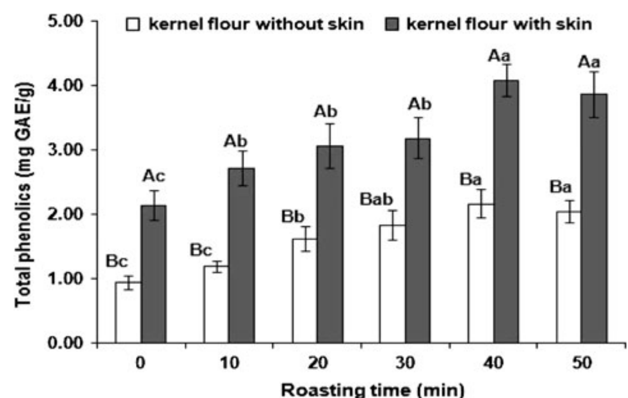

Fig. 1 Effect of roasting times on total phenolic contents (*TPC*) of peanut kernel flour with and without skin. Values with same small letters (*a*, *b*, *c*) are not significantly different ($p < 0.05$), among different roasting times. Values with same capital letters (*A*, *B*) are not significantly different ($p < 0.05$) between the samples

process did not exert significant effect on the TPC of buckwheat and apricot kernel [26, 27].

For peanut kernel flour with skin, TPC increased significantly ($p < 0.05$) as roasting times increased and maximum values, 4.08 and 3.86 mg GAE/g, were obtained for 40 and 50 min roasted samples, respectively. It can be seen that peanut kernel flour with skin contained significantly higher amount of total phenolics than peanut flour without skin. A higher phenolic content in peanut kernel flour with skin might be ascribed to the presence of certain phenolics such as proanthocyanidins (condensed tannins) in the skin. According to Karcheshy and Hemingway [28], peanut skin was found to be a rich source of proanthocyanidins that contributed 17% by weight of the skin. Of the identified proanthocyanidins, about 50% were low-molecular weight oligomers [28].

It can be expected that bound form phenolics with larger molecular weight, both in peanut kernel flour with and without skin, might have been liberated into simple free forms by heat treatment leading to enhancing over all TPC of the samples. Several studies reported that heat treatment is effective toward increasing the total phenolic content in different foods such as dry beans [29], carob powder [30], vegetables [31], and grape seeds [32]. Boateng et al. [29] explained that disruption of the cell wall through heating or by the breakdown of insoluble phenolic compounds as function of thermal treatments could lead to better extractability of phenolic compounds in dry beans. However, Lee et al. [14] revealed that simple heat treatment did not liberate covalently bound phenolic compounds from rice hull while far-infrared treatment broke the esterified phenolic bonds. This indicates that an effective processing method for liberation of such bound plant phenolics into simpler forms may differ from species to species [33]. In addition, the increase in total phenolics of peanut kernel flour in this study may also be linked to the development of Maillard reaction products that are reported to be formed during roasting process. Yu et al. [10] investigated that Maillard reaction products might lead to increase the amounts of total phenolics or phenolic-like complexes that further contribute to higher absorbance readings measured by Folin assay.

DPPH free radical-scavenging activity

The antioxidant activity of methanol extracts from roasted peanut kernel flour with and without skin, supported with two positive controls, i.e., BHA and α-tocopherol, was determined by measuring their DPPH· radical-scavenging capacity (Fig. 2). DPPH is a stable free radical that has ability to accept an electron or hydrogen from antioxidant compounds and is then converted to a DPPH stable molecule. The loss of DPPH radical is examined by the

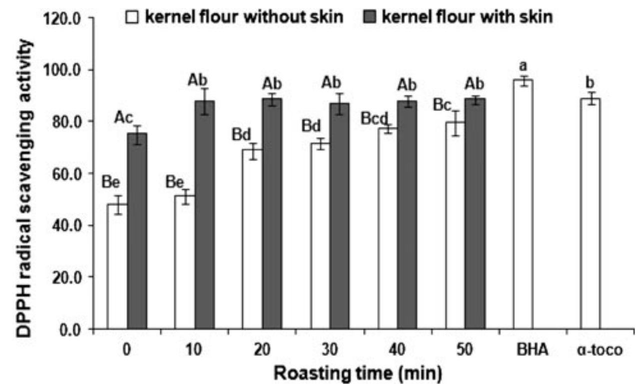

Fig. 2 Effect of roasting times on DPPH radical-scavenging activity of peanut kernel flour with and without skin. Values with same small letters (*a*, *b*, *c*) are not significantly different ($p < 0.05$), among different roasting times. Values with same capital letters (*A*, *B*) are not significantly different ($p < 0.05$) between the samples

decrease in the magnitude of absorbance of the antioxidant solution at 515 nm. In the present experiment, DPPH free radical-scavenging activity of the extracts from peanut kernel flour without skin linearly increased as function of roasting times. The extracts, from 20, 30, 40, and 50-min roasted samples of peanut flour without skin, showed significantly ($p < 0.05$) higher DPPH radical-scavenging activity (68.93, 71.70, 77.26, and 79.57%, respectively) than 10-min roasted (51.22%) and unroasted samples (48.09%). The roasting time was found to be a critical factor to determine the overall antioxidant activity of the peanut kernel flour without skin. The highest antioxidant activity, in terms of DPPH free radical-scavenging capacity, was exhibited between 20 and 50 min of roasting. On the other hand, the peanut kernel flour with skin, when roasted for 10–50 min, also showed good scavenging activity (86.96–88.61%), relative to that of unroasted sample (75.15%). The present scavenging ability values of roasted kernel flour with skin were comparable to that of positive control α-tocopherol (89.00%).

The potential health benefits of plant phenolics are mainly due to their free radical-scavenging activities through donating a hydrogen atom and or an electron from an aromatic hydroxyl group to free radicals [34]. In the present study, an enhancement in the radical-scavenging activity, observed in both the roasted peanut kernel flour with and without skin, might be attributed to their better ability to release some bound antioxidant phenolic compounds to act as free radical scavengers, from the cell matrix upon roasting [35]. On the other hand, other phenomenon may also involve, for example, better solubility of non-phenolic compounds (such as Maillard reaction products) following the thermal treatments, which may further enhance the free radical-scavenging properties of processed foods [35, 36]. Jeong et al. [37] also investigated

that roasting increased the radical-scavenging activity of sesame meal extracts and a positive linear correlation between melanoidin content and antioxidant activity of the roasted coffee brew fractions was established [38]. In our study, the increase in the radical-scavenging activity of peanut kernel flour, in relation to increasing roasting times, is in good agreement with the previous studies on apricot kernels [27] and okra seed flour [39].

Antioxidant activity in linoleic acid peroxidation system

The effects of roasting treatments on antioxidant activity of peanut kernel flour were also assessed by measuring percent inhibition of linoleic acid oxidation using a colorimetric method (Fig. 3). The peroxyl radicals, formed in the initiation step of oxidation as result of abstraction of hydrogen from a fatty acid and subsequent oxygen involvement, are good oxidizing agents. These peroxyl radicals oxidize ferric thiocyanate to ferrous thiocyanate yielding a colored complex, the intensity of which is measured at 500 nm and can be used as a basis for the measurement of peroxide level formed during the initial stage of lipid oxidation [40]. At a concentration of 500 µg in the final reaction mixture, the extracts from samples of roasted peanut kernel flour with skin inhibited 63.03–69.33% peroxidation of linoleic acid as against 26.86–56.29% for peanut kernel flour without skin, wherein, the roasting effects were followed in a time-dependent manner.

Interestingly, both raw (unroasted) and roasted peanut kernel flour with skin exhibited higher lipid peroxidation inhibition activity (65.91 and 63.03–69.33%, respectively), almost comparable to those of quercetin (65.71%), and tocopherol (73.05%) but lower than that of BHA (89.30%). An increase in the peroxidation inhibition magnitude of the extracts, from peanut kernel flour with skin, might be due to higher amounts of polyphenols present in peanut skin that contributed to inhibit the accumulation of oxidative products. Generally, it is believed that outer layers of seed such as peel, shell, and hull contain higher amounts of polyphenolic compounds to protect the seed from oxidative damage [41]. Similarly, as our present findings, the seed coat extracts of red and black bean, containing higher amount of phenolic substances, exhibited a stronger antioxidant activity against lipid peroxidation [42]. Nonetheless, the stability of antioxidants of roasted samples might be due to the formation of Maillard reaction products. During heat treatment, the formation of the Maillard reaction products is believed to be responsible for increasing the overall antioxidant capacity of the foods. Maillard pathway can produce several antioxidant compounds with strong reducing power such as reductones and amino-reductone that can inhibit low-density lipoprotein (LDL) oxidation, and the concentrations of Maillard type polymers are increased with increasing heat treatment [43].

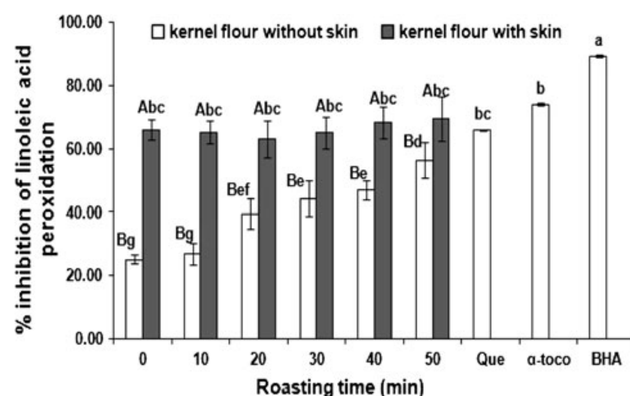

Fig. 3 Effect of roasting times on antioxidant activity of peanut flour with and without skin as measured by percent inhibition of linoleic acid peroxidation. Values with same small letters (*a*, *b*, *c*) are not significantly different ($p < 0.05$), among different roasting times. Values with same capital letters (*A*, *B*) are not significantly different ($p < 0.05$) between the samples

Antioxidant activity in TBA method

During the oxidation process, peroxides are gradually decomposed to lower molecular compounds, mainly malondialdehyde (MDA) [44] and their relative concentrations are measured by TBA method. Malonaldehydes, one of the lipid oxidation products can react with free amino group of proteins, phospholipid, and nucleic acids leading to structural modification, which induce dysfunction of immune systems [41]. At low pH and high temperature, MDA binds TBA to form red complex and the absorbance can be measured at 532 nm on the final day of the incubation period (1 day after the control reached maximum absorbance in inhibition of linoleic peroxidation assay).

Antioxidant activity (as measured by TBA test, the data given as absorbance values) of roasted peanut kernel flour with and without skin is displayed in Fig. 4. In this method, the lower the absorbance values the higher the antioxidant activity. Interestingly, also in this assay, the antioxidant activity of roasted peanut flour without skin was found to be higher than those of unroasted sample and the control. However, the present values were found to be significantly ($p < 0.05$) lower than those of positive controls (quercetin, tocopherol, and BHA). These results also support that the Maillard reaction products, probably formed due to roasting of peanut kernel, are able to decrease the lipid oxidation rate revealing antioxidant potential. The effect of Maillard reaction products on lipid oxidation in preheated model systems has been studied by Mastrocola and Munari [45]. In their studies, the simultaneous induction of the

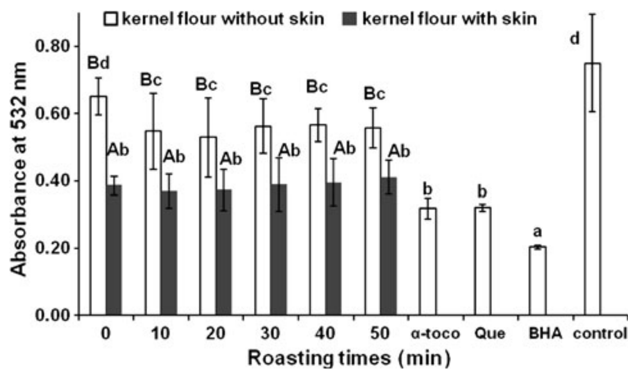

Fig. 4 Effect of roasting times on antioxidant activity of peanut kernel flour with and without skin measured by TBA method. Values with same small letters (*a*, *b*, *c*) are not significantly different ($p < 0.05$), among different roasting times. Values with same capital letters (*A*, *B*) are not significantly different ($p < 0.05$) between the samples

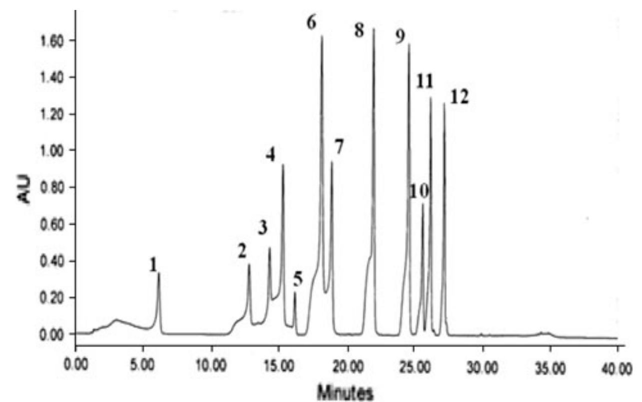

Fig. 5 HPLC chromatogram showing the separation of standard phenolic compounds (*1*) gallic acid, (*2*) *p*-hydroxybenzoic acid, (*3*) chlorgenic acid, (*4*) caffeic acid, (*5*) epicatechin, (*6*) *p*-coumaric acid, (*7*) ferulic acid, (*8*) resveratrol, (*9*) quercetin, (*10*) daidzin, (*11*) luteolin, and (*12*) kaempferol

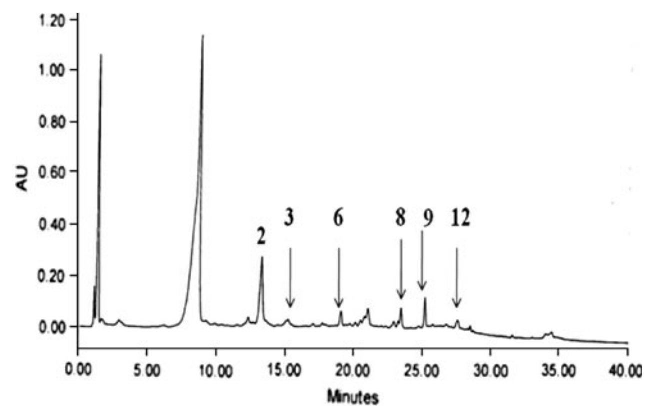

Fig. 6 A typical HPLC chromatogram showing the separation of phenolic compounds in roasted peanut kernel flour with skin (*2*) *p*-hydroxybenzoic acid, (*3*) chlorogenic acid, (*6*) *p*-coumaric acid, (*8*) resveratrol, (*9*) quercetin, and (*12*) kaempferol

Maillard reaction in heated lipid fractions models greatly affected the development of lipid oxidation by slowing the reaction and increasing the antioxidant activity of the system. Also in this assay, the antioxidant activity of roasted and unroasted peanut flour with skin was not significantly ($p < 0.05$) different from those of quercetin and tocopherol but lower than that of BHA. These results correlated well with those obtained previously using the linoleic acid emulsion method. It is well known that phenolic compounds act as hydrogen donors and reduce the extent of formation of hydroperoxides and lipid oxidation [46].

Individual phenolics composition measured by HPLC

Based on our preliminary trials, we observed that a number of complex and unidentifiable peaks appeared in non-hydrolyzed samples analyzed, which might be due to detection of bound (esterified form) phenolics in conjunction with carbohydrates and other related moieties. HPLC analysis of the hydrolyzed samples is the most recommended and appreciating approach. Therefore, for simplicity and clarity of the analysis, we used the hydrolyzed samples for detection of target phenolic compounds. The differences between control samples (non-hydrolyzed) and hydrolyzed samples referring to polyphenols were considered during the quantification of phenolics final amounts in the samples tested. HPLC chromatograms obtained for the separation of standard phenolic compounds and the phenolic compounds in roasted peanut kernel flour with skin are given as Figs. 5 and 6, respectively.

In the present study, three phenolic acids (*p*-hydroxybenzoic, chlorogenic, and *p*-coumaric), two flavonols (quercetin and kaempferol), and stilbene (resveratrol) were mainly detected in the tested samples as measured by RP-HPLC. The data generated showing the effects of different roasting times that affected the individual phenolic compounds of peanut flour, with and without skin, are summarized in Table 1. The results indicated that the contents of phenolic acids, flavonols, and resveratrol in roasted peanut kernel flour without skin were gradually increased up to 20 min of roasting. Thus, in case of peanut kernel flour without skin, after 20-min roasting, the concentration of *p*-hydroxybenzoic acid increased from 133.49 to 146.00 μg/g, chlorogenic acid 32.01–37.16 μg/g and *p*-coumaric acid 73.08–81.88 μg/g, respectively. Our finding are in agreement with the work of Talcott et al. [15] who reported that roasting increased the concentration of the predominant antioxidant phenolic acid (*p*-coumaric acid) in peanut that might be attributed to heat-catalyzed hydrolytic reactions of its native esterified or bound forms liberating into free forms. Naturally, polyphenols occur as in both free and bound forms. Some processing methods

Table 1 Phenolic compounds (μg/g) analyzed by HPLC in roasted peanut kernel flour with and without skin

Samples	Phenolic acids			Flavonols		Stilbene
	p-Hydroxybenzoic	Chlorogenic	*p*-Coumaric	Quercetin	Kaempferol	Resveratrol
Kernel flour without skin (min)						
0	133.49 ± 12.91^{abA}	32.01 ± 3.49^{aA}	73.38 ± 2.98^{aA}	104.46 ± 6.27^{bB}	1.56 ± 0.27^{bB}	0.11 ± 0.01^{abA}
10	141.00 ± 1.00^{aA}	34.00 ± 1.00^{aA}	76.00 ± 4.00^{aA}	110.00 ± 3.00^{bB}	2.60 ± 0.50^{aA}	0.12 ± 0.03^{abA}
20	146.00 ± 6.00^{aA}	37.16 ± 2.12^{aA}	81.88 ± 5.54^{aA}	133.00 ± 6.00^{aB}	2.68 ± 0.10^{aA}	0.13 ± 0.00^{aA}
30	141.50 ± 3.50^{aA}	33.79 ± 1.22^{aA}	75.79 ± 4.33^{aB}	134.41 ± 4.86^{aB}	2.37 ± 0.09^{aA}	0.08 ± 0.00^{bB}
40	132.50 ± 7.50^{abA}	35.27 ± 2.25^{aB}	59.44 ± 6.67^{bcB}	128.50 ± 1.50^{aB}	1.44 ± 0.05^{bcB}	0.08 ± 0.00^{bB}
50	112.80 ± 4.80^{bB}	23.04 ± 0.22^{bB}	51.62 ± 3.55^{cB}	81.56 ± 3.44^{cB}	0.96 ± 0.10^{cB}	0.03 ± 0.00^{cB}
Kernel flour with skin (min)						
0	131.30 ± 5.99^{aA}	32.60 ± 2.80^{bA}	61.86 ± 6.79^{bA}	121.47 ± 7.71^{cA}	2.31 ± 0.37^{abA}	0.13 ± 0.01^{abA}
10	136.48 ± 0.30^{aA}	38.15 ± 1.5^{abA}	60.09 ± 9.07^{bA}	136.37 ± 3.71^{cA}	2.70 ± 0.27^{abA}	0.14 ± 0.01^{abA}
20	137.11 ± 1.62^{aA}	34.32 ± 1.16^{bA}	60.09 ± 1.17^{bB}	164.99 ± 4.69^{bA}	2.19 ± 0.15^{bA}	0.14 ± 0.01^{abA}
30	136.47 ± 2.39^{aA}	35.63 ± 1.28^{abA}	93.62 ± 6.46^{aA}	168.70 ± 0.83^{abA}	2.99 ± 0.25^{aA}	0.13 ± 0.01^{abA}
40	143.74 ± 5.96^{aA}	43.17 ± 4.11^{aA}	92.40 ± 1.65^{aA}	178.05 ± 1.11^{aA}	2.85 ± 0.04^{abA}	0.14 ± 0.01^{abA}
50	144.06 ± 1.62^{aA}	44.42 ± 3.99^{aA}	92.83 ± 2.62^{aA}	171.31 ± 5.96^{aA}	2.14 ± 0.20^{bA}	0.16 ± 0.00^{aA}

Values with same small letters (a, b, c) are not significantly ($p < 0.05$) different among different roasting times. Values with same capital letters (A, B) are not significantly ($p < 0.05$) different between the samples

such as roasting have been shown to increase the polyphenolic content of foods probably due to releasing of bound form antioxidants into free forms and coupled with formation of Millard reaction products due to roasting effects. Dabrowski and Sosulski [47] studied free and hydrolysable polyphenols in ten oil seeds including peanuts wherein esterified forms of *p*-coumaric, ferulic, and caffeic acids were detected in defatted peanut flour. It has been revealed that the degree of roasting influenced the quality and antioxidant properties of peanut kernels, creating a complex environment for peanut quality assessment [15].

Flavonols (e.g., quercetin and kaempferol) are one of the important classes of flavonoids that are secondary metabolites in plants and have beneficial effects for human health because of their antioxidant, antiproliferative, and anticarcinogenic properties [23]. Recently, Wang et al. [23] reported that peanut kernels contained trace amount of kaempferol and higher amounts of quercetin than other legumes. The presently determined amount of quercetin (104.46 μg/g) in unroasted peanut kernel flour without skin and 121.47 μg/g in unroasted peanut kernel with skin were found to be lower than those reported by Wang et al. [23]; however, kaempferol content (1.56 and 2.31 μg/g, respectively) was comparable with that of Wang et al. [23]. In their report, peanut kernels (including skin) contained higher amount of quercetin (133–289 μg/g) and its concentration mainly depended on kernel seed-coat color, whereas kaempferol was 1.92–4.66 μg/g.

In the present study, the concentration of flavonols in peanut kernel flour without skin was also affected as result of roasting. The increase in concentration of quercetin from 104.46 to 133.00 μg/g and kaempferol from 1.56 to 2.68 μg/g as result of 20-min roasting may be linked to the breakdown of flavonol glycosidic bonds to respective aglycons under the thermal treatment. Zill-e-Huma et al. [48] reported a similar phenomenon where, thermal treatment led to the degradation of the quercetin glucosides and increased the concentration of free quercetin aglycone in microwave heated onion. It was also established that roasting caused a slight increase, from 0.11 to 0.13 μg/g, in the content of resveratrol. In contrary to our result, Sander et al. [49] reported that roasted Virginia and Spanish peanuts (176 °C) contained less amount of resveratrol than that of unroasted ones. The differences in data obtained in our study in comparison to those of Sander et al. [49] may be probably due to different roasting temperatures employed (160 °C vs. 176 °C).

On the other hand, in the present analysis, prolong roasting (>20 min) slowly decreased the contents of phenolics in peanut kernel flour without skin while a significant ($p < 0.05$) decline in the amounts occurred at 50 min roasting. It is understandable that practically up to certain temperatures, the concentration of phenolic components may increase due to improved cell wall rupturing, or due to other favorable reactions, leading to their higher recovery into the solvents; however, a prolonged heating may decrease the concentration of naturally occurring polyphenolics in food products [50]. A similar study on the effect of different roasting process on carob powder indicated that phenolic compounds were decreased after 75 min roasting at temperatures of 135, 150, and 165 °C [31]. The present results indicate that an appropriate

roasting time (<20 min) should be employed to enhance and/or retain optimum amount of phenolic compounds in peanut flour without skin.

Interestingly, in case of roasted peanut kernel flour with skin, the concentrations of phenolics, especially *p*-coumaric acid and flavonol (quercetin), were linearly increased as function of roasting times. As evident in Table 1, the original contents of *p*-coumaric acid (61.86 μg/g) and quercetin (121.47 μg/g) were notably ($p < 0.05$) enhanced after 30, 40, and 50 min roasting to levels as high as 93.62, 92.40, and 92.83 μg/g and 168.70, 178.05, and 171.31 μg/g, respectively. Improvement in phenolics contents and antioxidant activity of peanut during roasting can be supported from the literature. During heating process, compounds with free amino groups such as lysine can undergo a sequence of complex reactions with carbonyl compounds to produce intermediate Maillard reaction products such as furans and its derivatives like tetrahydrofuran (THF), melanoidins, pyrroles, pyrazines, and other heterocyclic compounds that not only impart color, flavor, and aroma to the heated end-use products but also contribute to enhance the antioxidant activity [16, 51, 52]. Melanoidins with high-molecular weight are also one of the end products of Maillard reaction and might have some antioxidant activity [52]. However, Yanagimoto et al. [53] reported that pyrazine formed during roasting of coffee had no antioxidant activity whereas furans and pyrroles exhibited minor antioxidant activity. As peanut contain considerable amounts of carbohydrates and amino acids, it is expected that Millard reaction derived products might have been the major contributor to enhancing the antioxidant attributes of peanut during roasting. Therefore, it is possible to say that while peanuts kernel were roasted with their skin intact, these Maillard reaction products may interact with the monomeric and oligomeric proanthocyanidins present in peanut skin generating some new antioxidant compounds [28, 54].

It was reported that various oligomeric bridged compounds were formed from the reaction between cyanidin-3-*O*-glucoside and Maillard reaction products such as furfural compounds that may play a major role in the flavanol polymerization process [55]. Yu et al. [56] investigated that proanthocyanidin (trimers and tetramers) content of peanut skin decreased after roasting whereas its monomers content increased. Saffan [57] also revealed that heat-stressed peanut seedlings at 40 °C exhibited significantly ($p < 0.05$) higher polyphenolic contents (phenolic acids and flavonoids) than those of the control. The other possible reason is that proanthocyanidin compounds most likely condensed tannins, in peanut skin, have degraded into simpler phenolics during heat treatment leading to increasing the overall contents of phenolics [58]. Similar phenomenon was reported by Rakic et al. [59] where, the contents of gallic acid increased significantly following thermal treatment of oak acorns, whereas those of hydrolysable tannins degraded resulting in an increase of simple phenolics such as gallic acid. Additionally, the significant increase in *p*-coumaric and quercetin contents at 30, 40, and 50 min roasting times in this work may also be explained either as a result of the breakdown of the molecular structures containing phenolic groups or by the thermal stability of these compounds.

Overall, in this research, the increase in the antioxidant capacity of roasted peanut flour, with and without skin, can be likely linked to better release of some antioxidant phenolics such as phenolic acids, flavonols, and degradation of tannins to simple phenolics as well as due to the contribution of Maillard reaction products following roasting process. Obviously, a mixture of such compounds might have contributed to enhanced antioxidant activity.

Conclusion

The results of this study revealed that roasting has significantly affected the antioxidant activity and the phenolic composition of peanut flour, with and without skin. Interestingly, the antioxidant activity and amounts of phenolic compounds in peanut kernel flour with skin were appreciably increased even up to 50-min roasting; however, the longer roasting time (>20 min) resulted in the degradation of phenolic compounds in peanut flour without skin. Therefore, we suggest that proper roasting timing should be taken into account to retain and/or enhance the natural antioxidant phenolics of peanut flour with or without skin.

Based on the high phenolics concentration and superior antioxidant capacity, the roasted peanut flour with skin can be recommended for uses as a potential source of valuable functional bioactives. Since peanut skin is often treated as an agro-waste, it can be explored as a cheap and renewable raw material for isolation of antioxidant compounds for protecting other lipid containing food products, especially the vegetable oils as well as an ingredient of functional foods and nutraceuticals. Furthermore, an in-depth study on structural elucidation of some novel antioxidants formed during peanut roasting process is highly recommended.

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