

VARIATION OF PHENOLICS AND ANTIOXIDANT ACTIVITY BETWEEN PEEL AND PULP PARTS OF PEAR (*PYRUS COMMUNIS* L.) FRUIT

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Abstract

The aim of present study was to appraise the antioxidant activity and phenolic contents in peel and pulp parts from two locally harvested varieties of pear. The extraction yield of antioxidant components obtained with 80% methanol (80:20 methanol-water v/v) was found to be 14.30-17.50% for peel and 11.50-13.00 g/100 g for pulp on dry weight basis. The total phenolic contents and total flavonoid contents in peel and pulp parts of the fruits tested varied over 601.50-619.25, 333.90-355.80 mg GAE/100g and 543.50-561.30, 270.50-290.50 mg CE/100g, respectively. Reducing power, in terms of absorbance values, of peel and pulp extract (at 12.5 mg/mL concentration) ranged between 0.56-0.58 and 0.30-0.32, respectively. DPPH radical scavenging activity and inhibition of linoleic acid peroxidation varied from 49.71-49.94% and 60.32-60.60% in peel and 27.89-28.29% and 34.15-34.45% in pulp. The results of our present study indicate that pear peel exhibited significantly higher antioxidant activity and phenolic contents compared to the pulp and thus, being an agrowaste, can be explored as a viable source of natural antioxidants for the functional food and pharmaceutical applications.

Introduction

Currently, much attention is being focused on the consumption of fruits because of their valuable constituents which contribute towards prevention of degenerative diseases caused by oxidative stress (Lopez *et al.*, 2007; Reddy *et al.*, 2010). Fruits contain a wide array of dietary phytonutrients such as flavonoids, phenolic acids, carotenoids and vitamins with strong antioxidant capacities (Oliveira *et al.*, 2009). Among fruits, *Pyrus* species of family Rosacea are reported to contain considerable amount of valuable compounds such as carotenoids, anthocyanins and phenolics that act as natural antioxidants and in turn impart health-promoting effects to the consumers (Gil *et al.*, 2002; Kim *et al.*, 2003).

The overall nutritional and functional food value of fruits can be better understood by assessing their antioxidants and bioactives profile which in turn may depend on the type of fruits and their cultivation conditions (Scalzo *et al.*, 2005). Similarly, the distribution of antioxidants may vary among different parts such as peel and pulp of the same fruit (Soong & Barlow, 2004; Manzoor *et al.*, 2012). Interestingly, the peel of several fruits such as citrus, mango and apple etc. have shown higher antioxidant activity than the pulp fractions (Jayaprakasha *et al.*, 2001; Manzoor *et al.*, 2012).

Pear (*Pyrus communis* L.) fruit is popular among consumers due to its high nutritive value, good taste and low caloric level. It has a low content of protein and lipids and is rich in sugars such as fructose, sorbitol, and sucrose. It has been found that pears contain 12.4% sugars, 0.5% protein, 0.3% lipids and 2.8% fiber (Barroca *et al.*, 2006). Apart from their richness in macronutrients, pears also possess others nutritional components such as vitamins and antioxidants that are important as health-beneficial compounds (Silos-Espino *et al.*, 2003; Hagen, 2006). Pear is recommendable substitute for diabetics and the obese; moreover, its dietary fiber together with phenolics helps reduce the risk of cardiovascular diseases (Colaric *et al.*, 2007; Nunes *et al.*, 2008).

In Pakistan different varieties of pear fruits are available including Nakh, Nashpati and Bartlett etc. According to estimates, the total area under pear cultivation in Pakistan is around 2.4 thousand hectares

which includes 0.1 Punjab, 2.4 NWFP and 0.1 thousand hectares Balochistan while the total production of pear in Pakistan is 30.7 thousand tons which includes 1.2 Punjab, 29.0 NWFP, 0.5 thousand ton in Balochistan (Agric. Stat. Pakistan, 2003-2004). As such there have been no any studies so far conducted regarding the variation of antioxidant attributes between different parts of locally cultivated pear fruits. The primary objective of this study was to investigate and compare the antioxidant activity and phenolics between peel and pulp parts of pear fruit consumed in Pakistan.

Materials and Methods

Samples: Fully ripened fresh fruits of two varieties namely Nakh, Nashpati of pear (*Pyrus communis* L.) were collected from the vicinity of Swat (Khyber Pukhtunkha), Pakistan during Summer-2011. Three different samples of each variety were randomly harvested. The fruit specimens were further identified and authenticated from the Department of Horticulture, University of Agriculture, Faisalabad, Pakistan.

Chemicals and reagents: Linoleic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, Folin-Ciocalteu, Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT) and gallic acid were procured from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and reagents (analytical grade) used in the present investigation were from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland), unless stated otherwise.

Sample preparation: After washing thoroughly with tap water, the fruits were peeled off to separate pulp and peel parts. The pulp and peel portions recovered were sliced into approx. 1 × 1 cm cubes using a steel knife.

Dry matter determination: Due to varying levels of moisture in the pear fruits of the two varieties, all calculations were done on dry matter basis. For dry matter determination, AOAC procedure (method 925.10) was

used. Briefly, known weight (5.0 g) of the sample was subjected to an electric-oven (Memmert, Germany) drying at 105°C, until a constant weight achieved.

Antioxidant activity of fruits

Extraction of antioxidant components: Homogenized fruit from each pear variety (each 20 g) was extracted with 200 mL of 80% aqueous methanol (methanol: water, 80:20, v/v) at room temperature for 8 h using an electric orbital shaker (Gallenkamp, UK). The residues and the extracts were separated by filtering through a filter paper; the residues obtained were re-extracted twice with the fresh portion of extraction solvent. The extracts recovered from three extractions were combined and excess of the solvent distilled-off in a vacuum rotary evaporator (EYELA, Tokyo, Japan) at 45°C. The semi-solid extracts obtained were quantitatively transferred to the extraction solvent and preserved at 4°C, until used for further experiments.

Determination of total phenolics content (TPC): A colorimetric method, based on Folin-Ciocalteu reagent, was used to appraise the amount of total phenolics (Singleton & Rossi, 1965). The process involved the mixing of 50 mg of crude extract with Folin-Ciocalteu reagent (0.5 mL) and deionized water (7.5 mL). After waiting for 10 min, 1.5 mL of 20% aqueous sodium carbonate (w/v) was added and then the mixture incubated at 40°C in a water bath for 20 min, followed by cooling in an ice bath. The absorbance of the final mixture was monitored at 755 nm (Spectrophotometer U-2001, Hitachi Instruments Inc., Tokyo, Japan). For calculation of TP amount, a standard gallic acid calibration curve, prepared by running solutions in the concentration range of 10-200 mg/L ($R^2 = 0.9980$), was constructed. The amounts of total phenolics were expressed as gallic acid equivalents (GAE) mg/100g of dry matter.

Determination of total flavonoid contents (TFC): The amounts of TF were determined colorimetrically. A previously described method (Zhishen *et al.*, 1999) was used wherein fruit extract (1-mL containing 0.1 mg/mL dry matter) was mixed with 4 mL of water in a 10 mL volumetric flask. At the start, 3 mL of aqueous 5% NaNO₂ were added to the volumetric flask, then at 5 min, 0.3 mL of 10% AlCl₃ and at 6 min, 2 mL of 1.0 M NaOH were added sequentially. Finally, the volume was raised up to 10 mL by adding more distilled water. The reaction mixture was mixed thoroughly in the flask for homogenization. The absorbance was noted at 510 nm using a spectrophotometer. TFC, calculated using a standard calibration curve, were reported as catechin equivalents (mg CE/100g of dry matter).

DPPH scavenging assay: The 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity of the extracts was assessed following a previously described procedure (Brands-William *et al.*, 1995). In brief, 5.0 mL of freshly prepared DPPH methanolic solution (0.025 g/L) and 1.0 mL of the extract (containing 25 µg/mL of dry matter in methanol) were mixed in a test

tube. Absorbance was recorded at different time intervals starting with 0 to 12 min at 515 nm using a Hitachi U-2001 spectrophotometer. The DPPH radical (DPPH) scavenging was calculated by standard formula given elsewhere (Maleeha *et al.*, 2012). Absorbance measured at 5th min was used for the comparison of the radical scavenging activity of the extracts.

Determination of antioxidant activity in linoleic acid system: The antioxidant activity of the tested peach fruit extracts was also determined following the measurement of inhibition of linoleic acid peroxidation (Osawa & Namiki, 1981). In this test, the fruit extract (5 mg of each pear variety) was mixed independently with an emulsion which contained solution of linoleic acid (0.13 mL), 10 mL of 99.8% ethanol and 10 mL of (0.2 M) sodium phosphate buffer (pH 7). The mixture was brought to 25 mL with distilled water and incubated at 40°C up to 360 h. The magnitude of linoleic acid oxidation was measured by peroxide formation according to the thiocyanate method as described by Yen *et al.*, (2000). A control, containing all reagents, except the fruit extracts, was also processed under similar conditions. Three synthetic antioxidant compounds, BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole and TBHQ, tertiary butyl hydroxyquinone were used as positive controls for comparison purposes.

Percent inhibition of linoleic acid oxidation was calculated as under:

$$\frac{100 - \text{Abs. increase of sample at 360 h}}{\text{Abs. increase of control at 360 h}} \times 100$$

Determination of reducing power: The reducing power of the fruit extracts was determined according to a previously described procedure (Oyaizu, 1986) with slight modifications. The extracts (2.5-12.5 mg/mL) were mixed with 5.0 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 5.0 mL of potassium ferricyanide (1.0%) in a test tube. The reaction mixture was placed in a water bath at 50°C for 20 min and then 5 mL of 10% trichloroacetic acid were added followed by centrifugation of the mixture at 980 g for 10 min at 5°C using a refrigerated centrifuge machine (CHM-17; Kokusan Denki, Tokyo, Japan). After centrifugation, the upper phase of the reaction mixture (*ca.* 5.0 mL) was collected and diluted further by adding 5.0 mL of distilled water and 1 mL of 0.1% ferric chloride (FeCl₃) solution. The absorbance of the final solution was read at 700 nm using a spectrophotometer.

Result and Discussion

Extract yields, total phenolics and total flavonoids content: The extract yield using 80% methanol of peel and pulp from two varieties of pear fruit is shown in Table 1. The extractable matter varied from 14.30-17.50 g /100g for peel and 11.50-13.00 g/100 g for pulp showing significant variation between the two parts analyzed. The peel of pear fruit gave higher extraction yield compared to that of the pulp.

Table 1. Percent extraction yield, total phenolic contents (TPC) and total flavonoid contents (TFC) of peel and pulp extracts from two varieties of pear (*Pyrus communis* L.) fruit.

Variety	Dry matter (%)		Extract yield (g/100g)		TPC (mg gallic acid equivalent/100g dry weight)		TFC (mg catechin equivalent/100 g dry weight)	
	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Nakh	37.82±0.71	25.06±0.52	17.50±0.35	13.00±0.26	619.80±12.40	355.80±7.12	561.30±11.23	290.50±5.81
Nashpati	32.52±0.68	22.57±0.47	14.30±0.29	11.50±0.23	601.50±12.03	333.90±6.68	543.50±10.87	270.50±5.41
Mean	35.17±0.69 ^a	23.81±0.49 ^b	15.90±0.32 ^a	12.25±0.24 ^b	610.65±12.21 ^a	344.85±6.9 ^b	552.40±11.05 ^a	280.50±5.61 ^b

Data are mean ± SD ($n=3 \times 3$, $p<0.05$)

Different superscript alphabets within the Mean's row indicate significant differences ($p<0.05$) of means between peel and pulp

The extractable components from various plant materials are strongly dependant on the nature of extractable solvents. Polar solvents usually aqueous mixture of methanol and ethanol are commonly employed for the recovery of polyphenolic compounds from various plant materials. Anwar *et al.*, (2006) extracted antioxidant compounds from various plant materials including rice bran, coffee beans, citrus peel and guava leaves using aqueous 80% methanol (methanol: water, 80:20 v/v).

The antioxidant activity of plant phenolics is due to the reactivity of phenol moieties (hydroxyl group on aromatic ring) which have the ability to scavenge free radicals via hydrogen donation or electron donation. Total phenolics were determined by Folin-Ciocalteu (FC) reagent method. In FC reagent method, phenols form a blue colored phosphomolybdic-phosphotungstic complex in the presence of alkaline solution (Ajila *et al.*, 2007). The total phenolics content in 80% methanolic extract of Nakh and Naspati peels was found to be 619.80 and 601.50, whereas 355.80 and 333.90 mg GAE/100g DW for pulp. Both peel and pulp parts of pear fruit var. Nakh exhibited higher TPC than those of var. Naspati. Literature reports also reveal that peels of most of the fruits contain higher content of total phenolics compared to the pulp parts (Cevallos-Casals *et al.*, 2003; Lata, 2007; Manzoor *et al.*, 2012).

The total flavonoids content of two varieties of pear peel was found to be 543.50-561.30 mg CE/100g, whereas, for pulp these amounts ranged from 270.50-290.50 mg CE/100g).

In general, TPC and TFC results for both the varieties of pear fruit showed that the peels contained higher phenolic contents than the pulps (Table 1). However, peels of Nakh contained more phenolic and flavonoid contents than Naspati.

DPPH radical scavenging activity: The scavenging of the stable DPPH radical is widely used to assess antioxidant activity in a short time compared with other methods. This assay allows comparison of the reactivity of powerful antioxidants such as BHT and ascorbic acid with those present in fruit methanolic extracts against DPPH radical. It has also an advantage of being unaffected by certain side reaction of polyphenolic compounds (Oliveira *et al.*, 2009). A freshly prepared solution of DPPH displays a deep violet color which gives maximum absorption within 515-528nm that gradually fades in the presence of radical scavengers/antioxidants.

In reaction mixture, BHT and BHA that acted as positive controls gave the fastest color change from purple to yellow. In this assay the absorbance was recorded at different intervals (1-12 min) from the beginning of reaction however maximum difference in scavenging activity was recorded at 5th min of reaction.

As for the TPC and TFC, the peels extract of both varieties of pear fruit exhibited higher scavenging activity ranging from 49.71-49.94% compared to those of the pulp extract 27.89-28.29% (Fig. 1). Pear fruit var. Nakh exhibited high scavenging activity compared to Var. Nashpati. Statistical analysis revealed no significant variation in radical scavenging activity between two varieties tested; however, difference was significant between the peel and pulp parts.

Antioxidant activity of pear peel and pulp extract in linoleic acid peroxidation system: Both peel and pulp of pear fruit demonstrated appreciable inhibition of linoleic acid peroxidation as shown in Fig. 2. The inhibition of peroxidation ranged from 60.32 to 60.60 % for the peel extract whereas 34.15 to 34.45 % for the pulp extract.

The peel and pulp of Nakh exhibited higher inhibition of peroxidation whereas lowest by Nashpati. Significant difference in this assay was observed between peel and pulp of two varieties of pear fruit whereas variation between different varieties was found to be non significant.

Reducing power of pear extracts: Assessment of reductive capability is an important parameter to assess antioxidant potential of fruit extracts. The presence of reducing agent in a sample causes the reduction of ferric to ferrous and thus reduction potential can be measured by monitoring the color intensity of Prussian blue complex formed at 700 nm.

The reduction potential measured at concentration rang of 2.5 to 12.5 mg/mL of peel and pulp extract of two varieties of pear fruit is depicted in Table 2. The reductive capability of peel and pulp extracts at 12.5 mg/mL, ranged from 0.56-0.58 and 0.30-0.32, respectively. The peel of var. Nakh exhibited higher (0.58) reductive capability compared to the Naspati (0.56). In case of pulp, var. Naspati executed higher (0.32) reduction potential whereas, lowest (0.30) by var. Nakh. The reducing power of peel extract was significantly higher compared to the pulp extract.

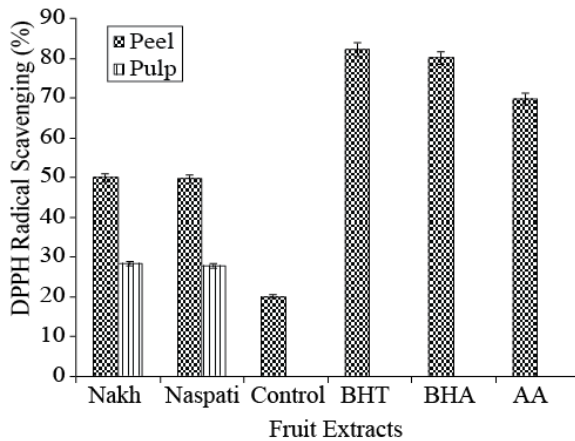


Fig. 1. DPPH radical scavenging activity of 80% methanolic extracts from peel and pulp of two varieties of pear fruit. Error bars indicate mean \pm SD ($n=3 \times 3$) BHT: butylated hydroxy toluene; BHA: butylated hydroxyanisole; AA: ascorbic acid.

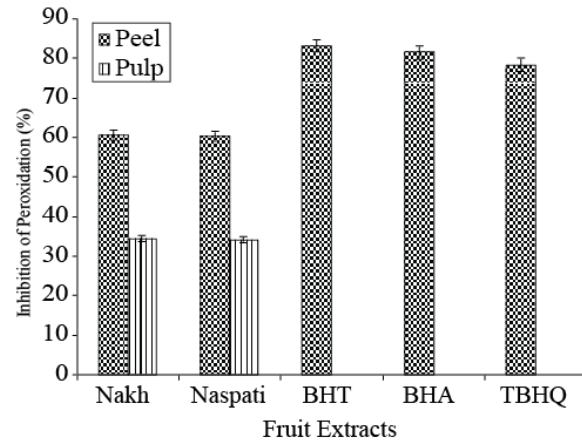


Fig. 2. Antioxidant activity of 80% methanolic extracts from peel and pulp of two varieties of pear fruit. Error bars indicate mean \pm SD ($n=3 \times 3$) BHT: butylated hydroxy toluene; BHA: butylated hydroxyanisole.

Table 2. Reducing power (absorbance values at 700 nm) of peel and pulp extracts from different varieties of pear (*Pyrus communis* L.) fruit.

Pear variety	Conc. (mg/mL)	Pulp	Peel
Nakh	0.0	0.004 \pm 0.0001	0.01 \pm 0.0001
	2.5	0.007 \pm 0.0004	0.03 \pm 0.0003
	5.0	0.02 \pm 0.001	0.07 \pm 0.004
	7.5	0.06 \pm 0.002	0.13 \pm 0.003
	10.0	0.13 \pm 0.004	0.27 \pm 0.006
	12.5	0.30 \pm 0.01	0.58 \pm 0.01
Mean		0.0868 \pm 0.0029	0.182 \pm 0.0039^b
Naspati	0.0	0.005 \pm 0.0001	0.009 \pm 0.0007
	2.5	0.009 \pm 0.0003	0.02 \pm 0.001
	5.0	0.03 \pm 0.001	0.05 \pm 0.002
	7.5	0.08 \pm 0.003	0.12 \pm 0.03
	10.0	0.15 \pm 0.05	0.25 \pm 0.04
	12.5	0.32 \pm 0.03	0.56 \pm 0.01
Mean		0.099 \pm 0.014^b	0.168 \pm 0.014^b

Data are mean ($n = 3$) SD \pm ($n = 3$, $p < 0.05$).

Different superscript alphabets within the mean's row indicate significant differences ($p < 0.05$) between peel and pulp

Conclusions

The results of the present study advocate that peel portion of two varieties of pear analyzed are rich in antioxidants. Besides, var. Nakh relatively exhibited superior antioxidant potential and amounts of phenolics. The uses of pear as a viable source for extraction of antioxidants can be recommended.

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(Received for publication 15 July 2012)