

Comparative Study of Antioxidant Activity of Apple and Pear Peel

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Abstract - Apple and pear are popular fruits consumed in Korea and are common fruit in daily diet. In order to compare the antioxidant activity of the apple and pear peels, total polyphenol contents, total flavonoid contents, ABTS⁺ free radical scavenging activity, and DPPH free radical scavenging activity were measured from hot water, ethanol, and methanol extracts of the two fruit peels. The total polyphenol and flavonoid contents were highest in 95% methanol extracts of the apple peels and 70% ethanol extract of the pear peels, respectively. Total polyphenol contents of the pear peels were higher than that of apple peels, and total flavonoid contents of the apple peels were higher than that of pear peels. The apple and pear peels had the highest ABTS⁺ and DPPH free radical scavenging activity in 95% methanol extracts and 70% ethanol extracts, respectively. ABTS⁺ and DPPH free radical scavenging activity of pear peels was higher than that of apple peels, and the DPPH free radical scavenging activity of apple and pear peels were detected in hot water, 95% methanol, and 70% ethanol extracts, respectively. Ascorbic acid, a synthetic antioxidant used as positive control, had significantly higher scavenging activity than the apple and pear peels. In conclusion, the apple and pear peel have great potential as natural antioxidants. Therefore, above results should be considered to provide the possibility for the development of high functional antioxidants.

Key words - ABTS, Apple peel, DPPH, Flavonoid, Pear peel, Polyphenol

Introduction

Apple, one of the most popular and important fruits in the world, occupies about 45 percent of the total area under fruit crops and the largest amount of production among the fruits in Korea (Lee, 2000). It is also rich in nutritional ingredients such as vitamin C, dietary fibers, carbohydrates as well as functional ingredients like phenol ingredients, effective against diseases such as diabetes, cancers, cerebrovascular diseases, and heart vascular diseases (Kroon and Williamson, 2005). It is not only popular as a fruit itself, but also used as processed food such as drinks, jam, canned and dried foods (Boyer and Liu, 2004).

Korean pear that belongs to *Pyrus* genus of the rose family is a deciduous arborescent tree and mostly grown in the middle and southern parts of Korean peninsula (Hong *et al.*, 2004). It is an important fruit next to apple, citrus fruit and grapes (Zhang *et al.*, 2003). It has been reported that the cell membranes of pear contain large amount of moisture and is

composed of 20-30% of cellulose, 25% of hemicellulose, 35% of pectin, 5-10% of glycoprotein and small amount of phenolic substances which are mutually connected in complicated structures (Fisher and Bennett, 1991). In ancient folk remedies, the peels are used as healing herbs for boil and skin diseases, flesh for treating constipation, cough, phlegm and hangover, and pear leaves as treatment for the acute gastroenteritis as a special remedy (Jang *et al.*, 2003).

Polyphenols that are contained much in apple and pear are antioxidation ingredients, and it has been reported that phenol contents in the peel is about 2-9 times more than in the flesh (Tsao *et al.*, 2003). Polyphenol is an aromatic compound which has more than two phenolic hydroxyl groups in a molecule, and it has been reported that polyphenol is concerned in strengthening immunity (Choi *et al.*, 2003) and bacterial activity (Boo *et al.*, 2012). The natural polyphenol compounds, which are plenty in plants, are majorly flavonoids, lignins, and tannins (Sharma and Sehgal, 1992).

Reactive oxygen species (ROS) produced during the metabolic processing of various bioenergies are noxious materials which are also produced in all organisms including human

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beings by free radical reaction, and degenerate biofunctions such as DNA degeneration, damaging cell membranes, and protein oxidation. ROS causes various adult diseases and accelerates aging (Valko *et al.*, 2007). Antioxidants such as ascorbic acid, tocopherol and carotenoid, play important roles in delaying or inhibiting the degeneration of biofunction (Lim *et al.*, 1996). The development of natural antioxidants has been undertaken continuously (Cort, 1974), but it requires high costs. The antioxidation effects of the natural antioxidants are lower than those of synthetic antioxidants. On the other hand, since the antioxidation effects of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are known to be higher and prices are also low, they are used largely in commercial purposes. However, these substances are harmful to human body, and hence studies on the natural antioxidants with high safety and antioxidation activity are continuously requested (Brannen, 1975).

Therefore, the objectives of this study were (1) to determine whether apple and pear peels can be used as antioxidants, and (2) to compare the antioxidation effects by measuring the total polyphenol contents, total flavonoid contents, ABTS free radical scavenging activity and DPPH free radical scavenging activity for different solvents hot water, 70% ethanol and 95% methanol.

Materials and Methods

Materials

The apple (trade name: Hongro) and pear (trade name: Singo) grown in Geochang Gyeongnam and Sangju Gyeongbuk respectively, were purchased from the local market, and only peels were used as materials in our study. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Fluka Biochemika AG (Buchs, Switzerland). 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS), ascorbic acid, and other chemicals were purchased from Sigma (St Louis, MO, USA). All chemicals were of analytical grade.

Preparation of the extracts

The apple and pear peels were dried in a vacuum oven at 40°C, ground into fine powder, and filtered using the sieve. For hot water extraction, 10 g of each powder sample was

boiled in 500 mL of distilled water at 100°C for 2 hrs using a heating mantle (Wisd, Korea), and then cooled and centrifuged at 8,000 rpm for 15 mins. The pellet collected by centrifugation was resuspended in 500 mL of distilled water, and centrifuged at 8,000 rpm for 15 mins. For ethanol and methanol extractions, after 10 g of each powder samples were soaked in 500 mL of 70% ethanol and 95% methanol, respectively, extracted at 25°C for 6 hrs using shaking incubator (Hanbaek, Korea) at 120 rpm, and then centrifuged at 8,000 rpm for 15 mins.

The supernatant solutions collected by three times centrifugation at 8,000 rpm for 15 mins, concentrated until 10 ml remained using a rotary evaporator (Eyela, Japan). The concentration temperature was at 65°C for hot water extraction and 50°C for ethanol and methanol extractions. Finally, freeze dried samples were stored at -70°C until further analysis.

Determination of total extraction yields

Total extraction yields of the apple and pear peels were calculated by the following equation:

$$\text{Yield (\%)} = A/B \times 100$$

A is amount of samples after freeze dry, and B is amount of samples used for extraction.

Determination of total polyphenol contents

The total polyphenol contents of the peel extracts of apple and pear were determined by Folin-Denis method (Folin and Denis, 1912). 60 µL of Folin-Ciocalteu reagent were added to 60 µL of the different extract solutions, and then stand for three min. Thereafter, 60 µL of 10% Na₂CO₃ was added to the mixture and then 1 hr incubation at room temperature, the absorbance was determined at 750 nm using ELISA microplate reader (Bio-Rad 680, USA). Gallic acid monohydrate as a standard phenol was used to prepare a standard curve. The total polyphenol contents of the extracts were expressed as gallic acid equivalents from the linear regression curve of gallic acid

Determination of total flavonoid contents

The total flavonoid contents of apple and pear peel extracts

were determined by the method of Moreno *et al.* (2000). 62.5 μ L of the different extract solutions were added to each well of plate, followed by addition of 1.08 mL of 80% ethanol, 30 μ L of 10% aluminum nitrate, and 30 μ L of 1 M potassium acetate, mixed well and reacted at room temperature for 40 mins. Absorbance of the reaction mixture was read at 415 nm using microplate reader. Rutin hydrate as a standard flavonoid was used to prepare the standard curve. Total flavonoid contents were expressed as Rutin Equivalents from the linear regression curve of rutin.

ABTS radical scavenging assay

Antioxidant activity of the peel extracts of apple and pear were measured in this assay as ability to ABTS radical scavenge according to Re *et al.* (1999). ABTS⁺ was produced by reacting 7 mM/L of ABTS [2,2'-azino-bis(3-ethylbenzothiazoline- δ -sulfonic acid)] solution with 2.45 mM/L of potassium persulfate, and the mixture would be kept in the dark at room temperature for 24 hrs. ABTS⁺ solution was diluted with PBS (phosphate buffer saline) to an absorbance of 0.70 \pm 0.02 at 732 nm. 20 μ L of the different extract samples diluted to 1000 μ g/mL were added to 180 μ L of ABTS⁺ solution and mixed vigorously. After reaction at room temperature for 1 min, the absorbance at 732 nm was measured using microplate reader. Ascorbic acid as a synthetic antioxidant, prepared in the same concentrations as the test extracts, was used as the positive controls for comparison. The ABTS⁺ scavenging effect was calculated by the following formula:

$$\text{Electron donating activity (\%)} \\ = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

A_{control} is the absorbance of control without sample and A_{sample} is the test sample with ABTS⁺.

DPPH radical scavenging assay

Antioxidant activity of the peel extracts of apple and pear were measured in this assay as ability to DPPH radical scavenge according to Blois (1958). 160 μ L of the different extract samples diluted to 1,000 μ g/mL were added to 40 μ L of 0.15 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) solution,

respectively and allowed to react in the dark at room temperature for 30 min. Absorbance of DPPH was measured at 517 nm using microplate reader. Ascorbic acid prepared in the same concentrations as the test extracts was used as the positive controls for comparison. DPPH free radical scavenging activity was calculated according to the following equation:

$$\text{Electron donating activity (\%)} \\ = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100$$

A_{control} is the absorbance of control without sample and A_{sample} is the test sample with DPPH

Statistical analysis

Activity data were analyzed using a one-way analysis of variance (ANOVA) accompanied with Tukey tests and Student's *t*-test (SPSS for Windows, Ver. 21), and presented as means \pm standard error. All measurements were replicated three times. P-values less than 0.05 were considered to be statistically significant.

Results

Total extraction yields of apple and pear peels

In apple peels, total extraction yields from the hot water, 70% ethanol, and 95% methanol were 59.91%, 81.94%, and 51.25%, respectively (Table 1). In pear peels, total extraction yields were 46.84%, 59.22%, and 46.09%, respectively

Table 1. Total extraction yields of apple peels

Sample	Total extraction yields (%)
Water extracts	59.91
70% EtOH extracts	81.94
95% MtOH extracts	51.25

Table 2. Total extraction yields of pear peels

Sample	Total extraction yields (%)
Water extracts	46.84
70% EtOH extracts	59.22
95% MtOH extracts	46.09

(Table 2). In both apple and pear peels, the highest extract yields were observed in 70% ethanol extract, and higher in the apple peel extracts.

Total polyphenol contents

The total polyphenol contents in apple peels were 16.41±0.23 mg/g, 15.32±0.09 mg/g, and 16.50±0.12 mg/g from the hot water, 70% ethanol and 95% methanol extracts, respectively. Statistically significant lower yields were observed in 70% ethanol extracts as compared to hot water and 95% methanol extracts, while the latter two showed no difference. In the case of pear peels, the contents from the hot water, 70% ethanol and 95% methanol extracts were 21.68±0.03 mg/g, 24.08±0.25 mg/g, and 22.17±0.08 mg/g, respectively. Statistical significance was similar to the apple peel samples (Fig. 1). The total polyphenols were maximized in 95% methanol extracts of the apple peels, and 70% ethanol extracts of the pear peels. The total polyphenol contents were higher in the pear peels than in the apple peels.

Total flavonoid contents

The total flavonoid contents in apple peels from hot water, 70% ethanol, and 95% methanol were 15.08±0.21 mg/g, 16.15±0.43 mg/g, and 22.52±1.16 mg/g, respectively. The highest content was observed in 95% methanol extracts. In the case of pear peels, the observed contents were 15.18±0.49 mg/g, 17.04±1.32 mg/g, and 14.03±0.31 mg/g, respectively. The highest content was observed in 70% ethanol extracts (Fig. 2). In apple peels, a significantly higher flavonoid contents were observed in 95% methanol extracts as compared to those from hot water and 70% ethanol extracts. No such differences were observed in the pear peels.

ABTS free radical scavenging activity

ABTS free radical scavenging activity of ascorbic acid was 94.29±0.03%, which indicates very high free radical scavenging activity. In the apple peel, ABTS free radical scavenging activity of the hot water, 70% ethanol and 95% methanol extracts were 19.14±9.57%, 10.56±5.28%, and 20.29±10.14%, respectively. The highest ABTS free radical scavenging activity was observed in 95% methanol extracts. ABTS free radical scavenging activity in the pear peel

extracts were 39.83±19.91%, 44.16±22.08%, and 43.50±21.75%, respectively. The pear peels had the highest ABTS⁺ free radical scavenging activity in 70% ethanol extracts. ABTS free radical scavenging activity of pear peels was higher than that of apple peels (Fig. 3). Statistical significant results were not observed among the extracts in both apple and pear peels, which implies low scavenging activity in comparison with ascorbic acid.

DPPH free radical scavenging activity

DPPH free radical scavenging activity of ascorbic acid was 34.23±1.59, and DPPH free radical scavenging activity of apple peels from hot water, 70% ethanol and 95% methanol were, respectively, 32.90±1.15%, 23.78±1.55%, and 38.93±1.70%, with 95% methanol being the highest scavenging activity. DPPH free radical scavenging activity of pear peels were 38.51±10.64%, 67.92±13.86%, and 49.83±4.66% for the same order of extracts. The 70% ethanol solvent showed

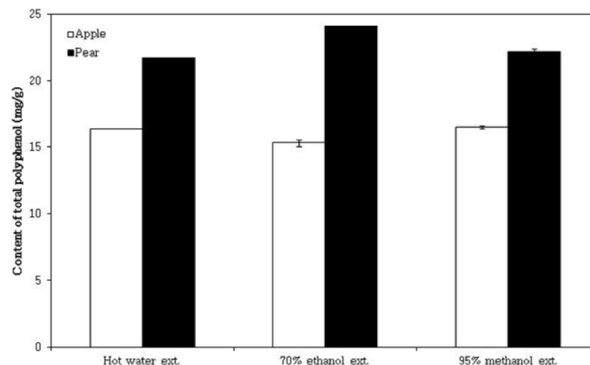


Fig. 1. Total polyphenol contents of apple and pear peels by solvents. The bars mean the standard error.

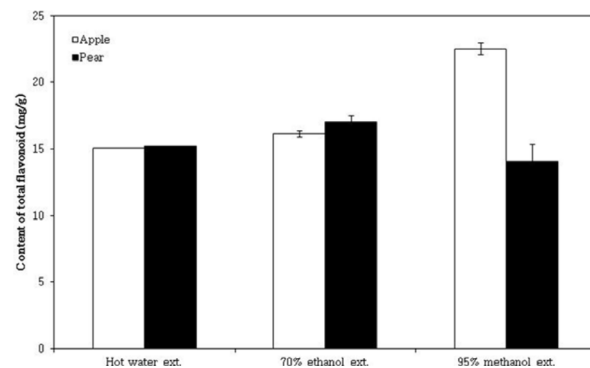


Fig. 2. Total flavonoid contents of apple and pear peels by solvents. The bars mean the standard error.

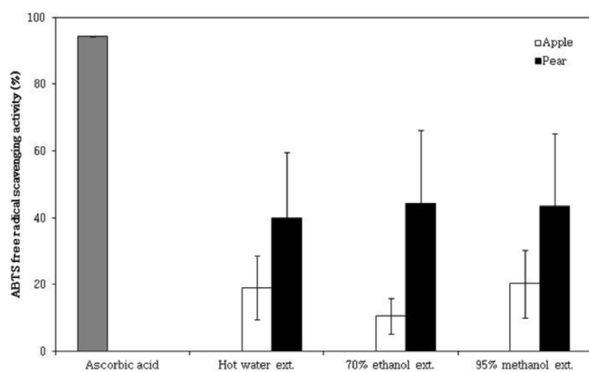


Fig. 3. ABTS radical scavenging activity of apple and pear peels by solvents. Ascorbic acid prepared in the same concentrations as the test extracts was used as the positive controls for comparison. The bars mean the standard error.

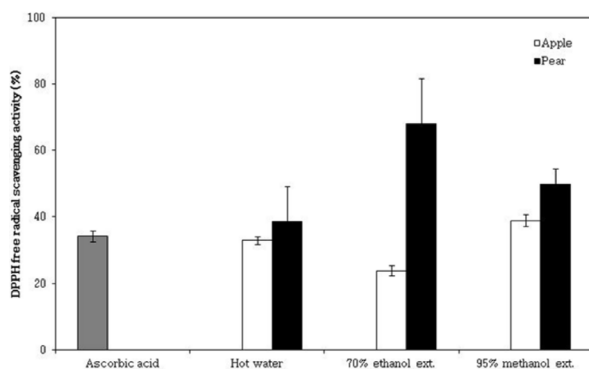


Fig. 4. DPPH radical scavenging activity of apple and pear peels by solvents. Ascorbic acid prepared in the same concentrations as the test extracts was used as the positive controls for comparison. The bars mean the standard error.

the highest scavenging activity (Fig. 4). In apple peels, the hot water showed statistically significant differences in DPPH free radical scavenging activity from 70% ethanol and 95% methanol, and in pear peels significant differences were observed between the hot water and 70% ethanol, and between the hot water and 95% methanol. Comparison with ascorbic acid, statistically significant results for DPPH free radical scavenging activity were not observed among the extracts in apple peels, whereas observed in 70% ethanol and 95% methanol extracts of pear peels.

Discussion

Our society is being progressed rapidly into aging society

as the levels of living and medical care are elevated. Accordingly, peoples are more and more interested in preventing infectious diseases and/or delaying the aging. Therefore, the necessity of antioxidation is being increased. Thus, present study aims at finding out the valuable resources for antioxidation and possibilities of practical uses of these resources by studying the effects of antioxidants in the peels of our representative fruits, apple and pear (Zhang *et al.*, 2003), widely distributed all over our country. Total extraction yields from 70% ethanol were highest in both apple and pear peels, and the yields were in the order of 70% ethanol, hot water and 95% methanol. Total extraction yields were higher in apple peels than in pear peels. However, it cannot be concluded that higher yield means higher antioxidation effect.

Phenolic compounds in plants, one of secondary metabolites, are known to have antioxidation function which is the property that phenolic hydroxyl group combines with large molecules such as enzymes (Husain *et al.*, 1987), and hence experiments on the total polyphenol contents, based on the oxidoreduction reactions, are performed, applying the phenomenon of changing the color blue when the extracts are reduced by polyphenol compounds (Ainsworth and Gillespie, 2007). Wolfe *et al.* (2003) reported that antioxidation effect in the peel of apple was higher than in the flesh. Thereafter, in the present study the total polyphenol contents were measured from apple peels only. The total polyphenol contents for the solvents did not show statistically significant differences although relatively higher polyphenol content was observed from 95% methanol extract. However, in apple peels the polyphenol contents from methanol extract was lower than $19.7 \pm 1.3\%$ reported by Cheign *et al.* (2011). In pear peels, relatively higher polyphenol content was observed in 70% ethanol extract. Park *et al.* (2007) reported that the polyphenol contents were determined from ethanol extracts of some Korean pear varieties. In the present study, relatively higher values of polyphenol were observed. As shown in a study on antioxidant activity of different parts of kiwi (Park *et al.*, 2008), flavonoid is yellowish and contained more in the peels rather than in the flesh. In apple peels, relatively high values of flavonoid, which can function as an antioxidant, were observed in 95% methanol extracts, and in pear peels in 70% ethanol extracts. It can be seen that the total

contents of polyphenol and flavonoid have the same trends. Similar results were reported in the pericarps of citron and trifoliolate orange (Park *et al.*, 2008). Similar results were also obtained in the present study.

ABTS free radical scavenging activity – a means of measuring antioxidation effects by using decolorizing reaction of bluish green color when ABTS cation absorbance produced by reacting with an oxidant, potassium persulfate, is scavenged (Myung and Hwang, 2008) – was used, and for which ascorbic acid were involved in the experiments. The higher scavenging activity was observed in ascorbic acid. In apple peels, the highest ABTS free radical scavenging activity was observed in 95% methanol extracts, and in pear peels it was highest in 70% ethanol extracts. According to the results of a study on antioxidant extracted from litchi peel (Jeong *et al.*, 2010), the radical scavenging activity was increased as the concentrations of extracts increased. Thus, it is considered that, in order to affirm the radical scavenging activity, precise examinations should be made at several levels by dividing the part that gives the highest scavenging activity such that distance between levels is small.

DPPH free radical scavenging activity – a means of measuring antioxidation effects by using the phenomenon: decolorizing reaction of purple color and scavenging free radicals when a certain extract containing antioxidant activity donates an electron to DPPH free radical – was used (Yen and Chen, 1995), and ascorbic acid, a synthetic antioxidant, was used as positive control in the measurement of DPPH free radical scavenging activity. DPPH free radical scavenging activity was lower than ABTS free radical scavenging activity in ascorbic acid. Relatively high scavenging activities were observed from 95% methanol extract in apple peels and from 70% ethanol extract in pear peels. Scavenging activity was higher in pear peels than in apple peels. Considerably higher scavenging activities were observed from 70% ethanol extract and 95% methanol extracts as compared to those from ascorbic acid, a synthetic antioxidant. Efficient substance of pomegranate for antioxidation was observed in the peels (Roh *et al.*, 2005), and in a study on the antioxidant activity of *Codonopsis lanceolate*, the highest antioxidant activity was observed in the peels (Kang, 2009). However, according to the reports of Park *et al.* (2007) on the measurement of the

antioxidant activity observed in ethanol extracts from Korean pears varieties, the antioxidant activity from whole fruits and flesh + peel were higher than those measured from the peel, which are different from the results reported by Jin and Song (2012), where the antioxidant activity in the peel was higher than in the flesh. The other study showed that free radical scavenging activity increased as the concentration of the extract from litchi peels increased (Jeong *et al.*, 2010), and free radical scavenging activity of the extracts from Campbell grape seeds and peels increased along with the extract temperature (Park *et al.*, 2003). Further studies are demanded to ascertain the free radical scavenging activities and to find out the optimum levels or combination of levels of extracting temperature and of solvent concentrations that gives the highest activity through well-designed experiments.

Consequently, the total polyphenol contents and the total flavonoid contents were similar in values, showing that, on average, higher polyphenol was observed in pear peels and higher flavonoid in apple peels. In the measurement of electron donating activity, ABTS and DPPH free radical scavenging activity were, on average, higher in pear peels than in apple peels. Since the compatible solvents are different for different fruits, it is considered that the solvents may affect the antioxidant activities. Pear peels are considered more valuable than apple peels for the development of natural antioxidants.

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