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Antioxidant Activities of Methanol Extracts and Phenolic Compounds in Asian Pear at Different Stages of Maturity

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Abstract Contents of phenolic compounds in peel, flesh, and core of three Asian pear cultivars, Hosui, Niitaka, and Chuwhangbae, were determined at different stages of maturity. Antioxidant properties of methanol extracts of peels at various fruit maturity stages were also evaluated. Total phenolic content decreased with maturity. Arbutin, chlorogenic acid, and epicatechin were major phenolic compounds in young fruits. Catechin, 4-hydroxymethyl benzoic acid, and caffeic acid were detected in peel and core of immature and mature pears. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activities of methanol extracts were 16.30 and 15.73 μg in peel of immature Hosui and Chuwhangbae pears, respectively, and 11.59 μg in mature Niitaka pears, which was significantly higher than those of other maturity stage in the same cultivar. Inhibitory activities on lipid oxidation of methanol extracts of three cultivars at all maturity stages were similar to that of α-tocopherol.

Key words: Asian pear, phenolic compounds, antioxidant activities, maturity

Introduction

Asian pear production has increased steadily along with improvement of quality of life, with domestic Asian pear cultivated area of 24,100 ha and a production of 316,600 tons per year, close to the apple production of 365,400 tons (1). Pear production in 2010 is forecast to increase to about 438,000 tons in cultivated area of about 30,000 ha (2). However, dramatic increase of cultivated area has brought about concerns of excess pear production with little loss, which resulted in low pear prices in years of large production. Recent occurrences of typoons have caused losses of fruits each year (1). Low quality fruits due to poor cultivation techniques or typoons have very limited processing applications and tend to be used only for pasteurized pear juice at a cheap price.

With consumers' interests focused on healthy living, demands have been increasing for natural and functional foods containing bioactive substances that can control biorhythm and aging and prevent disease (3). Among the bioactive substances, polyphenolics are known to have antioxidant activity (4). Fruits, including strawberry, grape, and apple, are the main sources of polyphenolics (5). Numerous researches have been conducted on polyphenolics and their antioxidant activities in fruits (6-9). Previous studies focused on quality issues of compositions and derivatives of polyphenols compounds causing browning (10-12), and structural recognition of phenol substances in pear (13, 14). Recent research activities also showed physiological activities in mature pear (15) and sorted fruits for utilization of under-utilized substances (16).

Because low-valued pears due to natural disaster and poor cultivation techniques are usually discarded, it will be possible to utilize these dropped or immature fruits to produce new food ingredients such as functional food ingredients. There have been few studies conducted in the area of physiological activities of pears at different maturity stages including immature fruits.

The objectives of this study were to investigate possible utilization of low-valued pears including dropped or immature fruits as functional food ingredients. Contents of phenolic compounds in peel, flesh, and core of pears at different maturity stages were measured, and the radical-scavenging activities and inhibitory effects on lipid oxidation by methanol extract of the fruit peel containing relatively higher levels of phenolic compounds were evaluated.

Materials and Methods

Materials Three cultivars of Asian pears used in this study were Hosui (Poong Su), Niitaka (Shin Go), and Chuwhangbae. They were grown and produced at Naju, Cholla Nam Do in 2003. Maturity of the fruit was divided into three stages and determined based on days after coming into full bloom as follows: 69 days (young fruit), 115 days (immature fruit), and 153 days (mature fruit) for Hosui; 73 days (young fruit), 119 days (immature fruit), and 179 days (mature fruit) for Niitaka; 73 days (young fruit), 134 days (immature fruit), 192 days (mature fruit) for Chuwhangbae. Seven to ten fruits from selected standard pear trees of the three cultivars were used for experiments.

Preparation of methanol extracts The pears were peeled, and their cores were removed. The peel, flesh, and core were separately homogenized with a homogenizer (BM-2, Nihonseiki Kaisha Ltd, Tokyo, Japan) with fivefold methanol for 2 min. The extracts were filtered through Whatman No 1 filter paper. The residues were extracted again with methanol using the same method as mentioned above. The extracts were combined and evaporated to dryness in a rotary evaporator (VV2011, Heidolph, Schwabach, Germany) at 40°C, dissolved in

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methanol, and used for determination of total phenolic concentration, and major phenolic compounds were analyzed.

To measure the antioxidant activity, the extract was dissolved in distilled water, washed with *n*-hexane repeatedly to remove chlorophyll and fats, and made up to final volume of 25 mL. Solid-phase extraction (SPE) was used to remove sugar. SPE tubes (Waters Sep-Pak C_{18} Cartridges) were preconditioned by washing with 5 mL methanol and water. Prepared extraction solution (2.5 mL) was transferred to the SPE tube, and the tube was washed with 5 mL water (pH 2.0) prior to elution of the phenolic fraction with 5 mL methanol. The methanol fraction was taken to dryness under nitrogen gas and stored at -20°C freezer until use (7).

Total phenolic contents and analysis of composition of phenolic compounds Total phenolic contents were determined using the Folin-Denis method (17). One hundred microliters of the methanol extract was added to about 50 mL distilled water. Five milliliters of Folin-Ciocalteu regent (Sigma Chemical Co., St. Louis, MO, USA) and 10 mL of 20% sodium carbonate solution were added, mixed, and made up to a final volume of 100 mL. The absorbance at 760 nm was measured (UV-1201, Shimadzu, Tokyo, Japan) after 30 min. The amount of total phenolic was calculated from standard curve of tannic acid (Yakuri Pure Chemicals Co., Ltd., Kyoto, Japan).

The extract sample was filtered through a 0.45-μm membrane filter and analyzed by an HPLC system. The separation of phenolic was performed on a Spherisorb ODS2 column with a particle size of 5 μm, 25.0×0.46cm. The solvent system used was a gradient of water and formic acid (19:1, v/v) (A) and methanol (B), starting with 5% methanol and installed a gradient to obtain 15, 25, 30, 35, 45, and 50% B at 3, 13, 25, 35, 39, and 42 min, respectively. The absorbance was measured at 280 nm with a UV/VIS detector (UV-975, JASCO, Tokyo, Japan) at solvent flow rate of 0.9 mL/min (18). Standards (Sigma Chemical Co) used in this study were as follows: chlorogenic acid, epicatechin, catechin, caffeic acid, arbutin, ferulic acid, sinapic acid, 4-hydroxy-methyl benzoic acid (4-HMBA), and *p*-coumaric acid.

DPPH free radical-scavenging activity A method studied by Abe *et al.* (19) was used to measure 1, 1-diphenyl-2-picryhydrazyl (DPPH) radicals. DPPH solution (0.9 mL, 1 $\times 10^{-4}$ M) dissolved in ethanol was mixed with 0.1 mL extract sample in a test tube and vortexed. The absorbance was measured at 517 nm after 10 min incubation in darkness. α-Tocopherol was used as the control. One hundred % DPPH free radical-scavenging concentration was set at when absorbance reading is stable with increasing concentration of α-tocopherol. The parameter SC_{50}, which reflects 50% depletion of DPPH free radicals, was expressed, and the following formula was used to measure free radical-scavenging activity:

Radical-scavenging activity (%) =

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Inhibitory activity on lipid oxidation Ferric thiocyanate (FTC) method (20) using linoleic acid model system was used to measure inhibitory activity on lipid oxidation. Mixture (0.5 mL) of methanol and extract sample, 0.5 mL of 2.51% linoleic acid, 1 mL of 50 mM phosphate buffer (pH 7.0), and 0.5 mL distilled water were mixed well in a capped test tube and incubated at 40°C. Sample aliquots (0.1 mL) were taken every 24 hr, dissolved in 9.7 mL of 75% ethanol, and placed in a capped test tube. Thirty percent ammonium thiocyanate solution (0.1 mL) was added to the sample suspension. After 3 min, 0.1 mL ferrous chloride solution (20 mM) dissolved in 3.5% HCl solution was added to the sample suspension as a color indicator. The absorbance was measured at 500 nm using a spectrophotomer for color indication until the absorbance of control reached the maximum value. Oxidation Index (OI) and antioxidant activity were measured as follows:

Oxidation index (OI) = $\text{Absorbance}_t / \text{Absorbance}_{t=0}$

Antioxidant activity (AA) =

$$100 - \frac{\text{Sample extract oxidation index}}{\text{Control oxidation index}} \times 100$$

Statistical analysis Results were expressed as mean values. Comparison of means was performed by Duncan's multiple range test using Statistical Analysis System (SAS).

Results and Discussion

Total phenolic contents and analysis of composition of phenolic compounds Total phenolic contents of peel, flesh, and core of pears at each maturity stage were shown in Table 1. The total phenolic contents decreased gradually as the fruit ripened, from 22.66, 22.98, and 20.61 mg/g for peels of young Hosui, Niitaka, and Chuwhangbae fruits to 2.96, 2.21, and 1.52 mg/g for those of mature fruits, respectively. The total phenolic contents in cores of young Niitaka and Chuwhangbae fruits were 24.61 and 37.96 mg/g and were higher than those in flesh. The total phenolic content of flesh was less than 1 mg/g except for young fruit of Chuwhangbae. The flesh of cultivars at each maturity stage had the lowest total phenolic contents. With the maturity of fruits, contents of polyphenols in the peel and flesh of all cultivars decrease. Polyphenols were high mainly in peel while low in flesh. Similar results were reported by Kim (21). Wilkinson (22) reported that polyphenols in fruit react with other substances and accumulate in different forms of polyphenols as the fruit matures. Kim (23) also reported that phenolic compounds content in fruit peel and flesh decreased as the fruit matures rapidly. Ryugo (24) reported that through color reaction tannins and polyphenols are localized about the vascular bundles, core line, and epidermal layers, where the stone cells are abundant. Machida (25) indicated that pulp cells containing polyphenols are distributed mostly in peel of fruit, while only some pulp cells contain polyphenols. Our results showed higher phenolic contents in peel and core of fruit.

Table 1. Contents of total phenolic compounds in Asian pear fruit at different growth stages (mg/g fresh weight basis)

Cultivar	Stage	Peel	Flesh	Core
Hosui	Young fruit	22.66[a]	0.87[a]	6.39[a]
	Unripe fruit	14.94[b]	0.45[b]	1.65[b]
	Ripe fruit	2.96[c]	0.46[b]	1.42[b]
Niitaka	Young fruit	22.98[a]	0.98[a]	24.61[a]
	Unripe fruit	16.49[b]	0.49[b]	5.69[b]
	Ripe fruit	2.21[c]	0.30[c]	1.78[c]
Chuwhangbae	Young fruit	20.61[a]	1.23[a]	37.96[a]
	Unripe fruit	10.86[b]	0.44[b]	5.04[b]
	Ripe fruit	1.52[c]	0.32[c]	1.43[c]

Young fruit: 69 (Hosui) and 73 (Niitaka and Chuwhangbae) days.
Unripe fruit: 115 (Hosui), 119 (Niitaka) and 134 (Chuwhangbae) days.
Ripe fruit: 153 (Hosui), 179 (Niitaka) and 192 (Chuwhanbae) days.
These days elapsed till harvest after full bloom.
Different letters within the same column indicate significantly different values ($p<0.05$).

Based on the composition analysis of phenolic compounds in peel, flesh, and core of pear (Table 2-4), the major phenolic compounds identified in flesh were arbutin, chlorogenic acid, and epicatechin, and the phenolics content decreased as the fruit matured. In peel and core of young fruit, arbutin, chlorogenic acid, and epicatechin were also identified as major phenolic contents. Although the phenolic contents were lower in immature and mature fruits than in young fruit, catechin, 4-HMBA, and caffeic acid were detected. The content of arbutin was highest in peel of mature fruit, 68.65, 93.74, and 74.51 mg/100 g in Hosui, Niitaka, and Chuwhangbae, respectively. Kang *et al.* (26) reported similar results that arbutin was the most abundant phenolic compound in the peel of Niitaka pear. Seo (12) and Blankenship *et al.* (10) showed different contents of phenolic compounds. In this study the content of chlorogenic acid was higher in the flesh and core of Hosui and Niitaka than epicatechin, while the that of epicatechin was higher in the peel than in chlorogenic acid. Similar results were reported by Sannomaru (27) in that the content of epicatechin was higher in apple peel during maturity than that of chlorogenic acid. In all three cultivars, *p*-coumaric acid, ferulic acid, and sinapic acid were not detected.

DPPH free radical-scavenging activity by methanol

Table 2. Contents of phenolic compositions in peel of Asian pear fruit at different growth stages (mg/100 g fresh weight basis)

Cultivar	Stage	Arbutin	Catechin	4-HMBA[1)]	Chlorogenic acid	Caffeic acid	Epicatechin
Hosui	Young fruit	335.54[a]	-[2)]	-	27.89[a]	-	66.77[a]
	Unripe fruit	120.99[b]	2.58[b]	-	11.43[b]	4.65[a]	39.42[b]
	Ripe fruit	68.65[c]	5.77[a]	13.01	8.53[c]	3.43[b]	9.33[c]
Niitaka	Young fruit	502.48[a]	-	-	49.10[a]	-	65.62[a]
	Unripe fruit	241.36[b]	5.74[a]	-	27.66[b]	6.25[a]	68.02[a]
	Ripe fruit	93.74[c]	5.80[a]	37.62	11.18[c]	3.77[b]	23.65[b]
Chuwhangbae	Young fruit	483.66[a]	-	-	103.88[a]	-	67.03[a]
	Unripe fruit	176.35[b]	6.44[a]	-	40.91[b]	4.04	52.21[b]
	Ripe fruit	74.51[c]	3.32[b]	-	9.74[c]	-	9.25[c]

[1)]4-hydroxymethyl benzoic acid.
[2)]Not detected.
Young fruit, unripe fruit and ripe fruit are same meaning in Table 1.
Different letters within the same column indicate significantly different values ($p<0.05$).

Table 3. Contents of phenolic compositions in flesh of Asian pear fruit at different growth stages (mg/100 g fresh weight basis)

Cultivar	Stage	Arbutin	Chlorogenic acid	Caffeic acid	Epicatechin
Hosui	Young fruit	11.41[a]	3.91[a]	0.14	3.47[a]
	Unripe fruit	1.96[b]	1.31[b]	-[1)]	0.89[b]
	Ripe fruit	1.50[b]	0.69[c]	0.07	-
Niitaka	Young fruit	16.55[a]	6.22[a]	-	4.04[a]
	Unripe fruit	3.52[b]	2.48[b]	-	1.28[b]
	Ripe fruit	1.88[c]	0.42[c]	-	-
Chuwhangbae	Young fruit	17.97[a]	5.26[a]	-	3.90
	Unripe fruit	2.12[b]	1.17[b]	0.07[b]	-
	Ripe fruit	1.64[b]	0.71[c]	0.12[a]	-

[1)]Not detected.
Young fruit, unripe fruit and ripe fruit are same meaning in Table 1.
Different letters within the same column indicate significantly different values ($p<0.05$).

Table 4. Contents of phenolic compositions in Core of Asian pear fruit at different growth stages (mg/100 g fresh weight basis)

Cultivar	Stage	Arbutin	Catechin	4-HMBA[1)]	Chlorogenic acid	Caffeic acid	Epicatechin
Hosui	Young fruit	51.85[a]	-[2)]	-	15.09[a]	0.36[a]	4.79[a]
	Unripe fruit	13.81[b]	0.11[a]	-	4.03[b]	0.21[b]	-
	Ripe fruit	9.17[c]	0.06[b]	0.80	2.34[c]	0.13[c]	0.83[b]
Niitaka	Young fruit	228.39[a]	-	-	269.46[a]	-	43.53
	Unripe fruit	62.34[b]	-	-	63.98[b]	-	-
	Ripe fruit	30.04[c]	-	0.87	21.98[c]	-	-
Chuwhangbae	Young fruit	530.35[a]	-	-	72.32[a]	-	117.03[a]
	Unripe fruit	26.16[b]	0.54	-	37.80[b]	0.60	-
	Ripe fruit	14.56[c]	0.46	1.66	17.00[c]	-	1.42[b]

[1)]4-hydroxymethyl benzoic acid.
[2)]Not detected
Young fruit, unripe fruit and ripe fruit are same meaning in Table 1.
Different letters within the same column indicate significantly different values ($p<0.05$).

Table 5. Amount of methanol extract from peel of Asian pear fruit necessary to reduce by 50% DPPH radical scavenging (SC_{50}) at different growth stages

Stage	Hosui		Niitaka		Chuwhangbae	
	SC_{50} (μg)	Fresh wt. Eq (mg)	SC_{50} (μg)	Fresh wt. Eq (mg)	SC_{50} (μg)	Fresh wt. Eq (mg)
Young fruit	20.27	2.03	18.57	1.86	18.76	2.09
Unripe fruit	16.30	1.81	15.10	1.67	15.73	2.24
Ripe fruit	17.33	3.46	11.59	2.32	17.55	3.42

Young fruit, unripe fruit and ripe fruit are same meaning in Table 1.
Different letters within the same column indicate significantly different values ($p<0.05$).

extracts during maturity The contents of phenolic compounds were high in pear peel compared with those of flesh and core. DPPH free radical-scavenging activity of the methanol extracts obtained from pear peel was measured during maturation of pears (Fig. 1). Immature and mature Hosui pears, and young and mature Chuwhangbae pears showed similar scavenging activity curves. The results of SC_{50}, which reflects 50% depletion of DPPH free radicals, are shown in Table 5. Scavenging activity by methanol extracts (SC_{50}) was considerably low (20.27 μg) in young Hosui pear, while relatively high (11.59 μg) in mature Niitaka pear. SC_{50} was higher (15.73 μg) in immature Chuwhangbae pear than in young and mature pears. When scavenging activities of the cultivars were compared within the same maturity stage, activity was higher in Niitaka than in Hosui and Chuwhangbae. SC_{50}, based on weight of fresh peel sample, and the total contents of phenolic compounds did not show significant correlation (r= -0.5613; $p>0.05$). Leontowicz *et al.* (8) reported that, using DPPH and β-carotene content, anti-oxidant activity and total contents of phenolic compounds in apple and pear showed significant correlation. Jinenez-Escrig *et al.* (5) reported a significant correlation between DPPH free radical-scavenging activity and total contents of phenolic compounds in guava fruit. However, Kahkonen *et al.* (28) and Marina Heinonen *et al.* (7) reported no significant correlations between phenolic compounds and antioxidant activities of plant extracts, berry, fruit wine, and liquor. Antioxidant activity can not be measured based on the total contents of phenolic compounds, because each phenolic compound has its own characteristic according to Folin-Ciocaltieu method, and its antioxidant activity varies depending on its chemical structure as determined by the methyl linoleate method (29). Therefore, in this study correlation between DPPH free radical-scavenging activity and the total contents of phenolic compounds was not observed. It is assumed that scavenging activity of pear cultivars and at maturity stages depends on not only the contents of phenolic compounds, but also the compositions of phenolic compounds.

Inhibitory activity on lipid oxidation at each maturity stage Oxidation Index (OI) of the methanol extract of fruit peel at each maturity stage was measured using linoleic acid model system using 0.5 mg/mL assay mixture (Fig. 2). OIs for three cultivars at each maturity stage were considerably lower than that of control sample, indicating that methanol extracts of pear peel have inhibitory activity on lipid oxidation. When OI value for control reached peak, antioxidant activity was measured (Table 6). Anti-oxidant activities of immature Hosui and Chuwhangbae extracts were 89.63 and 92.01%, respectively, which were higher than those of young and mature pears. Inhibitory activity on lipid oxidation of mature Niitaka extract was 89.97%, which was higher than those of young and immature Niitaka, and showed similar pattern to their DPPH free radical-scavenging activity. Inhibitory activity on lipid oxidation of the three cultivars ranged from 79.30 to 92.01%, which was lower compared with 97.92% of BHT and similar to 86.34% of α-tocopherol. In addition

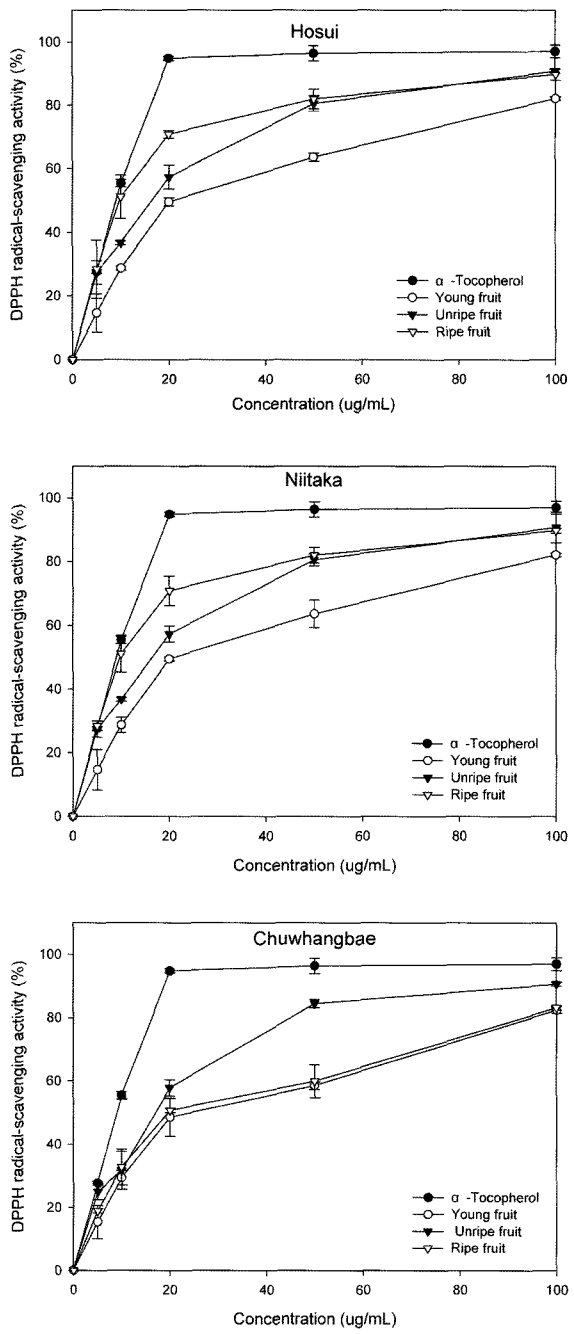

Fig. 1. DPPH radical scavenging activity of methanol extracts from peel of Asian pear fruit at different growth stages. Young fruit, unripe fruit and ripe fruit are same meaning in Table 1.

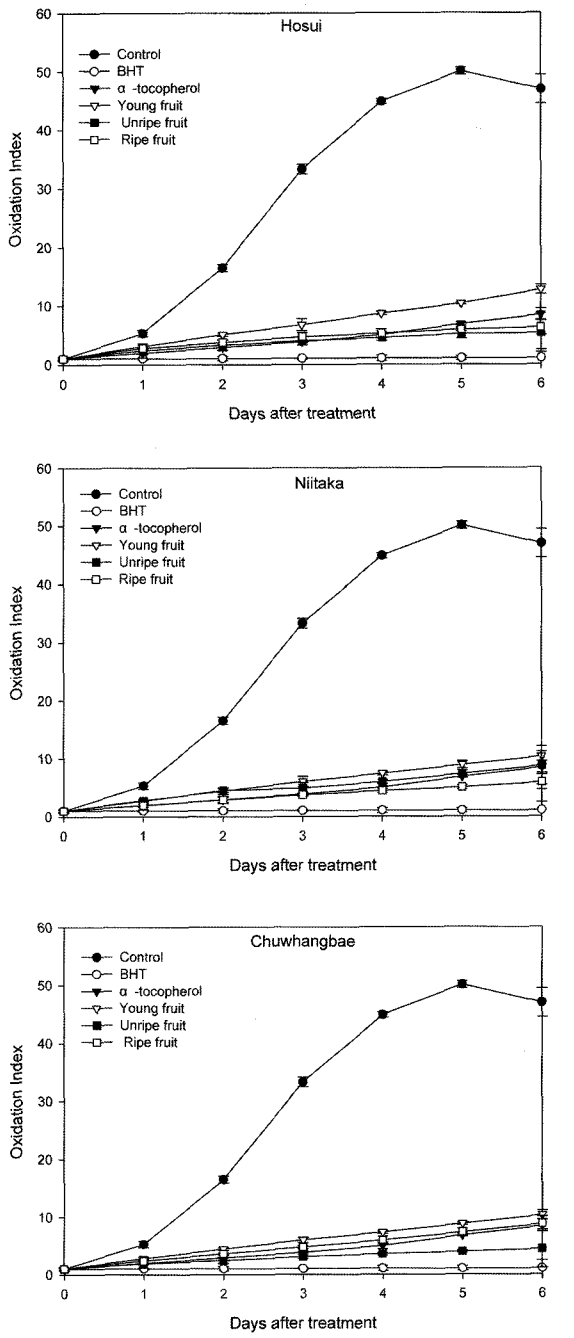

Fig. 2. Oxidation index of methanol extracts from peel of Asian pear fruit at different growth stages. Young fruit, unripe fruit and ripe fruit are same meaning in Table 1.

Table 6. Antioxidant activity of methanol extracts from peel of Asian pear fruit at different growth stages (%)

Stage	Hosui	Niitaka	Chuwhangbae
Young fruit	79.30[b]	82.26[c]	82.36[c]
Unripe fruit	89.63[a]	85.34[b]	92.01[a]
Ripe fruit	88.21[a]	89.97[a]	85.26[b]

Young fruit, unripe fruit and ripe fruit are same meaning in Table 1. Different letters within the same column indicate significantly different values ($p<0.05$).

inhibitory activity on lipid oxidation was higher than that of the peel extract of mango fiber and similar to that of the peel extract of pineapple fiber (20, 30).

Zheng *et al.* (9) reported that the role of phenolic compounds in berry's antioxidant activity mostly relies on their compositions and contents, and oxygen radical-scavenging activity in caffeic acid was higher compared with chlorogenic acid. Antioxidant activity using methyl linoleate method (31) and the experiment with lard as lipids (32) was greater in caffeic acid than in chlorogenic acid. However, other studies (33, 34) indicated that

scavenging activities in caffeic acid and chlorogenic acid were similar to trolox equivalent antioxidant activity and antioxidant activity in linoleic acid. Nakauweski (35) reported that phenolic compounds including chlorogenic acid, caffeic acid, catechin, and epicatechin in linoleic acid system have antioxidant activities similar to those of BHA and BHT. According to these data, antioxidant activity of each phenolic compound varies depending on the methods used. Results of this study showed chlorogenic acid, caffeic acid, catechin, and epicatechin extracted from peel could contribute to the antioxidant activity of pear peel. Ioku *et al.* (36) reported that arbutin using FTC method has antioxidant activity against lipids. Hisatomi *et al.* (37) indicated that arbutin showed antioxidant activity in linoleic acid. These results suggested that arbutin could play a role in the antioxidant activity of pear peel. Sanchez *et al.* (38) reported that there was a correlation coefficient of 0.46 between antioxidant activity in pear peel and chlorogenic acid contents, whereas very low correlation with flavonol and arbutin. Antioxidant activity cannot be expressed by contents of phenolic compounds. Inhibitory activity on lipid oxidation using methanol extracts from pear peel at each maturity stage may have correlation with the complicated roles of composition and content of phenolic compounds.

Low value pears such as immature fruits and dropped fruits, and pear peel, byproduct from processing, were evaluated for utilization as ingredients for functional foods. Three cultivars including Hosui, Niitaka, and Chuwhangbae pears were analyzed for contents of phenolic compounds and antioxidant activities of methanol extracts of the peel. Total contents of phenolic compounds decreased with pear maturity. Within the same maturity stage, the total contents of phenolic compounds were the lowest in flesh. Arbutin, chlorogenic acid, and epicatechin were the major components extracted from young fruits. Arbutin was the most abundant and its content decreased with the maturity of fruit. Catechin, 4-hydroxymethyl benzoic acid, and caffeic acid were extracted from peel and core of mature pear. In terms of DPPH free radical-scavenging activity of the methanol extract of pear peel, SC_{50} values were 16.30 and 15.73 μg in immature fruits of Hosui and Chuwhangbae, respectively, and 11.59 μg in mature fruit of Niitaka. No significant correlations were observed in SC_{50} values depending on fresh sample weight and total content of phenolic compounds. Inhibitory activity on lipid oxidation of extracts of immature fruits of Hosui and Chuwhangbae were 89.63 and 92.01%, which were higher than those of young and mature fruits. And that of mature fruits of Niitaka was 89.97%, which was higher than those of young and immature fruits, whereas similar to that of DPPH free radical-scavenging activity. Inhibitory activities on lipid oxidation by three cultivars were lower than that of BHT and similar to α-tocopherol. In conclusion, the peels of Asian pears obtained from processing plant as by-products could be good sources for use as ingredients of functional foods due to their antioxidant activity. Low-valued pears including immature fruits contain higher contents of phenolic compounds than mature fruit. Therefore, they may be more useful as materials for functional food ingredients.

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