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RESEARCH ARTICLE

Comparison of Bioactive Compound Contents and *In vitro* and *Ex vivo* Antioxidative Activities between Peel and Flesh of Pear (*Pyrus pyrifolia* Nakai)

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Abstract We compared chemical constituents and antioxidative activities between the flesh and peel of two Asian pears (*Pyrus pyrifolia* Nakai cv. Niitaka and Chuhwangbae). Total phenolic, flavonoid, and ascorbic acid contents in the peels were higher than those in the flesh. However, total tocopherol content between peels and flesh was not different. The peels exhibited higher free radical scavenging activities in the *in vitro* models of DPPH, $ABTS^+$, nitrite radicals, and reducing capabilities than those of the flesh. Pear fruit extracts significantly prevented 3T3-L1 cells from undergoing H_2O_2-induced oxidation and the effect was higher by the peel extract than by the flesh extract. In addition, blood plasma of rats administered the peel extract showed higher antioxidative activity than that of rats administered the flesh extract. These results suggest that consumption of unpeeled Asian pear fruit may effectively increase antioxidant activity in the body.

Keywords: *Pyrus pyrifolia*, pear, peel and flesh, phenolics, ascorbic acid, antioxidant activity

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Introduction

Fruit is generally composed of peel, flesh, and seeds. The peel plays an important role in protecting the flesh and seeds from various environmental biotic and abiotic stressors such as microorganisms, insects, and oxidative damage-induced factors (1,2). Phytochemicals such as phenolics, flavonoids, tocopherols, ascorbic acid, carotenoids, and triterpenoids contained in the peel (1-3) are produced and accumulated as part of the survival strategy for plants under various environmental stressors. Of them, many phytochemicals exert preventive effects against cancer, diabetes, and cardiovascular diseases in humans (4,5). Therefore, some fruits such as apple, peach, and persimmon are consumed frequently without removing the peels, although it does not provide the best taste and texture, because consumers already know that the biologically active compounds are richer in the peels rather than in the flesh (6).

Pear (*Pyrus* spp.) is a popular fruit in temperate regions. We recently performed a series of investigations on the isolation and structural determination of antioxidative active compounds contained in pear fruit peel. We reported various antioxidants such as seven hydroxycinnamoylmalic acids and their methyl esters (7), eight phenolic compounds (8), five coumaroyl quinic acid derivatives, nine phenolic containing flavonoids (9), and six triterpenes including three caffeoyl triterpenes (10) from the fruit peel of *P. pyrifolia* Nakai cv. Chuhwangbae, which is one of the most highly consumed pear fruits in Korea. In addition, the presence of sitosterols, quercitrin, and triterpenoids has been reported recently in Chinese pear (11,12). However, investigations on the constituents in Asian pears are limited compared to those of European pears. Recent studies (7-

12) on the biologically active compounds contained in Asian pears may provide important basic data for investigations on the beneficial effects of Asian pears.

Pear fruit is generally peeled for eating, because it has a thick peel that contains plenty of pectin and stone cells. European pear fruit possess thin peels and a low amount of pectin and fewer stone cells when compared to those in Asian pear fruit. Therefore, European pear fruits are eaten more often without peeling. However, most Asian pear fruits are consumed after peeling except for some pear-processed foods such as juices, jams, and jellies (13,14). In addition, the peel of Asian pear is used as folk medicine to treat constipation, diuresis, cough, and flu (15). The antioxidative activity of European pear peel extracts is superior to that of flesh extracts (16,17). Recent studies (12,18,19) indicate that Asian and European pears have a variety of chemical compounds. In addition, Asian pears are distinct from European pears in morphological traits and adaptation to a wide range of environments among *Pyrus* species (20,21). Moreover, recent molecular analyses of pears have shown that the restriction fragment length polymorphism patterns of chloroplast DNA are different between Oriental and occidental pears, suggesting that they may have evolved independently (20,22). Nevertheless, a comparison of chemical constituents and biological activity between the flesh and peel of Asian pears has not yet been fully performed. Therefore, we compared ascorbic acid, tocopherol, total phenolic, and flavonoid contents and antioxidative activities in various *in vitro* and *ex vivo* systems of rat blood plasma administered flesh and peel extracts of the two pears, which are cultivars of the most highly consumed pear fruits in Korea.

Material and Methods

Plant materials Fresh fruits of pear, *Pyrus pyrifolia* Nakai cv. Chuhwangbae (CHB) and cv. Niitaka (NTK), grown in Naju city, South Korea, were harvested in October, 2011. Voucher samples (NTK, no. JNU PE 20111015; CHB, No. JNU PE 20111017) were previously deposited in the herbarium of the laboratory. The fruits were hand-peeled with a peel thickness about 3 mm. The flesh samples were peeled and the core part was diced. The peel and flesh fractions were stored at −80°C until use.

Chemicals Folin-Ciocalteu phenol reagent and DPPH were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Aluminum chloride, iron (III) chloride, potassium ferricyanide, catechin, ABTS and 2',7'-dichlorofluorescin diacetate (DCF-DA) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ferulic acid (4-hydroxy-3-methoxycinnamic acid), potassium persulfate, ascorbic acid, and copper (II) sulfate ($CuSO_4$) were purchased from Wako Pure Chemicals Industries Ltd. (Osaka, Japan). Tocopherols were obtained from Fluka Co. (Buchs SG, St. Gallen, Switzerland). Dulbecco's modified Eagle's medium (DMEM) and trypsin were supplied by Welgene Inc. (Daegu, Korea). Bovine serum and penicillin/streptomycin were purchased from Gibco (Grand Island, NY, USA). CCK-8 was obtained from Dojindo Laboratories (Kumamoto, Japan), respectively. Mouse 3T3-L1 preadipocytes were obtained from the American Type Culture Collection (Manassas, VA, USA). All other chemicals and solvents were of analytical grade.

Extraction The fresh pear flesh (1,350 g) was homogenized with 80% EtOH (2,000 mL) and filtered (no. 2; Whatman International Ltd., Maidstone, U.K.). The extraction and filtering steps were repeated twice. The filtrates were combined and concentrated by vacuum evaporation at 38°C. The fresh pear peel (135 g) extracts were also prepared by the same method in 1/10 scale flesh. The extracts were divided into small amounts and stored at −80°C until use.

Determination of total phenolic content Each sample (5 mg) extracted with 80% EtOH (CHB flesh, 34.5 mg fresh wt. eq.; CHB peel, 36.2 mg fresh wt. eq.; NTK flesh, 34.7 mg fresh wt. eq.; NTK peel 34.7 mg fresh wt. eq.) were mixed with Folin-Ciocalteu phenol reagent (1 mL) and saturated Na_2CO_3 solution (1 mL) (23). The mixture was incubated at room temperature for 30 min and then filtered through a Millipore membrane (0.45-μm; Millipore, Bedford, MA, USA). The filtrate was analyzed at a 700 nm wavelength using a spectrophotometer (JP/V-550; Jasco, Tokyo, Japan). Total phenolic content of the samples was quantified from a calibration curve of gallic acid, which was used as the standard compound.

Determination of total flavonoid content The 80% EtOH extracts of each sample (CHB flesh, 275.9 mg fresh wt. eq.; CHB peel, 289.9 mg fresh wt. eq.; NTK flesh, 312.5 mg fresh wt. eq.; NTK peel 277.8 mg fresh wt. eq.) were mixed with 1 mL of distilled water followed by adding 60 μL 5% sodium nitrate (24). The mixture was allowed to react at room temperature for 5 min. Then, 0.1 mL of 10% aluminum chloride solution was added followed by adding 0.5 mL 1 N NaOH and 275 μL of distilled water. The mixture was filtered through a 0.45-μm Millipore membrane and analyzed at a wavelength of 517 nm using a spectrophotometer (JP/V-550; Jasco). Total flavonoid content was determined as percentage of catechin equivalents.

Determination of ascorbic acid content The samples (5 g) were mixed with 10% metaphosphoric acid (50 mL)

at 4ºC (25). After homogenizing for 1 min, the mixture was brought up to 100 mL with cooled 5% metaphosphoric acid solution and centrifuged at 890×*g* for 15 min. The supernatant (2 mL) was mixed with 2 mL MeOH. After filtration with a 0.45-μm membrane filter, the filtrate was subjected to HPLC equipped with Shiseido Capcell Pak column (4.6 mm i.d.×250 mm, 5 μm, Shimadzu,). Elution was achieved using 25 mM metaphosphoric acid solution in an isocratic system at a flow rate of 1.0 mL/min. The compound was monitored at 254 nm using a photodiode array detector (SPDM20A; Shimadzu). Ascorbic acid content in each sample was quantified by the chromatographic peak area of a standard. The calibration curve was plotted in the concentration range of 0.01-10 μg.

Determination of total tocopherol content The samples (5 g, fresh wt.) were homogenized with MeOH/$CHCl_3$=1:2 (v/v, 50 mL). After standing for 30 min, the homogenate was filtered through No. 2 filter paper (Whatman) (26). The residues were extracted with MeOH/$CHCl_3$=1:2 (v/v, 50 mL) using the same procedure. The extracted solutions were combined and brought up to 100 mL with MeOH/$CHCl_3$=1:2 (v/v). An aliquot (25 mL) of the MeOH/$CHCl_3$=1:2 (v/v) solution was concentrated in vacuum, and the concentrate was mixed to 0.5 mL 1% NaCl solution (0.5 mL) and 10 mL EtOH solution containing 3% (w/v) pyrogallol. After sonication, the mixture was added to 3 mL of 60% KOH, heated at 70ºC for 30 min, cooled to room temperature, and then filtered through No. 2 Whatman filter paper. The reaction mixture was added to 1% NaCl (22.5 mL) and partitioned with EtOAc/*n*-hexane=1:9 (v/v, 15 mL, 3 times). The upper layer (EtOAc/*n*-hexane=1:9, v/v) was concentrated in vacuum at 38ºC. The concentrate was dissolved with 4 mL of *n*-hexane and filtered through a 0.45-μm Millipore membrane. The filtrate was subjected to HPLC equipped with a fluorescence detector (RF-10A_{XL}; Shimadzu). Tocopherols were separated on a silica gel column (octyl-80Ts, 4.6 mm i.d.×250 mm, 5 μm; Tosoh, Tokyo, Japan) and monitored at an excitation wavelength of 295 nm and an emission wavelength of 325 nm. Elution was achieved using a solvent mixture of *n*-hexane/EtOAc/AcOH=97.3:1.8:0.9 (v/v/v) and a flow rate of 1.0 mL/min. Total tocopherol content was expressed as the sum of the contents of α-, β-, γ-, and δ-tocopherols.

Measurement of DPPH radical scavenging activity A 60% EtOH solution (250 μL) in 100 mM Tris-HCl buffer (pH 7.4, 250 μL) of each sample at different concentrations was added to a DPPH radical ethanol solution (250 μL, final concentration, 250 μM) (27). The solution was then mixed and allowed to stand for 30 min in the dark. The reaction solution was analyzed by HPLC (SPD-M20D; Shimadzu) operating at 517 nm. DPPH radical scavenging activity of each sample was evaluated from the difference in the peak area decrease of the DPPH radical detected at 517 nm between the blank and sample. The DPPH radicals remaining were analyzed on an Octyl-80Ts column (4.6 mm×150 mm, 5 μm; Shimazu). Elution was achieved using a solvent mixture of MeCN/H_2O=60:40 (v/v) as the mobile phase at a flow rate of 1.0 mL/min.

Determination of $ABTS^+$ radical scavenging activity An $ABTS^+$ radical ($ABTS^{\cdot+}$) solution was prepared by reacting a 7 mM ABTS solution and 2.5 mM potassium persulfate (95:5, v/v) for 12 h at 4ºC in the dark (28). The $ABTS^+$ radical solution was diluted to an absorbance of approximately 0.7±0.15 at 735 nm by adding EtOH. The pear extract samples (final concentration, 100 μg/mL) and ferulic acid (final concentration, 1 μg/mL) as the standard were added to the diluted $ABTS^+$ radical solution (0.5 mL). After the mixture was incubated for 30 min in the dark at room temperature, the solution was centrifuged for 5 min at 9,800×*g* and 4ºC. The absorbance (RS) of the supernatant was measured at 735 nm using a spectrophotometer.

Nitrite scavenging activity assay The pear extracts (final concentration, 1 mg/mL) and ferulic acid (final concentration, 10 μg/mL) were mixed with 0.1 mL of 1 mM $NaNO_2$ solution (29). The pH of the mixture was adjusted to 2.0 using 0.1 N HCl solution. Then, the mixture was brought up to 1 mL with distilled water and incubated at 37ºC for 1 h. Then, the solution was mixed with 5 mL 2% nitric acid and 0.4 mL Griess reagent. The mixture was reacted at room temperature for 15 min and then centrifuged for 5 min at 9,800×*g* at 4ºC. The absorbance of the supernatant was measured at 540 nm.

Determination of reducing power activity The pear extracts (final concentration, 600 μg/mL) and ferulic acid (final concentration, 15 μg/mL) were mixed with 0.5 mL 80% ethanol, 0.5 mL 0.2 M sodium phosphate buffer (pH 6.6), and 0.5 mL potassium ferricyanide solution. The mixture was incubated in a water bath at 50ºC for 20 min. Then, the reacted solution was mixed with 0.5 mL 10% TCA solution and centrifuged for 5 min at 9,800×*g* at 4ºC. The supernatant (0.75 mL) was diluted with 0.75 mL distilled water. Then, 0.1 mL 0.1% ferric chloride solution was added to the solution. After allowing the solution to stand for 10 min at room temperature, absorbance was measured at 700 nm.

Determination of protective effect of the pear extracts against oxidative stress of 3T3-L1 cells induced by H_2O_2 Mouse 3T3-L1 preadipocytes were seeded in DMEM containing 10% bovine serum albumin (BSA) and 1% penicillin/streptomycin. The cells were cultured in a

humidified 5% CO_2 incubator at 37°C. After reaching confluence, the cells were maintained in the same medium without insulin treatment. The medium was replaced with DMEM containing only 10% BSA, and was then changed with the same medium every 2 days. The effect of the Asian pear extract on cell viability was determined using the CCK-8 assay (30). Briefly, mouse 3T3-L1 preadipocytes were harvested in a trypsin-EDTA PBS solution containing 0.05% trypsin and 0.02% EDTA and washed with PBS solution. The cells were plated in 96-well plates at a density of 1×10^4 cells/well and cultured. After 24 h, the pear MeOH extract samples (0.5, 1, 2.5, 5, and 10 mg/mL) were added to the cell culture and incubated for 24 h. The final volume of solvent was <0.1% in the cell culture medium. After the incubation, the cell medium was removed and replaced with 100 μL of fresh cell culture medium. The cell cultures were mixed with 10 μL CCK-8 solution and reacted for 2 h. The absorbance was measured at 450 nm using a microplate reader (31). Cell viability (%) was calculated as the relative percentage of surviving cells relative to control cells without the Asian pear MeOH extract.

Mouse 3T3-L1 preadipocytes (1×10^4 cells/well) were incubated in various concentrations (0.5, 1.0, and 2.5 mg/mL) of MeOH extracts. After the incubation for 24 h, the cell culture was added to 1.0 μM H_2O_2 and then incubated at 37°C for 2 h. Then the cell cultures were mixed with 10 μL CCK-8 solution and reacted for 2 h. The absorbance was measured at 450 nm using a microplate reader (31). Cell viability (%) was calculated by the relative percentage of surviving cells relative to control cells without Asian pear MeOH extracts.

To investigate inhibitory effect of the pear extracts against reactive oxygen species (ROS) induced by H_2O_2 in 3T3-L1 cells, the cells cultured by the same conditions as cell viability were washed twice with PBS solution followed by incubation with 100 μL, 10 μM DCF-DA at 37°C for 12 h (32,33). The cell culture was analyzed with a fluorescence plate reader with an excitation wavelength of 480 nm and emission wavelength of 530 nm. The inhibitory effect of the pear extract on ROS production induced by H_2O_2 was evaluated by comparing the absorbance of control cells.

Inhibitory effect of the pear extracts against copper ion-induced oxidation in rat blood plasma Twenty-five Sprague-Dawley rats (males; 6 weeks of age, 180-200 g; Samtako Bio Korea, Osan, Korea) were kept at 23°C under a 12 h dark/light cycle and fasted for 12-15 h prior to oral administration of sample. Peel [44 mg (fresh wt. 300 mg eq.)/200 g BW] and flesh [500 mg (fresh wt. 3.7 g eq.)/200 g BW] extracts of CHB and NTK were orally administered to rats with 1 mL distilled water. After 1 h, the rats were anesthetized with diethyl ether, the abdomen was opened, and blood was collected from the abdominal aorta into heparinized tubes. Rat plasma was isolated by centrifugation (1,500×*g*, VS-15 CFN; Vision, Bucheon, Korea) at 4°C for 20 min. Blood plasma (250 μL) of each group (n=5) was combined and the mixture (1,250 μL) was diluted five-fold with PBS solution. The plasma mixture was oxidized by adding 240 μL $CuSO_4$ PBS solution (final concentration, 100 μM). The reaction mixture was incubated at 37°C for 10 h with continuous shaking. The cholesteryl ester hydroperoxide (CE-OOH) concentration was determined according to the method of Kim *et al*. (34). Briefly, aliquots (100 μL) of the incubation solutions were withdrawn at 30 min intervals and mixed with 3 mL MeOH containing 2.5 mM 2,6-di-tert-butyl-4-methylphenol (BHT). The mixture was sonicated (Power Sonic 4200; Hwashin, Ulsan, Korea) for 1 min, and then neutral lipids were extracted with 3 mL *n*-hexane by vortexing vigorously for 1 min. The upper layer (*n*-hexane) was collected and extraction of the lower layer with 3 mL *n*-hexane was repeated. The combined *n*-hexane phases were evaporated in a rotary evaporator at room temperature. The remaining lipids were dissolved in 100 μL MeOH/$CHCl_3$ (95:5, v/v), and aliquots were subjected to CE-OOH analysis by reverse phase-HPLC using a TSK-gel Octyl-80Ts column (Tosoh). The effluent was monitored by UV detection at 235 nm (Shimadzu SPD-10A). The mobile phase was a mixed solution of MeOH/H_2O (97:3, v/v), and the flow rate was constant at 1.0 mL/min. CE-OOH concentration was calculated from a standard curve of CE-OOH. Detailed procedures for preparation of the CE-OOH standard have been published previously (35). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Chonnam National University (no. CNU IACUC-YB-R-2013-4).

Statistical analysis Data are expressed as mean±standard deviation (n=3) using the Statistical Package for the Social Sciences (IBM, Armonk, NY, USA) 17.0 package. Total phenolic content, total flavonoid content, and radical-scavenging assay data were assessed by one-way analysis of variance, followed by the Turkey-Kramer test. Significant differences were considered at $p<0.05$. Cell viability and ROS production data in 3T3-L1 cells were evaluated by Student's *t*-test.

Results and Discussion

Total phenolic and flavonoid contents in CHB and NTK peel and flesh As shown in Fig. 1A, pear peel showed overwhelmingly higher total phenolic content (NTK, 246.7±8.8 mg GAE/100 g fresh weight; CHB, 198.3±5.4 mg GAE/100 g fresh weight) when compared to pear flesh (NTK, 10.4±0.1 mg GAE/100 g fresh weight;

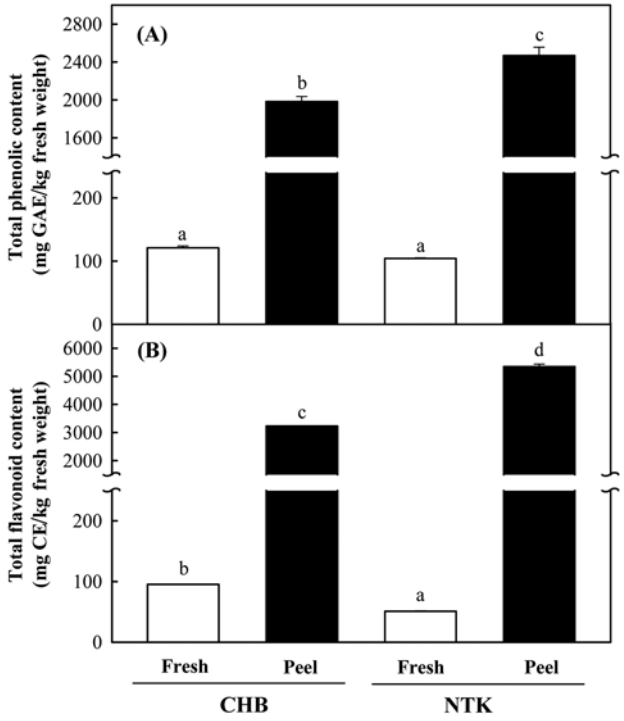

Fig. 1. Total phenolic (A) and total flavonoid (B) contents in fruit flesh and peels of two Asian pear cultivars. Values are mean±SD (n=3). Different letters indicate a significant difference among samples at p<0.05. CHB, Chuhwangbae; NTK, Niitaka; GAE, equivalent to gallic acid; CE, equivalent to catechin

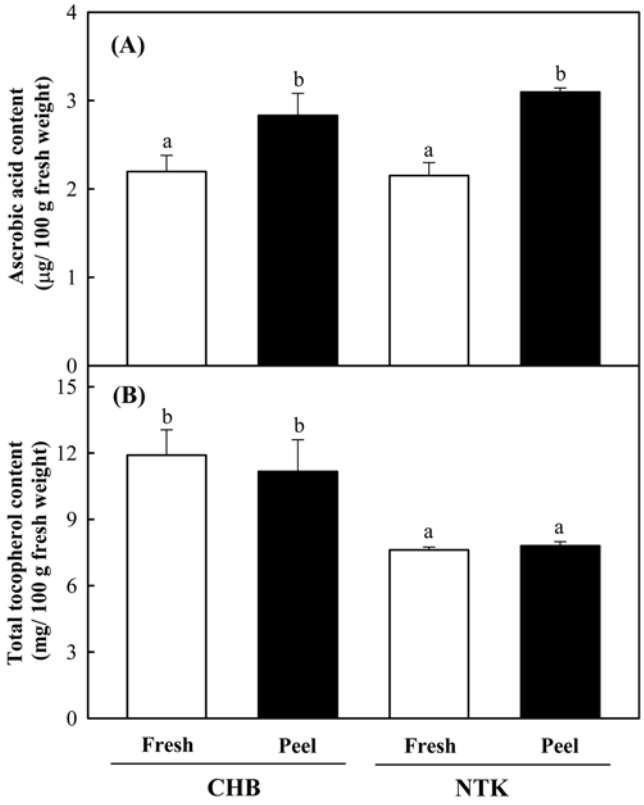

Fig. 2. Ascorbic acid (A) and total tocopherol (B) contents in fruit flesh and peels of two Asian pear cultivars. Values are mean±SD (n=3). Different letters indicate a significant difference among samples at p<0.05. CHB, Chuhwangbae; NTK, Niitaka

CHB, 12.1±0.3 mg GAE/100 g fresh weight). In addition, total phenolic content in the NTK peel was significantly higher (p<0.05) than that in the CHB peel. In contrast, total phenolic content in CHB flesh was slightly higher than that in NTK flesh, but no significant difference was observed.

Total flavonoid content in NTK pear peel (535.0±8.8 mg CE/100 g fresh weight) was significantly higher (p<0.05) than that in CHB peel (323.1±11.00 mg CE/100 g fresh weight) (Fig. 1B). In contrast, total flavonoid content in CHB flesh (9.5±0.6 mg CE/100 g fresh weight) was significantly higher than that in NTK flesh (5.1±0.7 mg CE/100 g fresh weight). Total flavonoid content in pear peel was predominantly higher than that in pear flesh.

Ascorbic acid and total tocopherol contents in CHB and NTK peel and flesh Ascorbic acid content in the peels (NTK, 3.10±0.05 μg/100 g fresh weight; CHB, 2.83±0.25 μg/100 g fresh weight) was slightly higher than that in pear flesh (NTK, 2.15±0.15 μg/100 g fresh weight; CHB, 2.19±0.18 μg/100 g fresh weight) (p<0.05) (Fig. 2A). However, ascorbic acid contents between the two cultivars were not different.

α-Tocopherol was detected on HPLC chromatograms of the peel and flesh extracts, whereas β-, γ-, and δ-tocopherols were not detected (detection limits of β-, γ-, and δ-tocopherols were 30, 25, and 30 pmol, respectively). Therefore, total tocopherol content was calculated only from α-tocopherol content. Total tocopherol content in CHB (11.17±1.44 mg/100 g fresh peel weight and 11.91±1.15 mg/100 g fresh flesh weight) was significantly higher (p<0.05) than that in NTK (7.81±0.19 mg/100 g fresh peel weight, 7.62±0.13 mg/100 g fresh flesh weight) (Fig. 2B). However, no significant difference in total tocopherol contents was observed between flesh and peel.

Free radical scavenging activities of CHB and NTK peel and flesh The free radical-scavenging activities of the pear samples are shown in Fig. 3. The pear peel extracts (NTK, 78.4±0.5%; CHB, 52.3±2.4%) exhibited apparently higher DPPH radical-scavenging activities than those of the flesh extracts (NTK and CHB,<2.0%) (Fig. 3A). NTK (78.4±0 .5%) had significantly (p<0.05) higher DPPH radical-scavenging activity when compared to CHB (52.3±2.4%) and ferulic acid (54.3±1.5%) at a concentration of 10 μg/mL, which was used as a positive control (Fig. 3A).

The pear peel extracts (NTK, 74.8±1.1%; CHB, 62.3±2.4%) at a concentration of 100 μg/mL also showed higher $ABTS^{+}$ radical-scavenging activities than those of the fruit flesh extracts (NTK and CHB,<0.5%) (Fig. 3B). However,

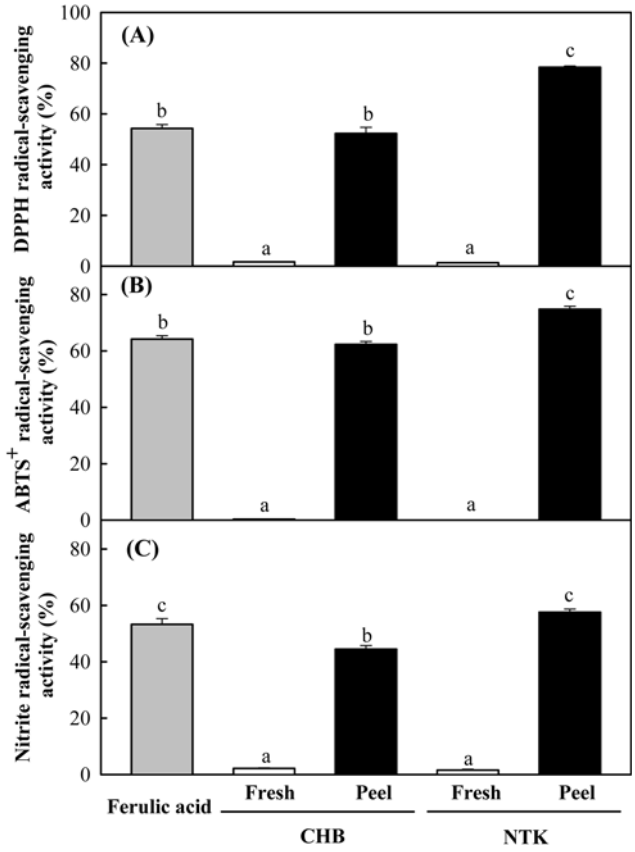

Fig. 3. DPPH (A), ABTS$^+$ (B), and nitrate (C) radical scavenging activities of two Asian cultivars fruit fleshes and peels. Values are mean±SD (n=3). DPPH radical solution (final concentration, 100 μM) was reacted with the 80% MeOH extract (final concentration, 500 μg/mL) samples and ferulic acid (final concentration, 10 μg/mL). ABTS$^+$ radical solution (0.5 mL, absorbance=0.7±0.15 at 735 nm) was reacted with 80% MeOH extract (100 μg/mL) sample and ferulic acid (1 μg/mL). Nitrate radical solution was reacted with 80% MeOH extract sample (1 mg/mL) and ferulic acid (10 μg/mL). Different letters indicate a significant difference among samples at p<0.05. CHB, Chuhwangbae; NTK, Niitaka

the NTK and CHB flesh extracts almost did not scavenge the ABTS$^+$ radical at a concentration of 100 μg/mL and their radical-scavenging activities was not significant (p<0.05) (Fig. 3B).

The pear peel extracts (NTK, 57.6±1.1%; CHB, 44.5±1.3%) at a concentration of 1.0 mg/mL also exhibited apparently higher nitrate radical-scavenging activities when compared to the flesh extracts (NTK, 1.6±0.3%; CHB, 2.2±0.2%) (Fig. 3C). The NTK peel exhibited higher nitrate radical-scavenging activity than that of the CHB peel, although the nitrate radical-scavenging activities of the flesh were not different (Fig. 3C).

Reducing power of CHB and NTK peel and flesh The reducing capabilities of the peel and flesh extracts from the two Asian pear cultivars are shown in Fig. 4. The reducing

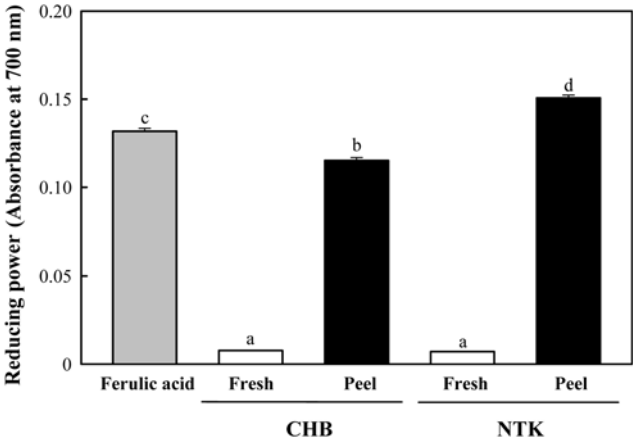

Fig. 4. Reducing power of two Asian cultivars fruit flesh and peels. Values are mean±SD (n=3). Different letters indicate a significant difference among samples at p<0.05. CHB, Chuhwangbae; NTK, Niitaka

power of the pear peel extracts was significantly higher than that of the pear flesh extracts. That is, at a concentration of 600 μg/mL, the NTK pear peel extracts (0.151±0.002, absorbance values at 700 nm) showed significantly (p<0.05) higher reducing capability than that of CHB (0.115±0.002) (Fig. 4). In addition, NTK (0.151±0.002, absorbance values at 700 nm) at a concentration of 600 μg/mL displayed significantly (p<0.05) higher reducing capability when compared to that of ferulic acid (0.132±0.002, absorbance values at 700 nm) (Fig. 4). The reducing capabilities (absorbance values at 700 nm) of the NTK and CHB pear flesh extracts were 0.007±0.0002 and 0.008±0.0001, respectively, but the values were not significantly different.

Protective effect of the pear peel and flesh extracts against cell death induced by H_2O_2 in 3T3-L1 cells When 3T3-L1 cells were treated with the pear flesh extracts at a concentration of 10 mg/mL, their viabilities were similar to the control group regardless of differences in the two pear cultivars (data not shown). However, pear peel extract treatment at higher concentrations than 5.0 mg/mL induced loss of cell viability in a dose dependent manner (data not shown). Therefore, the protective effects of pear peel and flesh extracts against H_2O_2-induced cell death in 3T3-L1 cells were examined at lower concentrations than 2.5 mg/mL (0, 0.5, 1.0, and 2.5 mg/mL). Cell viability decreased significantly to 55% after cells were exposed to 1.0 μM H_2O_2 for 2 h (Fig. 5). Pretreatment with the pear fruit peel and flesh extracts resulted in significantly (p<0.05 or p<0.01) higher protective effects against cell death induced by H_2O_2 than those in the control group exposed only to H_2O_2. However, the protective effects of both the pear peel and flesh did not increase proportionally depending on their concentrations (Fig. 5). Cell viability

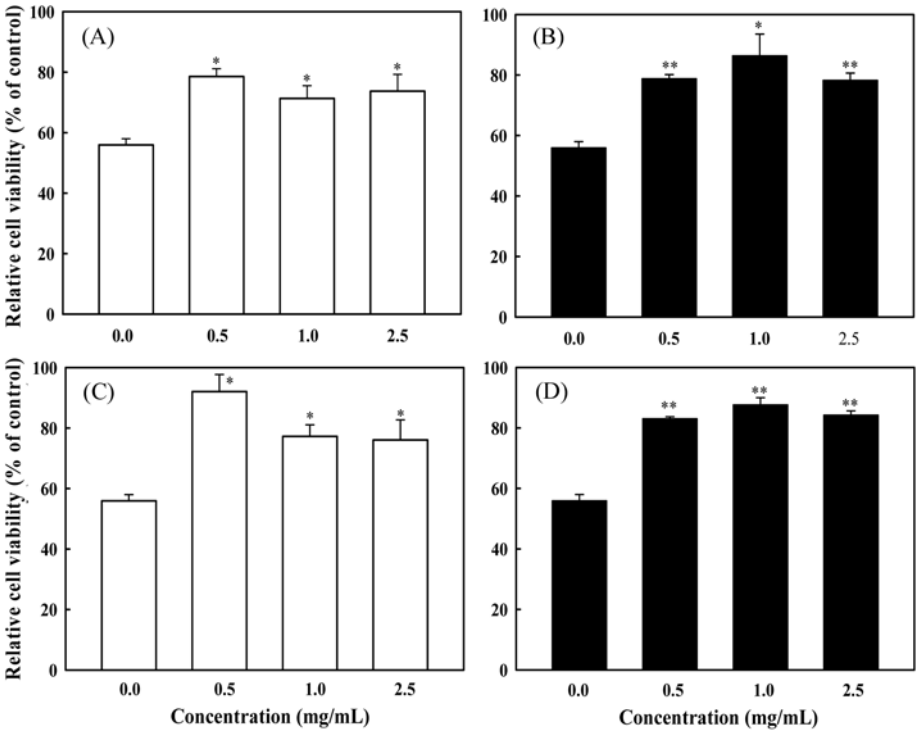

Fig. 5. Comparison of inhibitory effects against cell death induced by adding H_2O_2 (1 μM) after 3T3-L1 cells were treated with various concentration of CHB and NTK fruit flesh and peel extracts. (A), CHB flesh; (B), CHB peel; (C), NTK flesh; (D), NTK peel. Asterisk indicates a significant difference between control treated without sample extracts and groups treated with samples by Student's *t*-test (*p<0.05; **p<0.01; ***p<0.001).

after treatment with the pear peel (Fig. 5B, 5D) was relatively higher than that of the pear flesh treatment (Fig. 5A, 5C), although no significant difference between CHB and NTK was observed.

Inhibitory effect of the pear peel and flesh extracts against ROS production induced by H_2O_2 in 3T3-L1 cells ROS content produced after H_2O_2 treatment of 3T3-L1 cells for 2 h was determined using the DCF method (33). The results (Fig. 6) exhibited a similar pattern with those (Fig. 5) of the cell viability experiment. Pear peel (Fig. 6B, 6D) extract treatment resulted in a higher inhibitory effect against ROS production in H_2O_2-treated cell than those of pear flesh (Fig. 5C, 6A). Both CHB (Fig. 6B) and NTK (Fig. 6D) pear peel extracts showed similar inhibitory effects against ROS production in H_2O_2-treated cells. The CHB (Fig. 6A) pear flesh extract showed relatively higher inhibitory effect on ROS production in H_2O_2-treated cell than that of NTK (Fig. 6C) and its inhibitory effect increased proportionally in a dose-dependent manner. NTK also more effectively inhibited ROS production in H_2O_2-treated cells compared with that of the control group (Fig. 6), but no significant difference was between NTK and the control group.

Inhibitory effects of the pear peel and flesh extracts against copper ion-induced peroxidation of rat plasma After the pear peel and flesh extracts were administered to rats, blood plasma was collected from each group and pooled to the same volume. Oxidation of the 4-fold diluted plasma was initiated by adding copper ions (100 μM, final concentration), and the inhibitory effect was evaluated by measuring CE-OOH concentration (Fig. 7). The lag time for CE-OOH formation in rat plasma (control) treated without the pear extracts was 3 h, whereas the lag time in blood plasma of rats administrated the pear extracts was >4 h. In particular, the lag times (about 6 h) in plasma of the rat groups (▲ and ■) administered the pear peel extracts was significantly higher than those (about 4 h) of rat groups administered the pear flesh extracts (△ and □), although a slight difference was observed between the two cultivars. After the blood plasma of the rats administered the pear extracts was oxidized by copper ions for 10 h, the CE-OOH concentration decreased in the following order: control (6.37 mM)<CHB flesh (5.06 mM, □)<NTK flesh (4.28 mM, △)<NTK peel (3.62 mM, ▲)<CHB peel (3.03 mM, ■). That is, pear peel exhibited significantly higher inhibitory effect against CE-OOH formation than the pear fruit flesh. NTK pear flesh showed a higher inhibitory

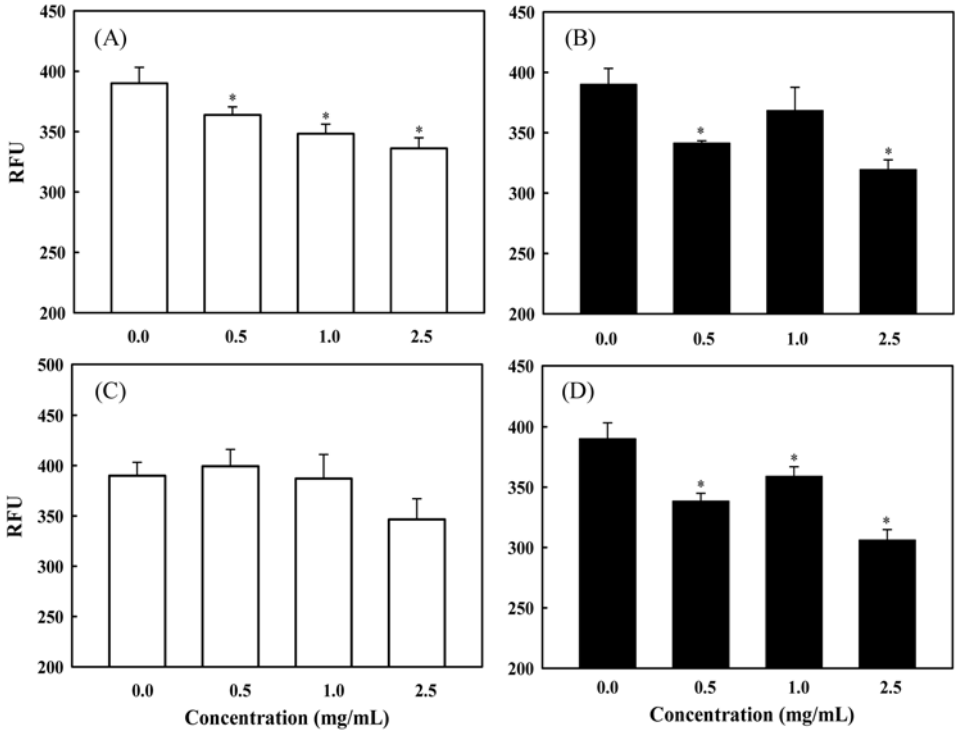

Fig. 6. Comparison of inhibitory effects against reactive oxygen species (ROS) production induced by adding H_2O_2 (1 μM) after 3T3-L1 cells were treated with various concentrations of pear fruit flesh and peel extracts. (A), CHB flesh; (B), CHB peel; (C), NTK flesh; (D), NTK peel. Asterisk indicates a significant difference between control treated without sample extracts and groups treated with samples by Student's *t*-test (*p<0.05; **p<0.01; ***p<0.001).

ability against CE-OOH formation than that of CHB. In contrast, the CHB pear peel displayed higher inhibitory ability against CE-OOH formation than that of NTK.

Total phenolic contents in the NTK and CHB peels were higher by about 24 and 16-folds, respectively, when compared to those of NTK and CHB flesh (Fig. 1A). In addition, total flavonoid contents in the NTK and CHB peels was about 105 and 36-fold than that in their flesh (Fig. 1B). Ascorbic acid content in the peels was slightly higher than that in the flesh. No significant difference was observed in total tocopherol contents between the peels and flesh. However, ascorbic acid and total tocopherol contents were found in relatively very small amounts in pear fruits when compared to that of phenolic compounds including flavonoids. In addition, the quantitative pattern of ascorbic acid and tocopherol in the flesh and peels differed from those of phenolics and flavonoids (Fig. 2). These results indicate that phenolics including flavonoids in pear are more plentiful in peel than in flesh. The antioxidative activities between the peels and flesh of two pear cultivars were measured using DPPH, $ABTS^+$, and nitrite radicals. The results of these three *in vitro* tests showed that the peels had predominantly higher antioxidative activity than that of the flesh regardless of cultivar (Fig. 3). In addition, the reducing power capacities of the pear peels and flesh were very similar to the results of three *in vitro* tests for antioxidative activities (Fig. 4). Interestingly, the free radical-scavenging activities and reducing capacities of the peels and flesh were proportionally correlated with their phenolic and flavonoid contents. These results suggest that the antioxidative activity of pear fruit peels may be mainly contributed by the phenolic compounds including flavonoids. The NTK peel was significantly superior to the CHB peel when we compared total phenolic and flavonoid contents, free radical scavenging activities, and reducing capacity between the two cultivars (p<0.05). However, it should be considered that these properties of the two pear cultivars might be influenced by factors such as cultivation method, climate, soil conditions, and farming systems. In contrast, no significant differences between NTK and CHB flesh were observed (Fig. 1, 2), except CHB flesh showing slightly higher total flavonoid and tocopherol contents than those in NTK (p<0.05). These results repeatedly suggest that the difference in antioxidative activity between the peels and flesh of pear fruit may be mainly related to phenolic compound content including flavonoids.

The pear extracts also significantly reduced the increase in intracellular ROS production and cell death when 3T3-L1 cells were exposed to H_2O_2 (Fig. 5, 6). The pear peel extracts (Fig. 6B, 6D) showed more effective antioxidative activities than those of the pear flesh extracts (Fig. 6A, 6C) regardless of cultivars. However, the antioxidative activities

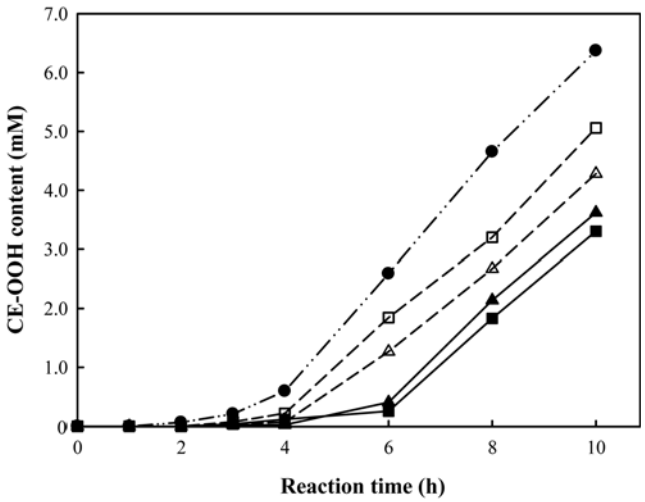

Fig. 7. Comparison of the inhibitory effects against CE-OOH formation in copper ion-induced oxidation of rat plasma 1 h after oral administration of the pear fruit flesh and peel extracts. ●, control; ■, CHB peel; □, CHB flesh; ▲, NTK peel; △, NTK flesh. The pear fruit extracts (~7 mg fresh wt., 14 g equivalent) were orally administered as a propylene glycol solution (1 mL). Plasma was pooled in the same volume from rats (n=5) of each group, diluted four times with PBS buffer (pH 7.4), and incubated with 100 μM (final concentration) $CuSO_4$ to induce CE-OOH formation. The reaction mixture was incubated at 37°C with continuous shaking. Data are representative of 2 experiments.

of the peel and flesh extracts in 3T3-L1 cells did not increase proportionally in a dose-response manner (Fig. 6). In addition, the antioxidative activities of the pear fruits on 3T3-L1 cells were not correlated with phenolic contents including the flavonoids. This result suggests that other biological active compounds besides phenolic compounds may contribute to antioxidative activity of pear fruit on ROS production in 3T3-L1 cells induced by H_2O_2.

The peel and flesh extracts of the two pear cultivars were orally administered to rats. The rats (200 g body weight) were orally administered each extract equivalent to 3.7 g of fresh flesh and 0.3 g of fresh peel. That is, when based on a 60 kg human, each rat was administered with 1,100 g flesh (extract, 145 g) and 90 g peel (extract, 13 g), which are equivalent to two whole pear fruits, respectively. One h after administration, plasma was pooled from rats (n=5) in each group, diluted four times with PBS buffer (pH 7.4), and incubated with 100 μM (final concentration) $CuSO_4$. CE-OOH produced from oxidation is present in healthy human plasma at approximately 3 nM and is very stable (36). Therefore, CE-OOH has been selected as an index of lipid peroxidation in blood plasma (37,38). The amount of CE-OOH in the reaction mixture was monitored in a continuous shaking incubator at 37°C. Administering the pear extracts more effectively inhibited CE-OOH formation in rat plasma regardless of cultivar compared to the control (Fig. 7). That is, the administration of pear extracts increased the lag time for CE-OOH formation in copper ion-induced rat plasma peroxidation by 1-3 h in comparison to control. After 10 h of copper ion-induced rat plasma peroxidation, the plasma samples of the pear peel and flesh administered groups had lower CE-OOH levels than that of the control group. These results suggest that consuming pears may increase antioxidative activity in blood plasma, and pear peels may be more effective this than pear flesh.

The pear fruit peels showed predominantly higher radical scavenging activities, reducing power capacity, and reduction effect of H_2O_2-induced oxidative stress in 3T3-L1 cells, when compared to those of the flesh. The high antioxidative activities of the pear fruit peels may be attributed to phenolic compounds including flavonoids. In addition, the antioxidant activity based on the inhibition of copper ion-induced lipid peroxidation in the blood plasma of rats administered the peel extracts was significantly higher than those administered the flesh extracts. Therefore, health beneficial compounds contained in pear fruit peel may more contribute to the blood antioxidant defense than those contained in pear flesh.

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