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## Antioxidant and whitening activities of five unripe pear cultivars

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### Summary

The present study was the first evaluation of the arbutin content, antioxidant activity, and whitening function of the unripe pears of five major Korean pear cultivars. Unripe pears were investigated 30 days after florescence for possible utilization as a whitening ingredient, instead of being thrown away for thinning out. Among the five cultivars tested, Gamcheonbae and Manpungbae had significantly higher total phenolics and arbutin contents, while Niitaka had lower values of both total phenolics and arbutin. For whitening activity related to tyrosinase and cellular melanin formation, Manpungbae also showed the strongest tyrosinase inhibition (4.9%), and achieved 74% reduction of the cellular melanin compared to non-treated cells. These results indicate that unripe pears, especially the Manpungbae cultivar, could be useful for application as a possible natural whitening additive with high arbutin content and excellent whitening activity.

### Introduction

Pear (*Pyrus* spp.) is one of the most widely consumed fruits in the world. It is typically eaten fresh, and is often found in processed foods such as juice, puree, jellies, and jams. For many years, pears have been used not only as one of the most common edible fruits, but also as an herbal medicine for relieving cough, embellishing lung, eliminating constipation, and relieving alcoholism (CUI et al., 2005). Various phenolic compounds have been found in pear fruits, including arbutin, chlorogenic acid, rutin, and procyanidins (FERREIRA et al., 2002; CHEN et al., 2007; LEE et al., 2011).

In particular, arbutin (4-hydroxyphenyl  $\beta$ -D glucopyranoside) is widely used as a whitening agent in cosmetic products (SUGIMOTO et al., 2004). Arbutin was also reported to inhibit the activity of mushroom tyrosinase, the biosynthesis of B16 mouse melanoma, and HMV-II human melanoma cells (CHAKRABORTY et al., 1998; NIHEI and KUBO, 2003; SUGIMOTO et al., 2004). Arbutin was found to be the most abundant phenolic compound in pear fruit (ZHANG et al., 2006), and has also been used as a specific marker to evaluate the authenticity of pear products (BRANCA et al., 2000).

Pear was reported to contain approximately 0.1~1% arbutin and chlorogenic acid, although the values vary depending on the cultivars and the ripening of the fruit (CUI et al., 2005; LEE et al., 2011). Several studies showed similar results in that the phenolic compounds and antioxidant activities of pears differed between cultivars and reduced with maturity (CUI et al., 2005; ZHANG et al., 2006). Oriental pear cultivars (Chinese or Asian) have a tendency to contain higher amounts of phenolic compounds including arbutin and chlorogenic acid than Occidental pears cultivars, although Oriental pear cultivars were also found to have variation in the arbutin level depending on the cultivar (GALVIS SANCHEZ et al., 2003; CHO et al., 2011a; LI et al., 2012; 2014). Among maturing stages, arbutin content was the highest in unripe pear before and after florescence, and rapidly decreased by 70% at 40 days after florescence (CUI et al., 2005; ZHANG et al., 2006).

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Large amounts of unripe pears are usually discarded due to natural disasters, poor cultivation techniques, or the thinning out of superfluous fruits. Thinning out is an especially high contributor, with 90% of unripe pears thrown away to obtain high quality pears. Therefore, it is necessary to find out how these unripe fruits could be utilized to produce new food ingredients, such as functional food ingredients, instead of wasting them.

So far, the previous studies have mostly focused on phenolic functional compounds and the physiological activities of pears between different cultivars or different parts of the pear during fruit development (GALVIS SANCHEZ et al., 2003; LI et al., 2012; 2014). In the present study, we investigated the possible utilization of five unripe pear cultivars at a certain developmental stage (30 days after florescence) as a whitening ingredient. This stage was chosen for investigation since most unripe pears are thrown away 30 days after florescence due to thinning out, even though they have peak arbutin level or antioxidant activity (CUI et al., 2005; ZHANG et al., 2006). The total phenolics and arbutin contents of the five unripe pear cultivars were measured, and further functional characterization was carried out with respect to the antioxidant activities, and inhibitory effects on tyrosinase as well as cellular melanin formation.

### Materials and methods

#### Reagents

Folin-Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS),  $\alpha$ -tocopherol, Trolox, sodium chloride, tannic acid, quercetin, and ascorbic acid were purchased from Sigma Aldrich-Fluka (Germany). Acetonitrile and formic acid (98% purity) were of HPLC grade, and were also purchased from Sigma Aldrich-Fluka (Germany). All other solvents and chemicals were of analytical grade.

#### Sample preparation

Five cultivars of Asian pear (*Pyrus pyrifolia*) were chosen for this study based on the highest produced in South Korea. These included Gamcheonbae, Manpungbae, Chuwhangbae, Hanareum, and Niitaka varieties, obtained from an orchard at the Naju National Pear Experimental Station, in Chonnam, Korea. Pear fruits of the five cultivars were collected 30 days after full bloom. Seven to ten fruits from each selected standard pear trees were used for the experiments, with uniformity in size and no defects. The pears were washed, rapidly cut into thin slices, and lyophilized by freeze-dryer (FD8512, Ilshin, Korea). The samples were then further grinded by pulverizer (FM-681C, Hanil Electric., Korea) and finally stored at -20 °C in polyethylene bags until further analysis. The frozen material of each pear cultivar (10 g) was previously homogenized in a Moulinex stirrer and then extracted with 80% ethanol by the soxhlet extraction method at 60 °C for 6h. The extracts were then filtered through Whatman No 1 filter paper. The residues were extracted again with 80% ethanol using the same method as mentioned above. The extracts were then combined and evaporated to dryness under vacuum. The

dried extract was prepared at the concentration of 0.1 g/mL of the freeze-dried pear in methanol. The methanol solution was used for analysis of the total phenolics, flavonoids, and antioxidant capacity.

#### Total phenolics and total flavonoids contents

The total phenolic content in the extracts of the five pear cultivars was determined using Folin-Ciocalteu's, reagent as described in CUI et al. (2005). The absorbance of each sample was measured at 765 nm with a UV/Visible Spectrophotometer (JP/U-3900, Hitachi, Japan.) after incubating at 25 °C for 2 h. Total phenolic content was calculated from a calibration curve, using tannic acid as the standard (160-960 µg per 100 g of dry weight). The flavonoid content was measured by the aluminum chloride colorimetric method, which was described by BOO et al. (2009). Quercetin was used as the standard. The flavonoid content was determined at 506 nm with a spectrophotometer. The data were expressed as milligram quercetin equivalents /100 g dry weight, and the calibration curve ranged from 40 to 840 µg.

#### Quantification of arbutin by HPLC analysis

The pear samples were filtered through 0.22-µm membrane filters and analyzed by an HPLC system. The separation of arbutin was performed on ACE C18 columns (4.6x250 mm, Advanced Chromatography Technologies, Aberdeen, UK). The solvent system used was a gradient of water and formic acid (19:1, v/v) (A) and methanol (B), starting with 5% methanol. A gradient was installed 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 Photodiode Array (PDA) detector (SPD-M20A, Shimadzu, Japan) at a solvent flow rate of 1 mL/min. Finally, arbutin was eluted at 25 min. Arbutin standard (mg/mL) was dissolved in 80% ethanol. This solution was diluted to the appropriate concentrations for construction of a calibration curve. The calibration curve was constructed by plotting the peak areas vs. the concentration of the standard.

#### DPPH and ABTS radical scavenging activity

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging activities of the pear extracts were determined by the method of BLOIS (1958). To 0.25 ml of sample (1 mg/mL), 0.8 ml of 0.15 mM DPPH (methanol) was added, and shaken vigorously, followed by incubation at room temperature for 30 min in the dark. The control sample was prepared with 80% ethanol instead of pear extract. The decrease in absorbance in presence of DPPH<sup>•</sup> was measured at 517 nm using a Spectrophotometer (JP/U-3900, Hitachi, Tokyo, Japan). Ascorbic acid was used as a reference compound. Radical scavenging activity was expressed as % inhibition of DPPH<sup>•</sup> radicals using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = 1 - (\text{sample absorbance} / \text{control absorbance}) \times 100$$

ABTS radical scavenging activity of the pear extracts was measured by the ABTS cation decolorization assay, as described by RE et al. (1999) with some modifications. The ABTS radical cation (ABTS<sup>•+</sup>) was produced by reaction of 7 mM stock solution of ABTS with 2.45 mM potassium persulfate, which was allowed to stand in the dark at room temperature for 12 h-16 h before use. The ABTS<sup>•+</sup> solution was diluted with methanol to give an absorbance of  $0.7 \pm 0.01$  at 734 nm. Pear extracts (1 mg/mL) were allowed to react with 2 ml of the ABTS<sup>•+</sup> solution for 1 min, after which the absorbance were measured at 734 nm. Trolox was used as the reference compound. Radical scavenging activity was expressed as % inhibition of ABTS radicals, using the following equation:

$$\text{ABTS radical scavenging activity (\%)} = (1 - \text{sample absorbance} / \text{control absorbance}) \times 100$$

#### In vitro Tyrosinase inhibition assay

Tyrosinase inhibitory activity was determined by spectrophotometry as described by MASAMOTO et al. (1980), with minor modifications. In brief, the incubation mixture (total volume = 190 µl) consisted of 50 U/ml mushroom tyrosinase in 140 µl phosphate buffered saline (PBS) (pH 6.8), and 50 µl of 15 mM L-DOPA (L-β-3, 4-dihydroxyphenyl alanine) solution, in PBS, with or without pear extracts. The mixture was incubated at 37 °C for 10 min, after which the absorbance was measured at 515 nm using a Benchmark microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). Tyrosinase inhibition rate was expressed as % inhibition of Tyrosinase using the following equation:

$$\text{Tyrosinase inhibition rate (\%)} = (1 - \text{sample absorbance} / \text{control absorbance}) \times 100$$

#### Cell viability and inhibition of melanin synthesis

Cell viability was assessed by the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, with a slight modification (SHIBATA et al., 2006). B16F10 mouse melanoma cells ( $1 \times 10^5$  cells/well) were seeded in a 96-well microtiter plate and allowed to adhere completely to the plate overnight. The next day, new media containing pear extracts at dosages of 10 to 500 µg/mL dosages was added to the plate, which was then incubated at 37 °C in a CO<sub>2</sub> incubator. After a total of 72 h of incubation, the media was removed and 50 µL of MTT solution (1.0 mg/mL) was added to each well. After further incubation for 4 h, the formazan was solubilized in dimethyl sulfoxide (DMSO) and the absorbance was measured at 450 nm (reference at 630 nm) using an MR-96A microplate reader. DMSO at a toxic concentration of 5% v/v was used as a negative control (CAO et al., 2007). Each treatment was performed in triplicate, and each experiment was repeated three times.

The release of extra-cellular melanin was measured according to the method of MAKPOL et al. (2009). In brief, melanoma cells were seeded in 24-well tissue culture plates at  $1 \times 10^5$  cells/mL and incubated for 24 h. Next, α-MSH (Melanocyte Stimulating Hormone, 0.1 µM) was added, and the cells were treated with 100 µg/mL doses of the pear extracts for a total of 72 h incubation. After washing twice with phosphate buffered saline, cells were dissolved in 1 mL of 1 N NaOH. For measurement of the melanin content, 100 µL aliquots of solution were then placed in 96-well plates, and the absorbance was measured at 450nm using a microplate reader. Inhibition of melanin synthesis was expressed as the percentage of melanin content in the cells treated with pear extract to that of untreated melanoma cells.

#### Statistical Analysis

All experiments were carried out with three replicates and results were expressed as mean ± standard deviation. The data were analyzed by one-way ANOVA, and the means of different groups were compared with the Duncan's multiple range test (DMRT) using the SPSS version 17.0 statistical software package. Values of P < 0.05 were considered as significant in all cases.

## Results and discussion

#### Total phenolics and total flavonoids contents

The total phenolics and flavonoids contents of the five cultivars of Asian pear (*Pyrus pyrifolia*) examined herein are presented in Tab. 1. The pears examined included five major Korean major cul-

tivars: Gamcheonbae, Manpungbae, Chuwhangbae, Hanareum, and Niitaka. The samples were collected in the early growth stage, 30 days after florescence, for analysis of the functional compounds since the phenolic contents in pear fruit were reported to be the highest before and after florescence, with rapid decrease from 40-70 days after florescence (CUI et al., 2005; ZHANG et al., 2006). The five pear cultivars tested were found to have total phenolic contents ranging from  $255 \pm 2.64$  to  $367 \pm 9.78$  mg/100g fresh weight (FW), while the flavonoid contents were between  $26.5 \pm 0.82$  and  $36.6 \pm 1.45$  mg/100g FW. Among the species tested, Gamcheonbae and Manpungbae had significantly higher total phenolics. Niitaka, the representative cultivar with above 70% domestic production, had a lower value for total phenolics. The flavonoid content followed a similar trend to that of total phenolics, with higher values of  $36.6 \pm 1.45$  mg/100 g FW in Gamcheonbae and Manpungbae, but lower values of  $26.5 \pm 0.82$  mg/100 g FW in Niitaka. Interestingly, total phenolics and flavonoids of Manpungbae showed higher content in the unripe fruits, but lower content in the ripe fruits compared to the other cultivars (data not shown). This is because Manpungbae has a relatively heavy weight than other cultivars, at more than 800 g, resulting in lower levels of polyphenols due to the larger fruit volume. With respect to total phenolic content, the values obtained (Tab. 1) were comparable with the contents in other kinds of fruit, such as guava (179 mg/100 g FW), banana (51 mg/100 g FW), and peach (112-126 mg/100 g FW) (VEBERIC and COLARIC, 2008; ARRANZ et al., 2009). The amounts in the five pears (255-367 mg/100 g FW) were similar to those of other fruits reported in many works (DU et al., 2009; SALTA et al., 2010).

This pattern agreed with a previous report concerning phenolic content among similar pear cultivars, but 1.7-3.2 times higher content was found in the ripe fruit, in that study compared to the unripe pears in the present study (ZHANG et al., 2006). Besides pears, unripe peach (HONG et al., 2006) and mandarin orange fruit (KANG et al., 2005) also showed higher levels of polyphenols than the ripe fruits, with variation of polyphenol contents among cultivars. This could be due to several factors. Since polyphenols are important protective agents in plants, high levels of polyphenols in unripe fruits are required to prevent chemical damage and invasion by damaging organisms (ZHANG et al., 2006). The polyphenols in fruit react with other substances and accumulate in different forms as the fruit matures (CHOI and LEE, 2013). This may be the reason why rapid maturity of the fruit results in decreased polyphenol content (CHOI and LEE, 2013).

#### Quantification of arbutin by HPLC analysis

Arbutin (4-hydroxyphenyl  $\beta$ -D glucopyranoside) is the main phenolic constituent in pear fruit. Arbutin has gained wide use as a whitening agent to prevent unnecessary spots and freckles through inhibitions of melanin synthesis and tyrosinase activity (MAEDA

and FUKUDA, 1996). The arbutin contents in ethanol extracts of the five pears were determined by HPLC/DAD and shown in Tab. 1. Manpungbae, Hanareum, and Nikita showed the higher levels of arbutin at 40-50 mg/100 g FW, but Gamcheonbae displayed lower levels at 26-27 mg/100 g FW. At the early growth stage, arbutin contents among cultivars seemed to be positively correlated with the trend of phenolic contents, except for Gamcheonbae, which contained relatively lower amounts of arbutin. This agrees with the report that Nikita contained greater arbutin concentration than other cultivars, including Chuwhangbae and Hosui (ZHANG et al., 2006). A number of studies have reported that arbutin content varies greatly among pear cultivars, ranging from 5.0-40 mg/100 g FW (LI et al., 2012), and also among pear parts, at 11-530 mg/100 g FW (CUI et al., 2005; ZHANG et al., 2006). In addition, comparison of the arbutin levels of Oriental pears and Occidental pears, during different growth stages, or in different parts of fruit was also carried out (CUI et al., 2005). The mean concentration of arbutin in the Oriental pear cultivars was twice as high as the Occidental pear cultivars, at 16.4 mg/100 g FW compared to 8.3 mg/100 g FW. During the growth stages of Yali pear, the arbutin levels were higher in the unripe fruit at 992 mg/100 g FW right after florescence, but rapidly decreased to approximately 200 mg/100 g FW 40 days after florescence before further dropping to 40 mg/100 g FW at maturity (CUI et al., 2005). Among the parts in the pear fruit, the concentration of arbutin was reported to be the highest in the peel (120 mg/100 g FW), at 3-5 times greater levels than found in the core and 10-45 times greater than in the flesh (CUI et al., 2005; ZHANG et al., 2006; LI et al., 2014).

#### DPPH and ABTS radical scavenging activity

Antioxidant activities of the five pear extracts were evaluated by DPPH $\cdot$  or ABTS $^{+}$  radical scavenging activity (Tab. 2). The antioxidant activity of pear ethanol extract was determined for 100 mg/mL concentrations. For each methods, DPPH $\cdot$  can only be dissolved in organic media (especially in alcohols), not in aqueous media, which causes limitation in interpreting the role of hydrophilic antioxidants. In contrast, ABTS $^{+}$  can be solubilized either in aqueous or organic media, allowing interpretation of the role of the hydrophilic and lipophilic antioxidants.

In terms of DPPH $\cdot$  scavenging activity, the five pear cultivars did not show any significant differences ( $P > 0.05$ ), with DPPH bleaching abilities of  $77.4 \pm 2.134\%$  to  $82.5 \pm 3.97\%$ . It was reported that the antioxidant capacities of pear extracts were similar among cultivars, ranging from 79.3% to 92.0% (ZHANG et al., 2006). Among the cultivars examined herein, Nikita had lower antioxidant activity, while Manpungbae and Chuwhangbae displayed higher activity. This result was similar with the study of ZHANG et al. (2006), in that the antioxidant activity of Nikita was lower than that of Chuwhangbae in the unripe fruits. Pear cultivars showed similar values

**Tab. 1:** Total phenolics, total flavonoids, and arbutin contents of five unripe pear cultivars.

| Cultivars   | Total phenolics<br>mg/100 g FW | Total flavonoids<br>mg/100 g FW | Arbutin<br>mg/100 g FW         |
|-------------|--------------------------------|---------------------------------|--------------------------------|
| Gamcheonbae | $354 \pm 6.17$ b <sup>z</sup>  | $35.2 \pm 0.97$ a               | $26.8 \pm 0.64$ c <sup>z</sup> |
| Manpungbae  | $367 \pm 9.78$ a               | $36.6 \pm 1.45$ a               | $53.9 \pm 0.72$ a              |
| Chuwhangbae | $258 \pm 2.64$ d               | $32.5 \pm 0.87$ b               | $27.1 \pm 1.06$ c              |
| Hanareum    | $304 \pm 5.92$ c               | $29.7 \pm 0.54$ c               | $50.1 \pm 2.84$ a              |
| Niitaka     | $255 \pm 3.75$ d               | $26.5 \pm 0.82$ c               | $40.6 \pm 0.97$ b              |

<sup>z</sup>Different letters within columns indicate significant differences of the means, as determined by Duncan's multiple range test at  $P < 0.05$ .

**Tab. 2:** DPPH and ABTS radical scavenging of five young pear cultivars.

| Cultivars   | DPPH radical scavenging<br>%   | ABTS radical scavenging<br>% |
|-------------|--------------------------------|------------------------------|
| Gamcheonbae | $80.9 \pm 3.38$ a <sup>z</sup> | $67.7 \pm 6.23$ bc           |
| Manpungbae  | $82.5 \pm 3.97$ a              | $74.2 \pm 2.99$ a            |
| Chuwhangbae | $81.8 \pm 2.46$ a              | $70.4 \pm 4.77$ ab           |
| Hanareum    | $77.4 \pm 2.13$ a              | $66.3 \pm 5.19$ bc           |
| Niitaka     | $77.4 \pm 1.09$ a              | $65.5 \pm 3.42$ c            |

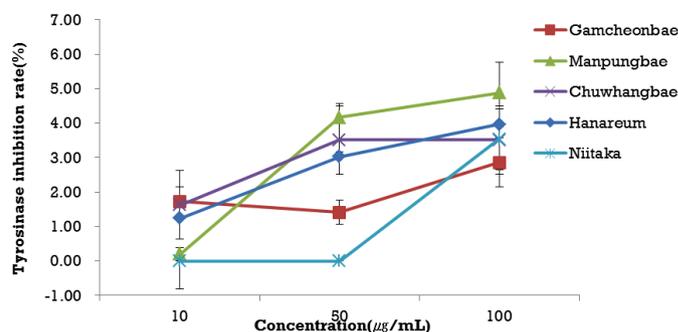
<sup>z</sup>Different letters within columns indicate significant differences of the means, as determined by Duncan's multiple range test at  $P < 0.05$ .

when the antioxidant capacities per serving (100 g) were compared (GALVIS SANCHEZ et al., 2003). The free radical scavenging activity of the pear cultivars increased linearly with increase in the sample concentration (data not shown). In comparison to other fruits, the pear fruits showed relatively higher antioxidant activity. Chinese plum (LEE et al., 2008) and peach (KIM et al., 2009) were reported to have antioxidant activities of 80.9% and 76.8%, respectively, at the same concentration of 100 mg/mL sample. The antioxidant activity of loquat greatly differed between parts, with 91.8% in the leaf, 5.0% in the seeds, and 14.2% in the flesh at the concentration of 500 mg/mL sample (PARK et al., 2008).

With regard to the ABTS<sup>+</sup> radical scavenging activity, the five pear cultivars examined showed similar values, ranging from 65.5 ± 3.41% to 74.2 ± 2.99% antioxidant activity, though some differences were observed among cultivars ( $P < 0.05$ ). For the five pear cultivars examined herein, the antioxidant capacities determined by the two methods showed good agreement. Chuwhangbae and Manpungbae presented the strongest ABTS<sup>+</sup> bleaching activities (70.4 ± 4.77% or 74.2 ± 2.99%), respectively, followed by Gamcheonbae, Hanareum, and Nikita at 67.7 ± 6.23%, 66.3 ± 5.19% and 65.5 ± 3.42%, respectively (Tab. 2). Manpungbae, with its high total phenolics, flavonoids, and arbutin contents, exhibited significantly higher antioxidant abilities than Nikita when assayed by the DPPH and ABTS<sup>+</sup> methods. Therefore, it can be deduced that total phenolics and arbutin contents make a major contribution to the antioxidant capacity of the pears. A number of studies have reported that phenolic compounds, including arbutin and chlorogenic acid, are the main phytochemicals responsible for the antioxidant capacity of vegetables and fruits (DU et al., 2009; SALTA et al., 2010). Phenolic compounds in pears made much greater contribution to the antioxidant capacity than vitamin C (GALVIS SANCHEZ et al., 2003).

### *In vitro* Tyrosinase inhibition assay

Tyrosinase is a key enzyme that catalyzes the initial steps in the formation of the pigment melanin. It catalyzes two major reactions, including the hydroxylation of tyrosine, and oxidation of L-DOPA (SANCHEZ-FERRER et al., 1995). Tyrosinase is responsible for the coloring of the skin, hair and eyes in animals, as well as for the molting process in insects, fruits and vegetables (WANG et al., 2006). Application of tyrosinase-inhibiting agents may be the least invasive procedure for maintaining skin whiteness. Recently, natural substances such as fruits and vegetables have been in increased demand as new agents for depigmenting, cosmeceutical, and skin lightening purposes (ABURJAI and NATSHEH, 2003). Accordingly, the inhibitory effects of the ethanol extracts of pear were evaluated on mushroom tyrosinase activity herein (Fig. 1). The five pear cultivars exhibited dose-dependent inhibition of tyrosinase based on the examination of



**Fig. 1:** The inhibition of mushroom tyrosinase *in vitro* among different pear cultivar extracts. Results are means ± SD (n=3). The inhibitory effects of five pears cultivars on tyrosinase were measured at three concentrations: 10, 50, and 100 µg/mL.

three different concentrations from 10-100 µg/mL. For the 100 µg/mL pear ethanol extracts, Manpungbae displayed the strongest inhibitory activity at 4.9 ± 0.7%, followed by Hanareum, Chuwhangbae, and Niitaka, at 3.5 ± 0.4%, 2.9 ± 1.0%, and 2.8 ± 0.7%, respectively. Gamcheonbae showed significantly lower inhibition than the other four cultivars, at 2.0 ± 0.7%. Phenolic compounds are good inhibitors of tyrosinase activity in melanoma cells. There are also reports that phenolic compounds may be used as depigmenting agents, since they have a similar chemical structure to tyrosine, the substrate of tyrosinase (BOISSY and MANGA, 2004). Manpungbae showed high tyrosinase inhibition due to its high phenolic content, while Niitaka displayed lower inhibition with low phenolic contents among the five cultivars. In terms of the mushroom tyrosinase inhibition of fruits, unripe pear extracts had relatively inhibition (up to 4.9%) compared to those of other fruit extracts. Although unripe peach and cucumber extract showed slightly higher inhibition at 4.6-8.5% with a dose of 100 µg/mL (KIM et al., 2012; YANG and BOO, 2013), other fruits required much higher doses to obtain good activities. For example, trifoliate orange and kiwi fruits showed 1.9-11.5% and 6.8-14.4% inhibition with doses of 2 mg/mL (LEE et al., 2010; PARK et al., 2008), while loquat fruits showed 16% inhibition at a dose of 4 mg/mL (PARK et al., 2008).

### Cell viability and inhibition of melanin synthesis

We measured the cytotoxicity of the pear extracts for 10-500 µg/mL dosages. As shown in Tab. 3, the five pear extracts showed relatively low cytotoxicity, with cell viabilities of 78.6 ± 6.74 - 98.8 ± 10.36% for the 500 µg/mL sample. This low cytotoxicity shows promise for possible clinical usefulness.

**Tab. 3:** Cell viability and melanin synthesis inhibition by pear ethanol extracts.

| Cultivars   | Dose µg/mL                 |                |                |                | Melanin content*<br>% |
|-------------|----------------------------|----------------|----------------|----------------|-----------------------|
|             | 10                         | 50             | 100            | 500            |                       |
| Gamcheonbae | 96.9 ± 1.61 a <sup>z</sup> | 92.9 ± 6.10 a  | 90.4 ± 3.37 ab | 88.8 ± 9.12 ab | 62.9 ± 7.51 a         |
| Manpungbae  | 90.9 ± 3.60 a              | 93.4 ± 13.49 a | 93.5 ± 7.17 a  | 98.8 ± 10.36 a | 16.9 ± 6.42 b         |
| Chuwhangbae | 90.3 ± 3.82 a              | 81.5 ± 5.59 a  | 77.6 ± 12.27 b | 78.6 ± 6.74 b  | 25.8 ± 8.96 b         |
| Hanareum    | 81.6 ± 6.03 b              | 85.8 ± 7.26 a  | 85.9 ± 4.99 ab | 92.7 ± 7.45 ab | 63.4 ± 11.23 a        |
| Niitaka     | 95.2 ± 6.70 a              | 91.6 ± 9.14 a  | 86.1 ± 7.66 ab | 81.8 ± 8.80 b  | 58.9 ± 9.00 a         |

<sup>z</sup> Different letters within columns indicate significant differences of the means, as determined by Duncan's multiple range test at  $P < 0.05$ .

\* Inhibition of melanin synthesis was expressed as the percentage of melanin content in the cells treated with pear extract (100 µg/mL) to that of untreated melanoma cells (64.8 ± 9.03% melanin content)

To investigate the effects of the five pear extracts on melanogenesis, B16F10 mouse melanoma cells were treated with pear extracts (100 µg/mL) and compared with untreated controls. The acid insoluble fraction was prepared from the cells, and the amount of melanin was quantitatively measured by the method of MAKPOL et al. (2009). The cellular melanin content was reduced by the addition of pear extract to the medium, as shown in Tab. 3. **Melanin synthesis** was significantly inhibited by Manpungbae (16.9 ± 6.42%), and Chuwhangbae (25.8 ± 8.96%), displaying 74% and 60% reduction, respectively, compared to the levels in the non-treated cells (64.8 ± 9.03%). The Gamcheonbae, Hanareum, and Niitaka pear extracts showed only slightly lower content of cellular melanin compared to the control, ranging from 58.9 ± 9.00% to 63.4 ± 11.23%, failing to meet significance at P < 0.05. Thus, these results indicate that the pear extracts had an inhibitory effect on melanogenesis at non-cytotoxic concentrations (100 µg/mL). Among the five cultivars (Tab. 1-3), **arbutin content and whitening effects were the highest** in Manpungbae, but the lowest in Gamcheonbae. Here, it can be suggested that Gamcheonbae may possess different functional compounds rather than those involved in whitening activity, since it showed relatively high total phenolics and flavonoid contents (Tab. 1-3).

Although many papers have studied the inhibitory effects of various fruit or plant extracts on melanin synthesis, the extracts from unripe pear in this study showed a more prominent reduction of the melanin content, with 74% inhibition, compared to the untreated control at a concentration of 100 µg/mL. Kojic acid, as a strong whitening agent, showed only 44.7% reduction of the melanin formation at 100 µg/ml (KIM et al., 1998). *Hippophae rhamnoides* L. fruit and ginseng extract showed 46% and 27% decreased melanin synthesis, respectively, compared to controls at the same concentration (KO et al., 2012; HWANG and CHOI, 2006). Extracts of persimmon leaves and gardenia fruit had similar effects, with 46.7% and 55.9% reduced melanin formation, respectively, by high doses of 10 mg/ml (CHO et al., 2011b; KWAK et al., 2004). Taken together, the test findings indicate that pear extract efficiently inhibited tyrosinase and melanin biosynthesis in mouse melanoma cells, suggesting use as a possible natural whitening additive.

## Conclusions

The findings of this study could be summarized as follows:

- Five unripe pear cultivars were measured for total phenolics and arbutin content, and further characterized with respect to antioxidant activities, and inhibitory effects on tyrosinase activity and cellular melanin formation.
- Cultivars included Manpungbae, Gamcheonbae, Chuwhangbae, Hanareum and Niitaka, which were harvested 30 days after florescence.
- Total phenolic and flavonoid contents of the five pear cultivars ranged from 255 + 2.64 to 367 + 9.78 mg, and from 26.5 + 0.82 to 36.6 + 1.45 mg per 100 g fresh weight, respectively. Among the five cultivars tested, Gamcheonbae and Manpungbae had significantly higher total phenolics and flavonoid contents, while Niitaka had lower total phenolics and flavonoids.
- Quantification of the arbutin content by HPLC/DAD analysis revealed Manpungbae to have the highest levels (53.9 + 0.72 mg/100 g FW), while Chuwhangbae and Gamcheonbae had only half as much.
- With regard to the antioxidant capability by ABTS or DPPH radical scavenging, Nikita showed lower activity (65% or 77%), while Manpungbae was the highest (74% or 83%).
- To examine whitening activity, pear extracts of five cultivars were tested for inhibitory effects on tyrosinase in vitro, and on cellular melanin formation. Manpungbae showed the strongest tyrosinase inhibition, at 4.9±0.7%. The pear extracts showed relatively low cytotoxicity, with cell viability ranging from 79~96% at concentrations of 500 µg/mL. Melanin formation (pears extract of 100 µg/mL) was significantly inhibited by Manpungbae (16.9%), and Chuwhangbae (25.8%), resulting in 74% and 60% reduction compared to the level in non-treated cells (64.8%).

7. These accessions indicate that unripe pears, especially the Manpungbae cultivar, could be valuable for application as a natural whitening ingredient, instead of being thrown away for thinning out.

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## References

- ABURJAI, T., NATSHEH, F.M., 2003: Plants used in cosmetics. *Phyto Therapy Res.* 17, 987-1000.
- ARRANZ, S., SAURA-CALIXTO, F., SHAHA, S., KROON, P.A., 2009: High contents of nonextractable polyphenols in fruits suggest that polyphenol contents of plant foods have been underestimated. *J. Agri. Food Chem.* 26(57), 7298-7303.
- BLIOS, M.S., 1958: Antioxidant determination by the use of a stable free radical. *Nature* 26, 1198-1204.
- BOISSY, R.E., MANGA, P., 2004: On the etiology of contact/occupational vitiligo. *Pigment Cell Res.* 17, 208-214.
- BOO, H.O., LEE, H.H., LEE, J.W., HWANG, S.J., PARK, S.U., 2009: Different of total phenolics and flavonoids, radical scavenging activities and nitrite scavenging effects of *Momordica charantia* L. according to cultivars. *Kor. J. Med. Crop. Sci.* 17, 15-20.
- BRANCA, M.S., PAULAR, B.A., 2000: Phenolic profile in the evaluation of commercial quince jellies authenticity. *Food Chem.* 71, 281-285.
- CAO, X.G., LI, X.X., BAO, Y.Z., XING, N.Z., CHEN, Y., 2007: Responses of human lens epithelial cells to quercetin and DMSO. *Invest. Ophthalmol. Visual Sci.* 48(8), 3714-3718.
- CHAKRABORTY, A.K., MARI KOMOTO, Y.F., ICHIHASHI, M., 1998: Effect of arbutin on melanogenic proteins in human melanocytes. *Pigment Cell Res.* 11, 206-212.
- CHEN, J.L., WANG, Z.F., WU, J.H., WANG, W., HU, X.S., 2007: Chemical compositional characterization of eight pear cultivars grown in China. *Food Chem.* 104, 268-275.
- CHOI, J.H., LEE, S.H., 2013: Distribution of stone cell in Asian, Chinese, and European pear fruit and its morphological changes. *J. Appl. Bot. Food Qual.* 86, 185-189.
- CHO, J., PARK, K., LEE, K., LEE, H., LEE, S., CHO, J., KIM, W., SHIN, S., PARK, K., MOON, J., 2011a: Recovery of arbutin in high purity from fruit peels of pear (*Pyrus pyrifolia Nakai*). *Food Sci. Biotech.* 20(3), 801-807.
- CHO, Y.J., AN, B.J., KIM, J.H., 2011b: Application of isolated tyrosinase inhibitory compounds from persimmon leaves. *J. Life Sc.* 21(7), 976-984.
- CUI, T., NAKAMURA, K., MA, L., ZHONG, J., KAYAHARA, H., 2005: Analyses of arbutin and chlorogenic acid, the major phenolic constituents in Oriental pear. *J. Agric. Food Chem.* 53, 3882-3887.
- DU, G.R., LI, M.J., MA, F.W., LIANG, D., 2009: Antioxidant capacity and the relationship with polyphenol and vitamin C in Actinidia fruits. *Food Chem.* 113, 557-562.
- FERREIRA, D., GUYOT, S., MARNET, N., DELGADILLO, I., RENARD, C.M., COIMBRA, M.A., 2002: Composition of phenolic compounds in a Portuguese pear (*Pyrus communis* L. var. *S. Bartolomeu*) and changes after sun-drying. *J. Agric. Food Chem.* 50, 4537-4544.

- GALVIS-SANCHEZ, A., GIL-LZQUIERDO, A., GIL, M.I., 2003: Comparative study of six pear cultivars in terms of their phenolic and vitamin C contents and antioxidant capacity. *J. Sci. Food Agric.* 83, 995-100.
- HONG, J.Y., NAM, H.S.N., KIM, W., SHIN, S.R., 2006: Change on the components of *Elaeagnus multiflora* fruits during maturation. *Kor. J. Food Preserv.* 13, 228-233.
- HWANG, E.Y., CHOI, S.Y., 2006: Quantitative analysis of phenolic compounds in different parts of *Panax ginseng* C.A. Meyer and its inhibitory effect on melanin biosynthesis. *Kor. J. Med. Crop Sci.* 14, 148-152.
- KANG, Y.J., YANG, M.H., KO, W.J., PARK, S.R., LEE, B.G., 2005: Studies on the major components and antioxidative properties of whole fruit powder and juice prepared from premature mandarin orange. *Kor. J. Food Sci. Technol.* 37, 783-788.
- KIM, K.H., KIM, D.M., YU, S.R., YOON, H.S., 2012: Antioxidant and whitening activities of various cultivars of Korean unripe peaches (*Prunus persica* L. Batsch). *J. Kor. Soc. Food Sci. Nutr.* 41(2), 156-160.
- KIM, M.S., KIM, K.H., YOON, H.S., 2009: The effects of gamma irradiation on the microbiological, physicochemical and sensory quality of peach (*Prunus persica* L. Batsch cv. *Dangeumdo*). *J. Kor. Soc. Food Sci. Nutr.* 38, 364-371.
- KIM, B.J., JO, B.K., 1998: A skin protection beauty expenses composite which contains the phyto sphingosine with a vitamin C to relieve. Korea Institute of Patent Information. 10-1998-0058217.
- KO, M.S., LEE, H.J., KANG, M.J., 2012: Antioxidant activities and whitening effects of extracts from *Hippophae rhamnoides* L. *J. East Asian Soc. Diet. Life.* 22(6), 812-817.
- KWAK, J.H., KIM, H.R., CHANG, C., PARK, W., HAN, Y.H., 2004: Inhibitory effect of gardenia fruit extracts on tyrosinase activity and melanogenesis. *Kor. J. Biotechnol. Bioeng.* 19, 437-440.
- LEE, S.A., KIM, K.H., KIM, M.S., PARK, N.K., YOON, H.S., 2008: Microbial and physico-chemical characteristics of a Maesil (*Prunus mume*) treated with low levels of gamma rays. *J. East Asian Soc. Diet. Life* 18, 989-996.
- LEE, K.H., CHO, J.Y., LEE, H.J., PARK, K.Y., MA, Y.K., KWON, J., 2011: Isolation and identification of phenolic compounds from an Asian pear (*Pyrus pyrifolia* Nakai) fruit peel. *Food Sci. Biotech.* 20, 1539-1545.
- LI, X., WANG, T., ZHOU, B., GAO, W., CAO, J., HUANG, L., 2014: Chemical composition and antioxidant and anti-inflammatory potential of peels and flesh from 10 different pear varieties (*Pyrus* spp.). *Food Chem.* 152, 531-538.
- LI, X., ZHANG, J.Y., GAO, W.Y., WANG, Y., WANG, H.Y., CAO, J.G., 2012: Chemical composition and anti-inflammatory and antioxidant activities of eight pear cultivars. *J. Agri. Food Chem.* 60, 8738-8744.
- MAEDA, K., FUKUDA, M., 1996: Arbutin: Mechanism of its depigmenting action in human melanocyte culture. *J. Pharmacol. Exp. Ther.* 276, 765-769.
- MAKPOL, S., ARIFIN, N.N.M., ISMAIL, Z., CHUA, K.H., YUSOF, Y.A.M., NGAH, W.Z.W., 2009: Modulation of melanin synthesis and its gene expression in skin melanocytes by palm tocopherol rich fraction. *Afric. J. Biochem. Res.* 3(12), 385-392.
- MASAMOTO, Y., LIDA, S., KUBO, I., 1980: Inhibitory effect of Chinese crude drugs on tyrosinase. *Planta Medicines.* 40, 361-365.
- NIHEI, K.I., KUBO, I., 2003: Identification of oxidation product of arbutin in mushroom tyrosinase assay system. *Bioorganic. Med. Chem. Letters.* 13, 2409-2412.
- PARK, Y.S., PARK, Y.J., KIM, H.J., IM, M.H., LEE, M.K., KIM, Y.M., CHO, J.Y., HEO, B.G., 2008: Physiological activity of ethanol extract from the different plant parts of loquat (*Eriobotrya japonica* Lindl.). *Kor. J. Hort. Sci. Technol.* 26, 75-80.
- RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M., RICE-EVANS, C., 1999: Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 26, 1231-1237.
- SALTA, J., MARTINS, A., SANTOS, R.G., NENG, N.R., NOGUEIR, J.M.F., JUSTINO, J., RAUTER, A.P., 2010: Phenolic composition and antioxidant activity of Rocha pear and other pear cultivars: A comparative study. *J. Funct. Foods.* 2, 153-157.
- SANCHEZ-FERRER, A., RODRIGUEZ-LOPEZ, J., GARCIA-CANOVAS, F., GARCIA-CARMONA, F., KUO-HSIEN, F., 1995: Tyrosinase: a comprehensive review of its mechanism. *Biochimica et Biophysica Acta.* 1247(1), 1-11.
- SHIBATA, S., OKANO, S., ONEMITSU, Y., 2006: Induction of efficient anti-tumor immunity using dendritic cells activated by recombinant Sendai virus and its modulation by exogenous IFN- $\beta$  gene. *J. Immun.* 177(6), 3564-3576.
- SUGIMOTO, K., NISHIMURA, T., NOMURA, K., SUGIMOTO, K., KURIKI, T., 2004: Inhibitory Effects of a-arbutin on melanin synthesis in cultured human melanoma cells and a three-dimensional human skin model. *Biol. Pharm. Bull.* 27(4), 510-514.
- VEBERIC, R., COLARIC, M., STAMPAR, F., 2008: Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. *Food Chem.* 106, 153-157.
- WANG, K.H., LIN, R.D., HSU, F.L., HUANG, Y.H., CHANG, H.C., HUANG, C.Y., LEE, M.H., 2006: Cosmetic applications of selected traditional Chinese herbal medicines. *J. Ethnopharm.* 106, 353-359.
- YANG, A.Y., BOO, H.O., 2013: Phenolic compounds, antimicrobial effects and tyrosinase inhibition activities of cucumber grown greenhouse according to cultivars and growth stages. *Kor. J. Plant Res.* 26, 645-651.
- ZHANG, X., KOO, J., EUN, J.B., 2006: Antioxidant activities of methanol extracts and phenolic compounds in Asian pear at different stages of maturity. *Food Sci. Biotechnol.* 15(1), 44-50.

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