Changes in Total Phenolic and Flavonoid Content and Antioxidative Activities during Production of Juice Concentrate from Asian Pears (*Pyrus pyrifolia* Nakai)

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Abstract The total phenolic and flavonoid content and antioxidative activities during production of pear juice concentrate (PJC) from two cultivars, Hwasan and Niitaka, were investigated. The main processing steps in PJC production are washing, pressing, pasteurization, clarification, filtration, evaporation, and packaging. Total phenolic and flavonoid content of end-product PJC from Niitaka decreased by 53.11 and 46.47%, respectively, while those from Hwasan decreased by 55.46 and 36.09%, respectively, compared to the phenolic and flavonoid content of original fresh fruit. DPPH radical-scavenging activities, reducing power and nitrate radical-scavenging activities showed a similar tendency as total phenolic and flavonoid content; that is, they decreased in juice concentrate made from both cultivars. Also, antioxidant activities of press cake waste (skin and seeds) from Niitaka and Hwasan pears were higher than fresh pears. In conclusion, antioxidant levels were significantly affected during processing of PJC, especially during the pressing step in which press cake waste retains the seeds and skin.

Keywords: pear juice concentrate, press cake, Niitaka and Hwasan, antioxidant activity

Introduction

The Asian pear is a very popular fruit because of its sweetness, crispness, and overall flavor. The flesh of Asian pears contains moisture and has very little fat, protein, or ash. Meanwhile, the peel of Asian pears is high in dietary fiber and possesses antioxidant activities. The consumption of pears has been proposed to protect against asthma and hypertension-related diseases (1-3). In addition, pear consumption leads to decreases in cholesterol levels because of its high dietary fiber content (4,5). Many different cultivars of Asian pear are grown in Korea, but the most-grown cultivar is the Niitaka. A new cultivar, Hwasan, was created by the Pear Research Station of the National Institute of Horticultural & Herbal Science in 1993 and its cultivation has increased since then. The Hwasan cultivar is a cross between the Hosui and Mansamgil cultivars. The Niitaka cultivar has developed major problems due to overproduction, and the Hwasan cultivar was made in order to overcome this issue (6). In addition, the Hwasan pear is a high-quality fruit because of its juiciness, enhanced flavor, and because it contains few stone cells.

Pear fruit is widely consumed both fresh and after being processed into various fruit juices, vinegar, fruit juice concentrates, jam, and wine products. Previous studies have investigated changes in sugar, acid, phenolic composition, volatile compounds, anti-hyperglycemia activities, and antihypertensive activities during pear juice production as well as during fermentation of pear juice and pear wine processing (7-10) and storage (9,10). However, only a few studies have examined the effect of every step of processing of pear juice or concentrate in regard to antioxidants. Therefore, this study aimed to investigate changes in the total phenolic, total flavonoid, and antioxidant activities during processing of Asian pear (Niitaka and Hwasan cultivars) juice concentrate and pinpoint the steps that affect the antioxidant activities of this major pear product.

Materials and Methods

Materials Fresh pear samples (*Pyrus pyrifolia* Nakai cv. Hwasan and *Pyrus pyrifolia* Nakai cv. Niitaka) were purchased from Gimcheon,

Korea. Monobasic dihydrogen phosphate, methanol, dibasic monohydrogen phosphate, sodium nitrite, hydrogen chloride, aluminium chloride, sodium hydroxide, sodium carbonate, acetic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, and L-ascorbic acid (100%) were supplied by Duksan Science (Seoul, Korea). Folin-Ciocalteu reagent, gallic acid, Griess reagent, rutin, L-ascorbic acid and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Production of pear juice concentrate The major processing steps in the formation of pear juice concentrate (PJC) have been described by Capanoglu *et al.* (11); we employed their methods with some modifications. PJC production steps included: selecting pears, washing twice with distilled water, separating into four fractions, pressing in order to separate juice from seeds and skin, pasteurizing using a water bath (JSSB-50T; JS Research Inc., Gongju, Korea) at 95°C for 5 min, clarification, filtration, evaporation, and packaging at 25°C. Juice clarification was accomplished by centrifuging (UNION 32R PLUS; Hanil Science Industrial Co., Kimpo, Korea) for 15 min at 3,500*xg* to remove solids. After being clarified, the pear juice was immediately filtered with filter paper (Whatman, No. 4; Whatman International Ltd., Maidstone, UK). Evaporation was done with a rotary vacuum evaporator (EyelaN-11; Tokyo Rikakikai Co., Tokyo, Japan) in order to solubilize the solids present in pear juice concentrate (68–70°Bx). Finally, the pear juice concentrate was packed in 500 mL glass bottle containers. The pH of pear concentrate from Niitaka and Hwasan cultivars were 5.27 and 5.30, respectively, while the acidity of concentrate from Niitaka and Hwasan cultivars were 0.15 and 0.22% tartaric acid, respectively.

The production of PJC includes several intermediate products: fresh pear, pressed cake, initial pressed juice, pasteurized juice, clarified juice, filtered juice, and the final pear juice concentrate (Fig. 1). After processing, samples were freeze-dried and ground to obtain a powder using a blender (MSM-515; Angel Co., Busan, Korea) and stored at –80°C until further analysis.

Moisture content Moisture content of the samples was measured with the AOAC method (12).

Extract preparation One gram of each freeze-dried sample was extracted using a reflux extraction process; the samples were boiled in methanol (80%, 9 mL) in a water bath three times (1 h, 30 min, and 30 min) at 60°C then cooled to 25°C and centrifuged (UNION 32R PLUS; Hanil Science Industrial Co.) at 3,500*xg* for 15 min. The supernatant was recovered and analyzed for total flavonoid content, total phenolic content, DPPH radical-scavenging activity, and nitrate radical-scavenging activity reducing power.

Total phenolic content The total phenolic content was determined using Folin-Ciocalteu reagent obtained from Eghdami and Sadeghi (13). 200 µL of sample extract was added to Folin reagent (800 µL)

and sodium carbonate (7%, 2 mL). Distilled water (7 mL) was added to the final mixture and it was kept in the dark for an hour to allow the reaction to finish. The absorbance of the mixture was analyzed at 765 nm by a UV spectrophotometer (Optizen 2120 UV; Mecasys Co., Daejeon, Korea). Gallic acid was used as a standard and results are expressed as mg gallic acid equivalent (GAE)/100 g dry weight (DW).

Total flavonoid content The total flavonoid content was measured as described by Eghdami and Sadeghi (13). 3 mL of distilled water was added to 1 mL of sample extract, followed by the addition of NaNO₂ (5%, 0.3 mL). The mixture was allowed to stand at 25°C for 5 min, then AlCl₃ (10%, 0.3 mL) was added. After 5 min, the reaction mixture was mixed with NaOH (1 M, 2 mL). Finally, 10 mL of distilled water was added to the reaction mixture and the absorbance was measured at 510 nm with a UV spectrophotometer. Rutin was used as a standard and results are expressed as mg rutin equivalent (RE)/100 g DW.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity

The DPPH radical-scavenging activity of samples was determined using the method published by Kang *et al.* (14) with some modifications. The methanolic extract (50 µL) was mixed with methanol (80%, 50 µL) and added to 2 mL of DPPH radical solution (0.2 mM). The absorbance of the mixture was analyzed at 517 nm using a spectrophotometer after the mixture was stored in the dark for 30 min. The scavenging rate (%) of DPPH radicals was calculated as: Scavenging activity (%) = $[1 - A_1/A_0] \times 100$, where A_0 is the absorbance of a control solution (50 µL of methanol (80%) in DPPH radical solution (2 mL)) and A_1 is the absorbance of samples.

Reducing power The reducing power of samples was determined using a spectrophotometric method (15). The methanolic extract (0.3 mL) was added to phosphate buffer (0.2 M, pH 6.6, 1.1 mL) and 1% potassium ferricyanide (0.6 mL), followed by incubation (IB-600M; Daihan Labtech Co., Namyangju, Korea) at 50°C for 20 min. After adding 10% trichloroacetic acid (1 mL), the mixture was centrifuged at 3,500*xg* for 15 min. The supernatant was mixed with distilled water (1 mL) and 0.1% ferric chloride (0.5 mL). Absorbance was analyzed at 700 nm using a UV spectrophotometer and the reducing power of samples is represented by the absorbance value.

Nitrate radical-scavenging activities The nitrate radical-scavenging activity was determined by a previously described spectrophotometric method (16) with some modifications. The methanolic extract (0.3 mL) was reacted with 1 mM sodium nitrite (0.2 mL). The mixture was added to 1 N HCl in order to obtain a pH of 1.2. Distilled water was added to produce a final volume of 1 mL. Mixtures were incubated for 1 min at 37°C and added to 2% acetic acid (3 mL) and 0.4 mL of Griess reagent. After vigorous vortexing (GENIE 2; Hanil Lab Tech Co., Yangju, Korea) for 1 min, the mixture was stored for 15 min at 25°C and then absorbance was measured at 520 nm using a UV. The

nitrite radical-scavenging activity (percent) was calculated as: Scavenging activity (%) = $[1 - (A - B)/C] \times 100$, where A is the absorbance of the methanolic extract in 0.4 mL of Griess solution, B is the absorbance of the methanolic extract in 30% acetic acid (0.4 mL) instead of Griess solution, and C is the absorbance of a control solution of distilled water and Griess solution (0.4 mL).

Statistical analysis All experiments were conducted twice and all measurements were analyzed in triplicate and presented as mean $\pm$ standard deviation. Statistical significance was assessed by ANOVA followed by Duncan's multiple-range test using SPSS version 18.0. The level of significance was set at $p < 0.05$.

Results and Discussion

Moisture content The moisture content of processed pear juice concentrate samples ranged from 32.5 to 85.4%. Processing of the fresh pear fruit into the final concentrate resulted in a significant decrease (approximately 52.9%) in moisture content ($p < 0.05$).

Total phenolic and flavonoid content A previous study about identifying and quantifying the phenolic compounds such as chlorogenic acid, caffeic acid, coumaric acid, arbutin, rutin, catechin, epicatechin, and procyanidins in Comice, d'Anjou and Bartlett pear juice concentrate has been demonstrated by Spanos and Wrolstad (10). In this study, total phenolic and flavonoid content of processed pear juice concentrate samples are shown in Table 1. The total phenolic content

of fresh Niitaka and Hwasan pears were found to be 189.60 and 211.08 mg GAE/100 g DW, respectively. The total flavonoid content of fresh Niitaka and Hwasan pears were 78.81 and 96.37 mg RE/100 g DW, respectively. The results revealed that fresh Hwasan pears are higher in phenol and flavonoid content than are fresh Niitaka pears. Total phenolic and flavonoid content of PJC from Niitaka significantly decreased by 53.11 and 46.47%, while those PJC from Hwasan significantly decreased by 55.46 and 36.09%, respectively, compared the fresh pears. After pasteurization, the total phenolic and flavonoid content did not significantly change further; there were no significant differences following the clarification, filtration, and evaporation steps. On the other hand, the press cake waste of Niitaka and Hwasan pears was relatively rich in total phenolic content (381.59 and 437.11 mg GAE/100 g DW, respectively) and total flavonoid content (161.54 and 194.66 mg RE/100 g DW, respectively) ($p < 0.05$), indicating that press cake waste, which consists of pear seeds and skin, contains most of the phenolic and flavonoid compounds. According to Capanoglu *et al.* (11) total phenolic and flavonoid concentrations decreased during processing steps, while in pressing steps, the press cakes contain high concentrations of these compounds because they contain seeds and skin. Also, Capanoglu *et al.* (17) demonstrated that total phenolic content was not significantly altered by tomato paste production. The seeds and skin of tomatoes have high concentrations of phenolic compounds, but phenols are present in similar amounts in the original fruit as in processed tomato paste. However, Tomas *et al.* (18) reported that total phenolic and flavonoid concentrations increased during processing of black mulberry juice, and phenolic

Table 1. Total phenolic (mg GAE/100 g DW) and total flavonoid (mg RE/100 g DW) content of pear juice concentrate after different processing steps

		Fresh fruit	Press cake	Initial pressed juice	Pasteurization	Clarification	Filtration	Concentrate
Niitaka	Total phenolics	189.60 $\pm$ 3.92 ^{b1)}	381.59 $\pm$ 5.69 ^a	118.69 $\pm$ 6.31 ^c	94.33 $\pm$ 5.97 ^d	91.05 $\pm$ 7.81 ^d	90.56 $\pm$ 10.69 ^d	88.91 $\pm$ 9.67 ^d
	Total flavonoids	78.81 $\pm$ 5.31 ^b	161.54 $\pm$ 7.56 ^a	61.03 $\pm$ 3.59 ^c	50.39 $\pm$ 2.98 ^d	45.69 $\pm$ 5.81 ^d	43.79 $\pm$ 9.41 ^d	42.19 $\pm$ 9.81 ^d
Hwasan	Total phenolics	211.08 $\pm$ 8.36 ^b	437.11 $\pm$ 11.69 ^a	146.19 $\pm$ 9.78 ^c	100.87 $\pm$ 3.58 ^d	97.36 $\pm$ 5.17 ^d	95.89 $\pm$ 7.31 ^d	94.01 $\pm$ 8.26 ^d
	Total flavonoids	96.37 $\pm$ 6.24 ^b	194.66 $\pm$ 10.87 ^a	78.59 $\pm$ 6.19 ^c	67.25 $\pm$ 4.94 ^d	63.89 $\pm$ 6.84 ^d	62.10 $\pm$ 6.89 ^d	61.59 $\pm$ 8.47 ^d

¹⁾ a-d Means followed by different letters in each row are significantly different ($p < 0.05$)

Fig. 1. Flow diagram of processing steps involved in the making of pear juice concentrate.

Fig. 2. DPPH radical-scavenging activities of pear juice concentrate following different processing steps. ^{a-d}Means followed by different letters on the bar are significantly different ($p < 0.05$).

Fig. 3. Reducing power of pear juice concentrate following different processing steps. ^{a-d}Means followed by different letters on the bar are significantly different ($p < 0.05$).

and flavonoid concentrations in black mulberry press cakes were relatively low. In this study, we found that processing affects total phenolic and total flavonoid concentrations and confirmed that the press cake (seeds and skin) contains high concentrations of phenolic and flavonoid compounds.

Antioxidative activity Antioxidative activity of the methanolic extracts was measured using three different methods. The different analyses showed the same results. Analysis of DPPH radical-scavenging activity, reducing power, and nitrate scavenging activity revealed that the antioxidant activity of samples was decreased significantly following processing. Also in this study, fresh Hwasan pears had higher antioxidative activity than did fresh Niitaka pears. DPPH radical-scavenging activities decreased by 54.89 and 55.40% from fresh Niitaka and Hwasan fruit to the final concentrated product, respectively (Fig. 2). The reducing power of end-product Hwasan and

Fig. 4. Nitrate radical-scavenging activities of pear juice concentrate following different processing steps. ^{a-c}Means followed by different letters on the bar are significantly different ($p < 0.05$).

Niitaka juice concentrate were 0.66 and 0.48, which were slightly lower than the reducing power of fresh pears of both types (Hwasan, 0.69 and Niitaka, 0.52) (Fig. 3). Nitrate radical-scavenging activities decreased in a similar fashion (Fig. 4). Antioxidative activities also decreased during production of grape juice concentrate (11). In initial juice pressing steps, DPPH radical-scavenging activity and nitrate radical-scavenging activity decreased by 37.91 and 37.71, and 28.07 and 25.45%, respectively, compared to scavenging activities in fresh Niitaka and Hwasan pears. Pasteurization extracts have slightly lower antioxidant values than initial pressed juice ($p < 0.05$). The high rate of antioxidant loss might be caused by native polyphenol oxidase catalyst oxidation of phenolic compounds reacting with oxygen produced by *o*-quinone during pressing steps (19). Also, Spanos and Wrolstad (10) reported without addition of sulfite could result in oxidation of phenolics compounds such as chlorogenic acid and caffeic acid in the processing of pear juice concentrate at pressing step, that formation of brown compounds. Therefore, in this study the antioxidative activities reduced in pressing step might be due to degradation of chlorogenic acid and caffeic acid. On the other hand, the press cakes, which contain skin and seeds, had higher concentrations of antioxidants than did fresh pears. According to previous studies, the major loss of antioxidative activity during processing steps suggest press cake waste was very well detached during the pressing steps (11). In this study, the processing steps affect antioxidative activity, which decreases greatly as fresh fruit is processed into concentrated pear juice. Because press cake waste remains high in antioxidants, recovery of antioxidants from these cakes may allow for utilization of antioxidants in the food industry.

In conclusion, large decreases in total phenolic and flavonoid concentrations and antioxidative capacity are seen during the production of pear juice concentrate. In this study, high antioxidant levels were seen in press cake wastes. In Korea, other fruit press cake wastes from industrial pear processing are commonly used as animal

feed, which may be regarded as a waste of such a rich resource. Waste cakes generated during processing of fresh pears into pear juice concentrate may be a valuable source of natural antioxidants to be used as food additives.

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Disclosure The authors declare no conflict of interest.

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