

# Enzymatic Browning Inhibition and Antioxidant Activity of Pear Juice from a New Cultivar of Asian Pear (*Pyrus pyrifolia* Nakai cv. Sinhwa) with Different Concentrations of Ascorbic Acid

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**Abstract** Different ascorbic acid (AA) concentrations of 0.16, 0.20, and 0.24% (w/v) were added to pear juice from the new cultivar *Pyrus pyrifolia* Nakai cv. "Sinhwa". Enzymatic browning reduction and antioxidant activity were analyzed after 24 h at 37°C. Juices with 0.20% added AA showed the highest inhibition of 78.8% of polyphenol oxidase (PPO) activity. L\* values of juices with 0.20 and 0.24% added AA decreased more slowly than controls lacking AA addition and juice with 0.16% added AA after storage for 24 h. Browning indices of juices with added AA were lower than for controls. However, indices increased after storage for 24 h. The DPPH radical-scavenging activity, reducing power, and nitrite scavenging activity of all juices with added AA were higher than for controls and decreased after storage for 24 h. Addition of 0.20% AA to pear juice from the new "Sinhwa" cultivar showed the highest browning activity reduction.

**Keywords:** new cultivar pear, juice, browning, antioxidant activity

## Introduction

A reduction in nutritional quality, change in sensory perceptions, and decreased consumer acceptance of food are caused by browning reactions of vegetables and fruits during processing and storage (1). Enzymatic browning is major factor that contributes to quality loss in beverages and foods (2). The browning phenomenon usually damages sensory evaluation of products due to changes in flavor, softening, and color (3). Enzymatic oxidation of phenolic compounds, such as polyphenol oxidase (PPO), can be found in browning of vegetables and fruits (3).

AA has been widely used as an anti-browning agent for processing of fruits and vegetables. AA is a strong antioxidant and chelating agent that inhibits the activity of PPO and reduces oxygen (4). The action of AA is based upon reduction of intermediate *o*-quinones to original phenolic compounds before they undergo further reaction to form pigments (2). The *o*-quinones react with AA to produce stable colorless compounds that are effective for inhibition of enzymatic browning of fruit products (2,5). Hence, AA has been used in many juices to improve color and shelf-life.

Niitaka pears grown in Korea are popular among consumers due to sweetness, crispness, and appearance. However, they should be eaten fresh and this has limited consumption without increasing

demand. To overcome such limitations, a new cultivar of Asian pears called *Pyrus pyrifolia* Nakai cv. "Sinhwa" was developed. This new cultivar has a higher pH, a higher soluble solid content, and a lower titratable acidity (data not shown). The pH of "Sinhwa" pears of pH=5.59 was higher the value for "Niitaka" pears at pH=5.23 reported by Lee *et al.* (6). Therefore, addition of reducing agents, such as AA, to pear juice can lower the pH with a suitable sugar-acid ratio.

The "Niitaka" pear contains polyphenols and flavonoids with antioxidant activities. Nine cultivars of Korean pears have been analyzed for polyphenol compounds and DPPH radical scavenging activities (7). Total phenolic and total flavonoid contents, and antioxidant activities of heated juice from "Niitaka" and "Chuwang" pears have been reported (8,9). However, the antioxidant activity of processed "Sinhwa" pear juice treated with AA has not been studied. The processing capacity of Asian pears in Korea was 8,037 tons in 2010, accounting for 2.6% of table fruits. However, the capacity was lower than the processing capacity of citrus (14%) and apples (6.1%) (10). Consumption of processed products has reduced demand for fresh pears. To overcome this problem, Asian pears are also used to make juice. However, pear juice can easily brown during the manufacturing process, thus lowering the value of the juice. Treatment with the anti-browning agent AA might solve the browning problem and improve the antioxidant activity of pear juice.

Therefore, the objective of this study was to determine whether AA can reduce browning and improve the antioxidant activity of juice of the new pear cultivar *Pyrus pyrifolia* cv. "Sinhwa" after incubation at 37°C for 12 and 24 h.

## Materials and Methods

**Materials** The new Asian pear cultivar *Pyrus pyrifolia* Nakai cv. "Sinhwa" was obtained from the Pear Research Station of the National Institute of Horticultural and Herbal Science from Naju, South Korea and stored at 4°C until use. Monobasic dihydrogen phosphate, dibasic monohydrogen phosphate, nitrite sodium, trisodium citrate dihydrate, citric acid monohydrate, acetic acid, potassium ferricyanide, trichloroacetic acid, ferric chloride, and L-ascorbic acid (100%) were supplied by Daesang Chemical Company, Gwangju, Korea. Griess reagent (1% sulfanilic acid and 1% naphthylamine=1:1), catechol, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

### Methods

**Juice extraction:** Pears of the "Sinhwa" cultivar were washed twice with distilled water and sliced to 3 cm to extract juice using a rack and cloth press (Model-1500; Samwoo Engineering Co., Seoul, Korea) at a pressure of 4,410 N/cm<sup>2</sup> for 2 min. Different AA concentrations of 0.16, 0.20, and 0.24% (w/v) were added to pear juices, which were immediately filtered through a 40 mesh sieve (Testing Sieve; Chung Gye Sang Gong Sa, Seoul, Korea) and analyzed.

**PPO activity:** The PPO (EC 1.14.18.1) activity was measured using a spectrophotometric method described by Duangmal and Apenten (11). Briefly, 50 µL of pear juice was added to 2.5 mL of 10 mM catechol in a 50 mM phosphate buffer, pH 6.5 substrate solution. The absorbance of the mixture was measured at 420 nm using a spectrophotometer (2120 UV; Optizen, Daejeon, Korea). The residual activity (%) of PPO was calculated as: Residual activity =  $A/A_0 \times 100$ , where A is the PPO activity of pear juice with different amounts of added AA, and A<sub>0</sub> is the PPO activity of control pear juice lacking added AA.

**pH and titratable acidity:** The pH of pear juice was measured using a pH meter (Model 8000; VWR-Scientific, West Chester, PA, USA). The titratable acidity of pear juice was expressed as a quantity of citric acid.

**Color:** The color of pear juice was determined using a colorimeter (CM-3500d; Minolta Co., Ltd., Tokyo, Japan). Results were expressed as lightness (L\* value), redness (a\* value), and yellowness (b\* value).

**Browning index:** Ten mL of pear juice was centrifuged (UNION 32R PLUS; Hanil Science Industrial. Co., Incheon, Korea) at 3,500×g for 15 min. Five milliliter of ethanol (95%) was added to the supernatant (5 mL) and centrifuged (UNION 32R PLUS; Hanil Science Industrial. Co.) at 3,500×g for 15 min. The absorbance of supernatant of a

centrifuged pear juice (5 mL) added to ethanol (5 mL) was measured at 420 nm using a UV spectrophotometer (2120 UV; Optizen) (12).

**Vitamin C content:** The vitamin C content of pear juice was determined using the 2,6-dichloroindophenol titrimetric method (13).

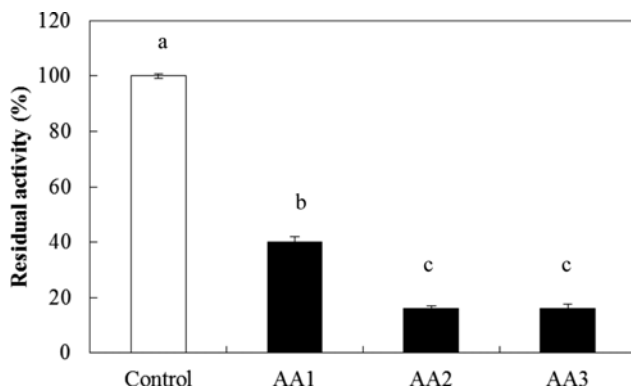
### **1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity:**

The DPPH radical-scavenging activity of pear juice was determined using the method of Kang *et al.* (14). Briefly, 0.1 mL of pear juice was mixed with 2 mL of a 0.2 mM DPPH radical solution. The mixture was stored in the dark for 30 min, then the absorbance of the mixture was analyzed at 517 nm using a spectrophotometer (2120 UV; Optizen). The scavenging rate (%) of DPPH radicals was calculated as: Scavenging activity (%) =  $[1 - A_1/A_0] \times 100$ , where A<sub>0</sub> is the absorbance of a control solution (0.1 mL of methanol (95%) in 2 mL of DPPH radical solution) and A<sub>1</sub> is the absorbance of pear juice with different amounts of added AA after 30 min of storage.

**Nitrate radical-scavenging activities:** The nitrate scavenging activity of pear juice was determined using a previous spectrophotometric method (15) with modification. Briefly, 0.3 mL of each pear juice was mixed with 0.2 mL of 1 mM nitrite sodium. The mixture was added to 8 mL of 0.2 M citrate buffer (pH 3.0, 4.2, and 6.0). After mixtures were incubated (DAI HAN LABTECH, Namyangju, Korea) at 37°C for 1 h, 1 mL of the mixture was withdrawn and added to 3 mL of a mixture of 2% acetic acid and 0.4 mL of Griess reagent. After vigorous vortexing (GENIE 2; Hanil Lab Tech CO., Ltd. Yangju, Korea) for 1 min and the mixture was placed at 25°C for 15 min and the absorbance was measured at 520 nm using a UV spectrophotometer (2120 UV; Optizen). The nitrite scavenging activity (percent) was calculated as: Scavenging activity (%) =  $[1 - (A - B)/C] \times 100$ , where A is the absorbance of pear juice in 0.4 mL of Griess solution, B is the absorbance of a pear juice sample with 0.4 mL of 30% acetic acid added instead of Griess solution, and C is the absorbance of a control solution of distilled water and 0.4 mL of Griess solution.

**Reducing power:** The reducing power of pear juice was measured using a previous spectrophotometric method (16) with modification. Briefly, 0.3 mL of pear juice was mixed with 1.1 mL of phosphate buffer (0.2 M, pH 6.6) and incubated (DAI HAN LABTECH) with 1% potassium ferricyanide (0.6 mL) at 50°C for 20 min. At the end of the incubation, 1 mL of a 10% trichloroacetic acid solution was added, followed by centrifugation (UNION32R plus; Hanil Science Industrial Co.) at 3,500×g for 15 min. The supernatant was mixed with 1 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride solution. The absorbance was measured at 700 nm using a UV spectrophotometer (2120 UV; Optizen). An increased absorbance value of the reaction indicated and increased reducing power of juice.

**Statistical analysis** All experiments were conducted in duplicate with two repetitive experiments. Data were presented as mean ± standard deviation. Statistical significance was determined using SPSS version 18.0 at  $p < 0.05$ .



**Fig. 1.** The residual activity of PPO in pear juices with different concentrations of added AA. <sup>a-c</sup>Means followed by different letters in each column are significantly different ( $p < 0.05$ ); Control, Pear juices lacking added AA; AA1, Pear juices with a concentration of 0.16% added AA; AA2, Pear juices with a concentration of 0.20% added AA; AA3, Pear juices with a concentration of 0.24% added AA.

## Results and Discussion

**PPO activity** Residual activities of PPO in pear juice with different concentrations of added AA are shown in Fig. 1. Increased AA concentrations decreased the residual activity of PPO. A reduced residual activity suggested stronger inhibition of PPO, which could cause complete enzyme inactivation even at low concentrations. Compared to pear juice without added AA, the residual activity of PPO of pear juice was not decreased significantly ( $p > 0.05$ ) when AA concentrations were more than 0.20%, compared with controls. The PPO activity was decreased to 23.5% with 0.12% added AA. The PPO activity was decreased to 48.8% when the added AA concentration was 0.16%. Treatment with 0.20% AA showed the strongest inhibition of PPO at 75.8%, indicating that PPO in pear juice can be efficiently inactivated by AA. Arias *et al.* (17) reported that AA can reduce the browning reaction of PPO of Spain pears (*Pyrus communis* L. cv. Conferencia). In addition, 0.1 mM AA added to Niitaka pear juice completely inhibited PPO browning (18). The inhibitory activity of AA in this study was similar to the report of Ozoglu and Bayyindirhy (19) in which the anti-browning effect of 1.8 mM AA in cloudy apple juice lasted for no more than 4 h. In addition, Unal (20) reported that addition of 0.2 and 0.8 mM ascorbic acid resulted in 99 and 100% inhibition of banana PPO, respectively. A concentration of 0.20% AA was the most effective level for inhibition of PPO in pear juice.

**pH and titratable acidity** The pH and titratable acidity of pear juice with different AA concentrations after storage at 37°C for 12 and 24 h are shown in Table 1. After AA concentrations of 0, 0.16%, 0.20%, and 0.24% were added, the pH of pear juices ranged from 4.61 to 5.59 at time=0, from 4.61 to 5.32 at 12 h after incubation, and from 4.61 to 4.81 after storage for 24 h. The pH of control pear juice lacking added AA was decreased from 5.59 to 5.32 after 12 h of storage. After 24 h, the pH of control pear juice was decreased from

**Table 1.** pH and titratable acidity of pear juices with different concentrations of added AA after storage at 37°C for 12 and 24 h

	AA concentrations (%)	Storage times (h)		
		0	12	24
pH	Control	5.59±0.01 <sup>Aa</sup>	5.32±0.01 <sup>Ba</sup>	4.77±0.01 <sup>Cb</sup>
	0.16	4.83±0.01 <sup>Ab</sup>	4.81±0.01 <sup>Ab</sup>	4.81±0.01 <sup>Aa</sup>
	0.20	4.73±0.01 <sup>Ac</sup>	4.72±0.01 <sup>Ac</sup>	4.72±0.02 <sup>Ac</sup>
	0.24	4.61±0.01 <sup>Ad</sup>	4.61±0.01 <sup>Ad</sup>	4.61±0.01 <sup>Ad</sup>
Titratable acidity	Control	0.04±0.01 <sup>Cc</sup>	0.12±0.00 <sup>Bc</sup>	0.16±0.02 <sup>Aa</sup>
	0.16	0.13±0.00 <sup>Ab</sup>	0.13±0.00 <sup>Ab</sup>	0.13±0.00 <sup>Ab</sup>
	0.20	0.15±0.01 <sup>Aa</sup>	0.15±0.01 <sup>Aa</sup>	0.15±0.01 <sup>Aa</sup>
	0.24	0.15±0.01 <sup>Aa</sup>	0.15±0.00 <sup>Aa</sup>	0.15±0.00 <sup>Aa</sup>

<sup>A-c</sup>Means followed by different letters in each row are significantly different ( $p < 0.05$ ); <sup>a-d</sup>Means followed by different letters in each column are significantly different ( $p < 0.05$ ); Control, Pear juices lacking added AA; AA, Ascorbic acid.

5.59 to 4.77. In addition, the flavor of control juice was bad with a strong acid smell due to microbiological spoilage (data not shown). Rivas *et al.* (21) also reported that the pH of carrot juice decreased after storage. However, the pH of pear juice with added AA did not significantly ( $p > 0.05$ ) change after storage for 24 h, compared with controls.

The titratable acidity of pear juice with added AA concentrations of 0, 0.16, 0.20, and 0.24% ranged from 0.04 to 0.15 at time=0. After storage at 37°C for 12 and 24 h, the titratable acidity of control juice lacking added AA increased. However, there was no significant ( $p > 0.05$ ) difference in the titratable acidity of pear juices with added AA after storage for 12 and 24 h, compared with controls (Table 1). Similar results were observed in orange juice with an increase in total acidity after storage at 4 and 10°C (22).

**Color** The color of pear juices with different concentrations of added AA after storage at 37°C for 12 and 24 h are shown in Table 2. The L\*, a\*, and b\* values of pear juices with 0.16, 0.20, and 0.24% added AA ranged from 46.41 to 46.57, 0.71 to 0.77, and 12.65 to 12.73, respectively. There were no significant ( $p > 0.05$ ) differences among pear juices with the 3 concentrations of added AA. However, L\* value of control pear juice decreased rapidly after 24 h of storage. Compared with control juice, the values of L\*, a\*, and b\* of pear juices with added AA decreased slowly after storage. Pear juices with 0.20 or 0.24% concentrations of added AA had higher L\* values than the control juice and pear juice with 0.16% added AA.

Addition of AA could prevent formation of browning pigments in pear juice via reactions with quinone to form stable colorless products. Pear juice from the Sinhwa cultivar was as effective as a 0.20% AA concentration for inhibition of pear juice browning after storage at 37°C for 12 h. Wu (23) reported that grape juice treated with glutathione had higher L\* values and less enzymatic browning than grape juice without glutathione during storage. However, in this study, a\* values increased after 24 h of storage while b\* values decreased when concentrations of added AA were increased (Table

**Table 2.** Color of pear juices with different concentrations of added AA after storage at 37°C for 12 and 24 h

Color Value	AA concentration (%)	Storage time (h)		
		0	12	24
L*	Control	46.41±0.09 <sup>Aa</sup>	41.88±0.07 <sup>Bc</sup>	37.64±0.00 <sup>Cc</sup>
	0.16	46.43±0.05 <sup>Aa</sup>	43.22±0.06 <sup>Bb</sup>	40.83±0.02 <sup>Cb</sup>
	0.20	46.57±0.07 <sup>Aa</sup>	43.38±0.01 <sup>Ba</sup>	41.36±0.04 <sup>Ca</sup>
	0.24	46.55±0.04 <sup>Aa</sup>	43.60±0.10 <sup>Ba</sup>	41.38±0.09 <sup>Ca</sup>
a*	Control	0.71±0.08 <sup>Ca</sup>	3.86±0.01 <sup>Ba</sup>	4.90±0.03 <sup>Aa</sup>
	0.16	0.74±0.01 <sup>Ca</sup>	1.17±0.01 <sup>Bb</sup>	1.27±0.06 <sup>Ab</sup>
	0.20	0.77±0.00 <sup>Ba</sup>	1.00±0.02 <sup>Ac</sup>	0.95±0.01 <sup>Ac</sup>
	0.24	0.75±0.01 <sup>Ca</sup>	0.95±0.02 <sup>Bd</sup>	1.04±0.04 <sup>Ac</sup>
b*	Control	12.65±0.04 <sup>Aa</sup>	12.32±0.04 <sup>Ba</sup>	9.79±0.01 <sup>Cc</sup>
	0.16	12.68±0.04 <sup>Aa</sup>	11.94±0.04 <sup>Bb</sup>	10.12±0.06 <sup>Cb</sup>
	0.20	12.72±0.08 <sup>Aa</sup>	11.82±0.04 <sup>Bb</sup>	10.33±0.06 <sup>Cab</sup>
	0.24	12.73±0.06 <sup>Aa</sup>	11.65±0.04 <sup>Bc</sup>	10.39±0.16 <sup>Ca</sup>

<sup>A-C</sup>Means followed by different letters in each row are significantly different ( $p < 0.05$ ); <sup>a-c</sup>Means followed by different letters in each column are significantly different ( $p < 0.05$ ); Control, Pear juices lacking added AA; AA, Ascorbic acid.

2). Since most PPO in pear juice was inhibited with 0.20% added AA, color changes of pear juices with 0.20% added AA were reduced. Thus, the browning reaction of pear juice was slower than for the control during storage.

**Browning index** The browning index of pear juice was determined and the control juice ranged from 0.168 to 0.629 (Table 3). For pear juices with 0.16, 0.20, and 0.24% added AA, browning indices increased from 0.169 to 0.395, 0.379, and 0.378, respectively, after 12 h of storage. After 24 h of storage, the browning indices of pear juices with 0.16, 0.20, and 0.24% added AA increased from 0.169 to 0.451, 0.421, and 0.420, respectively (Table 3).

Browning indices of juices have been reported to increase when storage time is increased due to enzymatic browning reactions (24). In this study, pear juice with 0.20 and 0.24% added AA inhibited browning more than for the control juice and for pear juice with 0.16% added AA, in agreement with Johnson *et al.* (25). In food systems, AA is used as an antioxidant due to effectiveness for inhibition of enzymatic browning (20). Herein, the browning index of pear juice with a concentration higher than 0.20% added AA was lower than for controls after storage.

**Vitamin C content** The vitamin C content of pear juices with different concentrations of added AA are shown in Table 4. The vitamin C content of pear juice increased when concentrations of AA increased. For control pear juice, the vitamin C content was decreased from 4.76 to 1.30 mg/100 g after storage for 24 h. For pear juices with 0.16, 0.20, and 0.24% added AA, 63.2, 47.4, and 49.3% of vitamin C was degraded, respectively, after storage for 24 h. In a report of Nagy and Smoot (26), a temperature of more than 27°C increased AA degradation in orange juice. However, grapefruit juice

**Table 3.** Browning index of pear juices with different concentrations of added AA after storage at 37°C for 12 and 24 h

AA concentrations (%)	Storage time (h)		
	0	12	24
Control	0.168±0.002 <sup>Ca</sup>	0.530±0.003 <sup>Ba</sup>	0.629±0.004 <sup>Aa</sup>
0.16	0.169±0.001 <sup>Ca</sup>	0.395±0.002 <sup>Bb</sup>	0.451±0.011 <sup>Ab</sup>
0.20	0.169±0.002 <sup>Ca</sup>	0.379±0.004 <sup>Bc</sup>	0.421±0.004 <sup>Ac</sup>
0.24	0.169±0.002 <sup>Ca</sup>	0.378±0.001 <sup>Bc</sup>	0.420±0.001 <sup>Ac</sup>

<sup>A-C</sup>Means followed by different letters in each row are significantly different ( $p < 0.05$ ); <sup>a-c</sup>Means followed by different letters in each column are significantly different ( $p < 0.05$ ); Control, Pear juices lacking added AA; AA, Ascorbic acid.

**Table 4.** Vitamin C content of pear juices with different concentrations of added AA after storage at 37°C for 12 and 24 h (Unit: mg/100 g)

AA concentrations (%)	Storage time (h)		
	0	12	24
Control	4.76±0.00 <sup>Ad</sup>	3.50±0.10 <sup>Bd</sup>	1.30±0.08 <sup>Cd</sup>
0.16	82.16±0.05 <sup>Ac</sup>	61.35±0.15 <sup>Bc</sup>	30.25±0.10 <sup>Cc</sup>
0.20	102.16±0.10 <sup>Ab</sup>	78.25±0.13 <sup>Bb</sup>	53.68±0.20 <sup>Cb</sup>
0.24	121.16±0.05 <sup>Aa</sup>	90.68±0.13 <sup>Ba</sup>	61.35±0.20 <sup>Ca</sup>

<sup>A-C</sup>Means followed by different letters in each row are significantly different ( $p < 0.05$ ); <sup>a-d</sup>Means followed by different letters in each column are significantly different ( $p < 0.05$ ); Control, Pear juices lacking added AA; AA, Ascorbic acid.

retained more AA than orange juice under similar conditions (27).

The vitamin C content is an important aspect of nutrition that is related to flavor change and color change of pear juice. Moore *et al.* (28) reported a connection between loss of AA and browning of orange juice. In this study, polymerization of AA degradation products, such as dehydroascorbic acid and amino acids, pear juice can result in color changes after storage for 24 h, Hayashi *et al.* (29) reported that the yellow product due to reaction of dehydroascorbic acid with amino acids. And red pigment produced also observed by Kurata *et al.* (30). Thus, storage time can affect the vitamin C content and reduce the quality of pear juice.

**DPPH radical scavenging activities** DPPH radical scavenging activities of pear juices with different concentrations of added AA ranged from 66.67 to 87.27% (Table 5). Addition of AA improved the antioxidant effects of pear juice. However, the concentration of added AA did not significantly ( $p > 0.05$ ) affect the antioxidant effect of pear juice, compared with controls (Table 5). A DPPH radical scavenging activity of 86.33% for pear juice with 0.16% added AA without storage was higher than for control pear juice in this study. When the concentration of AA added to pear juices was higher than 0.16%, there was no significant ( $p > 0.05$ ) difference in the scavenging effect against the DPPH radical, compared with controls.

Similar observations were obtained for antioxidant activities of pear juices with 0.20 and 0.24% added AA, perhaps due to scavenging effects against the hydroxyl radical instead of a hydrogen-donating ability (31). The DPPH radical scavenging activity of pear juice with

**Table 5.** DPPH radical scavenging activity, reducing power, and nitrate scavenging activity of pear juices with different concentrations of added AA after storage at 37°C for 12 and 24 h

	AA concentrations (%)	Storage time (h)		
		0	12	24
DPPH radical scavenging activities (Unit: %)	Control	66.67±1.97 <sup>Ab</sup>	59.49±1.64 <sup>Bb</sup>	49.54±0.66 <sup>Cb</sup>
	0.16	86.33±1.32 <sup>Aa</sup>	84.87±1.32 <sup>Aa</sup>	81.17±2.62 <sup>Aa</sup>
	0.20	86.81±0.33 <sup>Aa</sup>	84.26±0.65 <sup>ABa</sup>	82.55±0.87 <sup>Ba</sup>
	0.24	87.27±0.33 <sup>Aa</sup>	85.65±1.97 <sup>ABa</sup>	82.70±0.55 <sup>Ba</sup>
Reducing power	Control	0.72±0.01 <sup>Ab</sup>	0.60±0.01 <sup>Bb</sup>	0.42±0.01 <sup>Cb</sup>
	0.16	2.11±0.07 <sup>Aa</sup>	2.06±0.08 <sup>Aa</sup>	1.85±0.04 <sup>Ba</sup>
	0.20	2.10±0.03 <sup>Aa</sup>	2.09±0.04 <sup>Aa</sup>	1.87±0.03 <sup>Ba</sup>
	0.24	2.14±0.02 <sup>Aa</sup>	2.11±0.04 <sup>Aa</sup>	1.87±0.05 <sup>Ba</sup>
Nitrate radical scavenging activities (Unit: %)	Control	11.00±1.00 <sup>Ab</sup>	9.35±0.64 <sup>Bb</sup>	5.80±0.57 <sup>Cb</sup>
	0.16	63.15±0.49 <sup>Aa</sup>	61.27±0.41 <sup>Ba</sup>	59.17±0.73 <sup>Ca</sup>
	0.20	63.85±0.92 <sup>Aa</sup>	61.84±1.34 <sup>ABa</sup>	59.60±0.42 <sup>Ba</sup>
	0.24	64.20±0.85 <sup>Aa</sup>	61.26±0.83 <sup>ABa</sup>	59.61±1.09 <sup>Ba</sup>

<sup>A-C</sup>Means followed by different letters in each row are significantly different ( $p < 0.05$ ); <sup>a-b</sup>Means followed by different letters in each column are significantly different ( $p < 0.05$ ); Control, Pear juices lacking added AA; AA, Ascorbic acid.

added AA did not significantly ( $p > 0.05$ ) change after 12 h of storage, compared with controls. However, after storage for 12 and 24 h, the DPPH radical scavenging activity of control pear juice was significantly ( $p < 0.05$ ) decreased, compared with time=0 (Table 5). Piga *et al.* (32) reported a slight decrease in the Trolox equivalent antioxidant activity obtained using DPPH for orange juice after storage at 4°C for 15 days. Therefore, AA addition inhibited a decrease in the DPPH radical scavenging activity of pear juice during storage.

**Reducing power** Reducing power values of control, 0.16, 0.20, and 0.24% added AA juices increased when the concentration of AA added to pear juice was increased (Table 5). The reducing power of pear juices with 0.16% added AA was higher than for control juice. However, no significant ( $p > 0.05$ ) differences in reducing powers were observed among pear juices with 0.16, 0.20, and 0.24% added AA. Reducing power of pear juices also decreased after storage for 24 h. The reducing power of control juice and juices with 0.16, 0.20, and 0.24% added AA were reduced by 41.6, 12.4, 11.0, and 12.7%, respectively (Table 5). In agreement with results reported herein, Klimczak *et al.* (33) reported that the reducing power of orange juice decreased after storage at 38°C. The decrease in the vitamin C content after storage followed the same trend as the reducing power, suggesting that addition of AA and the storage time both affected the reducing power of pear juice.

**Nitrate radical scavenging activity** The nitrate radical scavenging activities of pear juices with different concentrations of added AA after storage for 12 and 24 h at 37°C are shown in Table 4. Nitrate scavenging activities of pear juices with added AA were higher than for control pear juice. The nitrate radical scavenging activities of pear juices with 0.16, 0.20, and 0.24% added AA were 63.15, 63.85, and 64.20%, respectively. The concentration of AA added to pear juices did not significantly ( $p > 0.05$ ) affect the nitrate radical scavenging

activity, compared with controls. Yoo (34) demonstrated that the nitrate content of vegetables treated with 0.01% AA was lower than nitrate contents of non-treated vegetables, with decreases of 5.34–31.55%. The nitrate scavenging activities of control juice were decreased after storage for 12 and 24 h. Similarly, the nitrate scavenging activities of pear juices with 0.16, 0.20, and 0.24% added AA were decreased by 59.17, 59.60, and 59.61%, respectively, after storage for 24 h. Thus, the vitamin C content and the nitrate scavenging activity of pear juice with added AA decreased after storage, probably due to AA degradation.

In summary, addition of 0.20% AA to pear juice can be used to prevent enzymatic browning. In addition, AA can improve the antioxidant activities of pear juice. Therefore, treatment with AA can improve the quality and extend the shelf life of “Sinhwa” pear juice.

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