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Analyses of Arbutin and Chlorogenic Acid, the Major Phenolic Constituents in Oriental Pear

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The HPLC retention time, photodiode array UV spectrum analysis, and LC/MS results indicated that arbutin and chlorogenic acid are the main phenolic constituents in Oriental pear. The two compounds exist in different organs of the Yali pear, which is one of the major cultivars of *Pyrus bretschnrideri*. The contents of arbutin in the leaf bud, floral bud, flower, and young fruit were 11.9, 12.4, 8.29, and 9.92 mg/g fresh weight (FW), respectively. Chlorogenic acid amounts in the same organs were 2.26, 3.22, 5.32, and 3.72 mg/g FW, respectively. During development, the concentration of the two compounds in Yali pears was the greatest in young fruit (9.92 mg/g FW of arbutin and 3.72 mg/g FW of chlorogenic acid), and then declined swiftly with fruit growth to less than 0.400 and 0.226 mg/g FW, respectively, in mature fruit. Large differences existed in the distribution of the two compounds in parts of the mature fruit of 14 Oriental pear cultivars. The greatest concentration of arbutin was found in the peel (1.20 mg/g FW), which was 3−5 times greater than that found in the core and 10−45 times greater than the level in the pulp. The concentration of chlorogenic acid in the core was greater than that in the peel. The compounds in 17 cultivars of Oriental pear, including *P. bretschnrideri, Pyrus pyrifolia*, *Pyrus ussuriensis*, and *Pyrus sinkiangensis*, were compared with those in 5 cultivars of Occidental pear (*Pyrus communis*). The mean concentration of arbutin in the Oriental pear cultivars was 0.164 mg/g FW, greater than the 0.083 mg/g FW found in the Occidental pear cultivars. The greatest arbutin content was 0.400 mg/g FW, found in the Yali pear. However, the mean concentration of chlorogenic acid in the Oriental pear was 0.163 mg/g FW, less than that found in the Occidental pear (0.309 mg/g FW).

KEYWORDS: Pyrus; Oriental pear; Yali pear; arbutin; chlorogenic acid; HPLC

INTRODUCTION

Pyrus species are thought to originate in the western mountainous area of China, and include the Oriental pear and Occidental pear, which developed separately due to geography. Oriental pears are distributed mainly around Eastern Asia, including China, the Korean peninsula, and Japan; major cultivated species include *Pyrus bretschnrideri* Reh., *Pyrus pyrifolia* Nakai, *Pyrus ussuriensis* Maxim., and *Pyrus sinkiangensis* Yu. In China, the pear has been considered not only a fruit but also an herbal medicine with antitussive, antiinflammatory, and diuretic effects, used for 1700 years. According to a well-known traditional Chinese medicine book (*1*) (Shi-zhen Li, 1518−1593), the flower and fruit of the pear also could eliminate black speckle on the face. Chemical and HPLC studies have revealed that the fruit of the Occidental pear contains phenolic compounds (*2*−*5*) such as chlorogenic acid (**Figure 1**), rutin, procyanidins, and arbutin (4-hydroxyphenyl-β-D-glucopyranoside, **Figure 1**). These phenolic compounds were investigated for their activity as antioxidants (*6*−*8*) or as coloring factors in the fruit and their products (*8*, *9*). Chlorogenic acid was the most important antioxidant-active constituent in pears (*7*). As a potential chemopreventive agent, it might promote the prevention of chronic diseases, such as cancer and cardiovascular disease (*10*−*12*). Recent studies have reported that chlorogenic acid possesses physiological activity, including anticancer activity (*13*−*16*), immune system enhancement (*17*, *18*), an ability to reduce the toxic effects of chemotherapy drugs (*19*, *20*), an influence on the sleep−wake cycle (*21*), and antioxidant capacity (*13*, *22*, *23*). Arbutin, another important phenolic compound in pear fruit (*5*, *24*), was initially identified as an antibiotic substance in fire blight resistance (*25*) and later as a specific marker of pear products for the evaluation of product authenticity (*26*). It has attracted attention for its antitussive and antibacterial effects (*27*). Extracts from the leaves of *Arctostaphylos uva-ursi*, containing arbutin as the major

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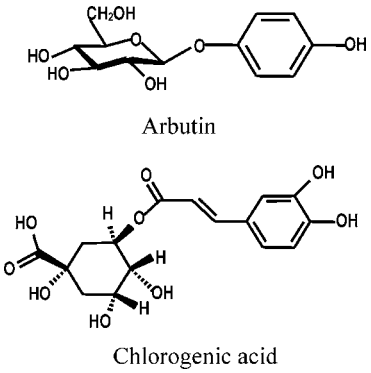

Figure 1. Structures of arbutin and chlorogenic acid.

active constituent, are used to treat infections of the urogenital tract (*28*). Furthermore, arbutin inhibits biosynthesis of the human pigment melanin (*29*, *30*) and is used as a lightener in cosmetics. Although some reports have indicated that extracts of pear flower (*31*) and young branches (*32*) contain arbutin as a natural antimicrobial substance, the existence and distribution of arbutin and other active phenolic constituents in Oriental pear have not been investigated in detail. Our previous study reported that a high concentration of arbutin and chlorogenic acid exists in the fruit of Oriental pear (*24*). The hydrolysis product of arbutin had potent tyrosinase inhibitory activity, while chlorogenic acid possessed superoxide-scavenging activity (*33*). In the present study, the distributions and concentrations of arbutin and chlorogenic acid in Oriental pear were evaluated, including changes in content during fruit development and a comparison among species and cultivars.

MATERIALS AND METHODS

Plant Material. The Yali, a main cultivar of *P. bretschnrideri*, was chosen for the examination of the arbutin and chlorogenic acid contents in different organs (leaf bud, floral bud, flower, and young fruit) during different growth periods. Five-year-old trees were randomly selected from the Experimental Farm of Hebei Agriculture University. Samples of leaf buds, flower buds, flowers, and young fruit were collected on April 1, April 2, April 12, and May 2 of 2003, respectively. To analyze variations in the content of phenolic constituents during different growth periods, more than 8 fruits from each of 5 trees were collected from 20 days after florescence until maturity, at 20 day intervals. For analysis of the distribution of the two compounds in different parts of the pear fruit, 14 cultivars of 4 primary cultivated species of Oriental pear (shown in **Table 1**) were sampled. To compare their contents in whole fruit of different species and cultivars, 17 cultivars of 4 species of Oriental pear and 5 cultivars of Occidental pear (*Pyrus communis*) (shown in **Table 2**) were sampled. The cultivars Yali, Xuehua, Daguo, Zaohuangjin, Huangjin, Huangguan, Dangshansu, Xueli, Chili, Xinxue, Jingbaili, Harrow Delight, and Green Bartlett were grown in the Heber province of China and provided by the Chinese Research Centre of Pears (China), cultivars Kuerlexiangli and Mantianhong were obtained from a fruit mart in Baoding of the Hebei province (China), and cultivars Housui, Kousui, Nansui, Gold 20 seiki, Max-Red Bartlett, Aurora, and Bartlett were provided by the Experimental Farm of the Agriculture Graduate School of Shinshu University (Japan).

Sample Preparation. Flowers, buds, and young fruits were cut into pieces; whole fruit samples at different developmental periods were mashed. Mature fruits also were divided into three parts: the peel, pulp, and core. The peel was removed at a thickness of 1 mm with a peeler and was cut into round pieces 8 mm in diameter. The pulp and core were mashed with an equal amount (1:1, w/w) of 95% ethanol (precooled to −18 °C) containing 0.4% phosphoric acid. Each 2 g sample was ground into a cream with 2 mL of 95% ethanol (precooled to −18 °C) and 50 μL of phosphoric acid in a cold (0 °C) mortar. The suspension was transferred to a 25 mL volumetric flask using 95% ethanol and brought to volume after being processed in an ultrasonic homogenizer for 5 min. The solution was centrifuged at 1340*g* for 5 min, and 1 mL of supernatant was evaporated to dryness under vacuum at 40 °C. The residue was dissolved in 1 mL of water and filtered through a 0.45 μm membrane before HPLC analysis.

Chemicals. Arbutin, epicatechin, chlorogenic acid (3′-caffeoylquinic acid), rutin, and quercetin were purchased from Sigma Chemical Co., St. Louis, MO; *p*-coumaric acid and ferulic acid were purchased from Wako Pure Chemical Industries, Japan. Procyanidin B2 was purchased from Funakoshi Co., Japan. The methanol and water used for the HPLC mobile phase were HPLC grade, and those used for other purposes were analytical grade.

HPLC/PDA Analysis. The chromatography instrument used was a Shimazu LC-10A_{VP} equipped with two LC-6AD solvent delivery units, a CTO-10A_{VP} column oven, an SPD-M10A_{VP} UV−vis photodiode array detector, a DGU-14 degasser, and an SCL-10A_{VP} system controller. Data were processed using a Shimazu Multi-PDA Class VP 5.3 HPLC data system on a Fujitsu computer. Chromatographic analysis was performed on a COSMOSIL 5C_{18}-MS-II column (150 × 4.6 mm, 4.4 μm) at 30 °C. Formic acid at a concentration of 0.04% (v/v) was employed as mobile phase A and methanol as mobile phase B. The gradient procedure was 0−3 min, 2% B; 3−4 min, 2−25% B; 4−11 min, 25% B; 11−16 min, 25−50% B; 16−26 min, 50−80% B; 26−33 min, 80% B; 33−35 min, 80−2% B; 35−40%, 2% B; followed by 10 min of reequilibration of the column before the next run. The flow rate was 0.8 mL/min, detection $\lambda = 280$ nm, and the scanning range was 200−400 nm. Concentrations of standard solutions were as follows: arbutin, 120 μg/mL; coumaric acid and ferulic acid, 20 μg/mL; all others, 80 μg/mL. Injection volumes were 5 μL for standards and 8 μL for test samples. The results were expressed as milligrams of arbutin or chlorogenic acid per gram fresh weight (FW).

HPLC/MS Analysis. HPLC/MS analyses were performed using an Agilent 1100 Series LC/MSD trap system (Agilent Technologies, Palo Alto, CA). The settings of HPLC conditions were the same as described for HPLC/PDA analyses. Mass spectra were acquired in ESI mode using nitrogen gas at a temperature of 350 °C, flow rate of 10 L/min, nebulizer pressure of 50 psi, quadrupole temperature of 30 °C, and capillary voltage of 3500 V. The mass spectrometer was operated in positive mode at 80 eV, target $m/z = 300$, scanning range 50−800 m/z.

RESULTS AND DISCUSSION

Qualitative Analysis of the Main Phenolic Components by HPLC. Figure 2 shows characteristic chromatograms of phenolic standards and a Yali pear fruit sample. Previous studies (*4*, *7*, *32*, *34*) reported using a stronger elution intensity of the mobile phase, but this was unsuitable for separation of arbutin in pear samples. Therefore, an isocratic mobile phase containing 2% methanol was employed for the initial 3 min. Then, the methanol content was increased to 25%, which was suitable for separation of hydroxycinnamates such as chlorogenic acid. Methanol at 25−50% separated the flavonoids and was followed by washing for 7 min with 80% methanol to cleanse the column for the next injection. Eight phenolic compounds were used as standards for this experiment, including arbutin (a strong polar compound), procyanidins and hydroxycinnamates (medium polarity), and flavonoids (weak polarity). Under these conditions, all of the major phenolic components in pears could be measured. The two largest peaks in the pear sample chromatograms were identified as arbutin and chlorogenic acid on the basis of retention times and UV spectra (right of **Figure 2**). In the HPLC/MS spectra, the peak corresponding to arbutin possessed an $m/z = 291$, consistent with the $[M + H_2O + H]^+$ of arbutin. Chlorogenic acid produced MS peaks at $m/z = 355$ and 163, which corresponded to the $[M + H]^+$ of chlorogenic acid and its caffeoyl moiety [caffeic acid $- H_2O + H]^+$. Thus, the mass spectra also supported arbutin and chlorogenic acid as the two primary phenolic compounds in Yali pear fruit. In a previous report (*2*), chlorogenic acid was isolated from

Table 1. Distribution of Arbutin and Chlorogenic Acid in the Fruit of Different Cultivars of Oriental Pear (mg/g FW ± SD)

	arbutin			chlorogenic acid		
species and cultivar	peel	core	pulp	peel	core	pulp
P. bretschnrideri						
Yali	1.189 ± 0.007	0.296 ± 0.036	0.029 ± 0.004	0.383 ± 0.017	0.446 ± 0.046	0.063 ± 0.005
Chili	2.520 ± 0.026	0.783 ± 0.031	0.026 ± 0.005	0.494 ± 0.034	1.009 ± 0.006	0.113 ± 0.004
Huangguan	0.647 ± 0.048	0.319 ± 0.003	0.062 ± 0.007	0.880 ± 0.033	0.690 ± 0.059	0.060 ± 0.008
Xuehua	0.610 ± 0.002	0.169 ± 0.009	0.007 ± 0.003	0.683 ± 0.041	0.861 ± 0.006	0.052 ± 0.003
Dangshansu	4.510 ± 0.009	nd[a]	0.110 ± 0.007	0.566 ± 0.023	0.750 ± 0.018	0.067 ± 0.007
Zaohuangjin	2.840 ± 0.006	0.478 ± 0.0 19	0.130 ± 0.011	0.131 ± 0.046	0.203 ± 0.068	0.036 ± 0.010
av	1.627	0.341	0.051	0.523	0.660	0.065
P. pyrifolia						
Huangjin	0.422 ± 0.040	0.031 ± 0.004	0.009 ± 0.005	0.073 ± 0.010	0.163 ± 0.018	0.004 ± 0.002
Xinxue	3.601 ± 0.061	nd[a]	0.025 ± 0.002	0.401 ± 0.011	1.992 ± 0.006	0.096 ± 0.018
Xueli	1.003 ± 0.060	0.928 ± 0.071	0.086 ± 0.024	0.522 ± 0.036	2.323 ± 0.009	0.024 ± 0.007
Daguo Shuijing	0.640 ± 0.058	0.247 ± 0.033	nd[a]	0.180 ± 0.028	2.730 ± 0.002	0.007 ± 0.001
Housui	0.159 ± 0.075	0.532 ± 0.024	0.012 ± 0.003	0.066 ± 0.001	0.350 ± 0.044	nd[a]
av	1.165	0.348	0.026	0.248	1.020	0.026
P. ussuriensis						
Jingbaili	nd[a]	nd[a]	0.131	nd[a]	nd[a]	0.278
P. sinkiangensis						
Kuerlexiangli	0.746 ± 0.016	0.143 ± 0.058	0.006 ± 0.002	0.066 ± 0.015	0.313 ± 0.012	0.025 ± 0.003
Mantianhong	0.490 ± 0.015	0.156 ± 0.039	0.086 ± 0.004	0.196 ± 0.027	0.278 ± 0.064	0.077 ± 0.019
av	0.618	0.150	0.046	0.131	0.296	0.051
av of 14 cultivars of Oriental pear	1.201	0.291	0.051	0.332	0.689	0.064

[a] Nd = not detected.

Table 2. Comparison of Arbutin and Chlorogenic Acid Contents in the Whole Fruit of Different Species and Cultivars of Oriental Pear (mg/g FW ± SD)

species and cultivar	arbutin	chlorogenic acid
Oriental Pear		
P. bretschnrideri		
Yali	0.400 ± 0.022	0.226 ± 0.011
Chili	0.288 ± 0.101	0.270 ± 0.022
Huangguan	0.046 ± 0.020	0.185 ± 0.024
Xuehua	0.106 ± 0.007	0.253 ± 0.012
Dangshansu	0.184 ± 0.046	0.225 ± 0.031
Zaohuangjin	0.117 ± 0.021	0.083 ± 0.004
av	0.190	0.207
P. pyrifolia		
Huangjin	0.067 ± 0.022	0.011 ± 0.006
Xinxue	0.110 ± 0.022	0.365 ± 0.008
Xueli	0.204 ± 0.019	0.431 ± 0.021
Daguo Shuijing	0.059 ± 0.006	0.013 ± 0.010
Housui	0.182 ± 0.011	0.162 ± 0.018
Kousui	0.216 ± 0.015	0.039 ± 0.006
Nansui	0.216 ± 0.035	0.006 ± 0.002
Gold 20 seiki	0.128 ± 0.019	0.080 ± 0.011
av	0.148	0.120
P. ussuriensis		
Jingbaili	0.102 ± 0.017	0.213 ± 0.027
P. sinkiangensis		
Kuerlexiangli	0.233 ± 0.023	0.111 ± 0.009
Mantianhong	0.105 ± 0.011	0.093 ± 0.006
av	0.169	0.102
av of Oriental pear	0.164	0.163
Occidental Pear		
P. communis		
Harrow Delight	0.119 ± 0.006	0.590 ± 0.043
Max-Red	0.077 ± 0.012	0.146 ± 0.025
Bartlett	0.057 ± 0.014	0.127 ± 0.015
Aurora	0.059 ± 0.007	0.193 ± 0.012
Green Bartlett	0.101 ± 0.050	0.487 ± 0.011
av of Occidental pear	0.083	0.309

P. communis and identified as 5′-caffeoylquinic acid by FAB-MS and chromatographic methods. In addition, flavonoids and procyanidins have been reported in Occidental pears (*2*−*5*, *8*). However, UV spectra of several unidentified small peaks such as peaks 9, 10, 11, and 12, shown in **Figure 2**, indicated that they were not procyanidins or the flavonoids.

Content of Arbutin and Chlorogenic Acid in Young Organs. Samples of floral buds, leaf buds, flowers, and young fruit were analyzed by HPLC according to the procedure described previously; the results are shown in **Figure 3**. Arbutin contents were high (approximately 1%) in young organs before and after florescence. Its concentration was highest in floral buds (12.4 mg/g FW), while the content in flowers was 8.29 mg/g FW. These results agree well with those reported in previous studies (*31*, *32*). In young fruits, the arbutin content was 9.92 mg/g FW, much higher than that found in ripe fruit (see later). The chlorogenic acid content was lower than the arbutin content in all organs, especially buds. Phenols are important protective agents found in plants (*35*). A high phenolic content in living tissues and young organs may prevent chemical damage and invasion by damaging organisms.

Changes in Arbutin and Chlorogenic Acid Levels during Fruit Development. Yali pear fruits were picked during development and weighed individually. Analytical results of the phenolic compounds are shown in **Figure 4**. During development, the fruit weight increased in a typical "S"-shaped curve. The arbutin and chlorogenic acid contents decreased continuously during this period. The arbutin level was highest in young fruit (9.92 mg/g FW); it rapidly decreased to approximately 2 mg/g FW in the 40 days after florescence. At maturity, the arbutin level was 0.400 mg/g FW. The chlorogenic acid content followed a trend similar to that of arbutin, and decreased from 3.72 mg/g FW in young fruit to 0.226 mg/g FW in ripe fruit. Variations in arbutin and chlorogenic acid levels were inversely correlated with the growth curve, reflecting the trend of phenolic constituents. This pattern agreed with a previous report concerning phenolic acids in European pear, but was different from those reported in apples, which peaked 30 days after florescence (*36*). The accumulation of arbutin in the fruit of Yali pears was greatest (89.0 mg per fruit) 140 days after florescence, while chlorogenic acid accumulation was greatest (72.2 mg per fruit) at 100 days. In addition, the content of arbutin in Yali pear fruit was approximately 2 times higher than that of chlorogenic

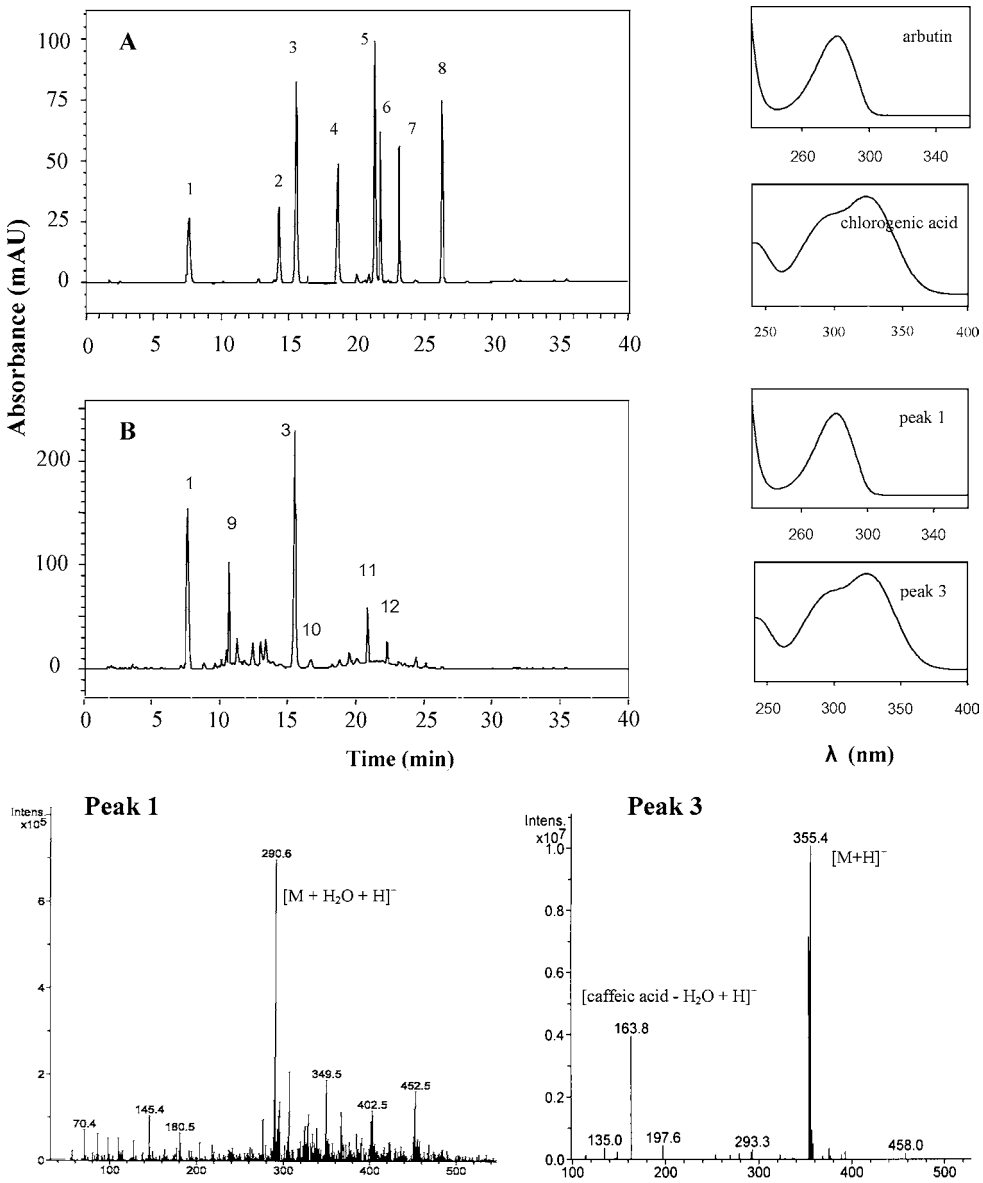

Figure 2. HPLC profiles and LC/MS spectra: (**A**) standards, (**B**) pear sample (*P. bretschnirideri*, cultivar Yali), (1) arbutin, (2) procyanidin B2, (3) chlorogenic acid, (4) epicatechin, (5) *p*-coumaric acid, (6) ferulic acid, (7) rutin, (8) quercetin, (9) arbutin derivative, (10) chlorogenic acid isomer, (11, 12) unknown hydroxycinnamic acids.

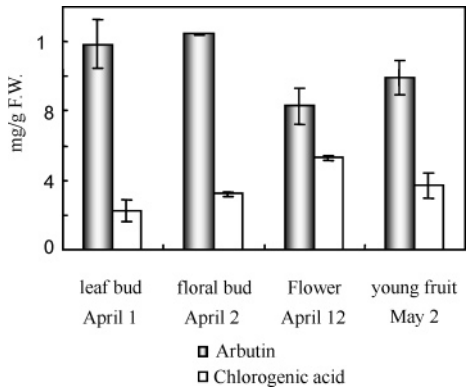

Figure 3. Contents of arbutin and chlorogenic acid in different organs of Yali pear (mg/g FW ± SD, $n = 5$).

acid during the entire development period. The fruit is the reproductive organ of plants. Before seed maturity, a high concentration of phenolic components is of value in defending against damaging organisms. Once the seed reaches maturity, the accumulation of phenols is no longer necessary.

Distribution of Arbutin and Chlorogenic Acid in Pear Fruit. The contents of arbutin and chlorogenic acid in different parts of the pear fruit of 14 cultivars of Oriental pear were determined and are shown in **Table 1**. In *P. bretschnrideri*, *P. pyrifolia*, and *P. sinkiangensis*, the total concentrations of the two compounds were highest in the peel, followed by the core and pulp. This agrees with research (*7*, *37*) reporting concentrations of polyphenols in the peels of apple and European pear that were much greater than in the pulp. However, in different parts of the plants, the concentration trend of the two compounds showed a few differences. Generally, arbutin was highest in the peel (1.20 mg/g FW), approximately 3−5 times greater than in the core and 10−45 times greater than in the pulp. The concentration of chlorogenic acid in the core (0.689 mg/g FW) was greater than that in the peel (0.332 mg/g FW). The ratio of arbutin to chlorogenic acid ranged from 3:1 to 5:1 in the peel, was nearly 1:1 in the pulp, and ranged from 1:2 to 1:3 in the core. This indicates that the primary phenolic in the peel was

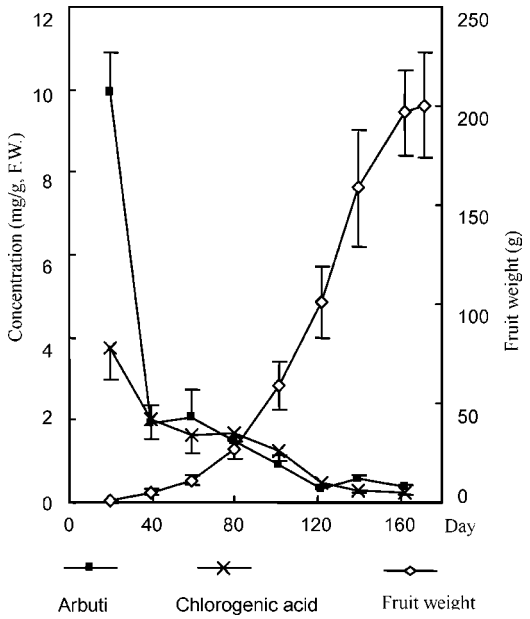

Figure 4. Changes in the contents of arbutin and chlorogenic acid in the fruit of Yali pears during development (mg/g FW ± SD, $n = 5$ for concentration, $n = 10$ for fruit weight).

arbutin; however, in the core, chlorogenic acid was more abundant. The presence of abundant arbutin in the peel may be cytotoxic to bacteria and thus prevent invasion. It is reasonable that chlorogenic acid, which possesses a strong antioxidant capacity and is involved in the cell wall of seeds, is plentiful in the core. Some cultivers, such as Zaohuangjin, Hosui, and Huangguan, did not quite conform to these results. In Jingbaili pear (*P. ussuriensis*), an unusual distribution of arbutin and chlorogenic acid was found, with concentrations of 0.131 and 0.278 mg/g FW, respectively, in the pulp. No detectable amounts of the compounds were found in the core or peel.

Comparison of Arbutin and Chlorogenic Acid Contents among Species and Cultivars. Seventeen cultivars of four species of Oriental pear and five cultivars of Occidental pear were analyzed, and the contents of the two compounds in mature whole fruit are shown in **Table 2**. The analysis revealed obvious differences in the content of the two phenolic constituents among different species and cultivars. The five pear species contained arbutin concentrations in the following decreasing order: *P. bretschnrideri* > *P. sinkiangensis* > *P. pyrifolia* > *P. ussuriensis* > *P. communis*. The average concentration of arbutin in Oriental pear was 0.164 mg/g FW, an amount higher than that found in Occidental pear (0.083 mg/g FW). Arbutin levels in Yali and Chili pears reached 0.400 and 0.288 mg/g FW, respectively. The content of chlorogenic acid in Oriental pear was 0.163 mg/g FW, lower than the level found in Occidental pear (0.309 mg/g FW). Their concentration order was *P. communis* > *P. sinkiangensis* >*P. bretschnrideri* > *P. pyrifolia* > *P. ussuriensis*.

Phenolic compounds as secondary metabolites are widely distributed in plants. Phenolics found in fruits and vegetables are considered important sources of dietary phenols (*10*), and are reported to function as antioxidants (*35*), antibacterials (*38*), antivirals (*39*), and cardiovascular disease and cancer preventive agents (*10*). Chlorogenic acid is a typical example of a functional nonnutrient food factor (*11*, *12*, *35*), and is the primary antioxidant in mature pears (*7*). Arbutin, a whitening substance with tyrosinase inhibitory activity, was present in a wide array of *Pyrus* species, except for Jingbaili pear (*P. ussuriensis*), and was concentrated in young organs such as the floral bud, leaf bud, and young fruits in Oriental pears. The high level of arbutin in the floral bud is a reason it has been utilized in Chinese medicine for its whitening effect. We expect development of additional uses for *Pyrus* as a good source of arbutin and chlorogenic acid.

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