JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Phenolic Compounds and Chromatographic Profiles of Pear Skins (*Pyrus* spp.)

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A standardized profiling method based on liquid chromatography with diode array and electrospray ionization/mass spectrometric detection (LC-DAD-ESI/MS) was used to analyze the phenolic compounds in the skins of 16 pears (*Pyrus* spp.). Thirty-four flavonoids and 19 hydroxycinnamates were identified. The main phenolic compounds (based on peak area) in all of the pear skins were arbutin and chlorogenic acid. The remaining phenolics varied widely in area and allowed the pears to be divided into four groups. Group 1, composed of four Asian pears (Asian, Asian brown, Korean, and Korean Shinko), contained only trace quantities of the remaining phenolics. Yali pear (group 2) contained significant amounts of dicaffeoylquinic acids. Fragrant pear (group 3) contained significant quantities of quercetin glycosides and lesser quantities of isorhamnetin glycosides and the glycosides of luteolin, apigenin, and chrysoeriol. The remaining 10 pears (group 4) (Bartlett, Beurre, Bosc, Comice, D'Anjou, Forelle, Peckham, Red, Red D'Anjou, and Seckel) contained significant quantities of isorhamnetin glycosides and their malonates and lesser quantities of quercetin glycosides. Red D'Anjou, D'Anjou, and Seckel pears also contained cyanidin 3-*O*-glucoside. Thirty-two phenolic compounds are reported in pear skins for the first time.

KEYWORDS: Pear skins; *Pyrus* spp.; varieties and cultivars; flavonoids; hydroxycinnamtes; polyphenol profile; LC-DAD-ESI/MS

INTRODUCTION

Pears, the fruits of pear trees (*Pyrus communis* L. and other spp., their varieties, and cultivars) (Rosaceae), are one of the common, highly consumed fruits in the world. Ten varieties of pears were found in local food markets in Maryland. Forelle, Peckingham's (Peckham), Comice, Bosc, d'Anjou, red d'Anjou, Bartlett, Beurre, Seckel, and red pears belong to varieties of *P. communis* L. (*1, 2*). Yali or Chinese Yali pears belong to *Pyrus bretschneideri* Reh., while fragrant pear or Kuerle fragrant pear (Chinese name, Xiejiang Xiali, grows in Xiejiang, China) are from *Pyrus serotina* Reld (*3, 4*). Asian or oriental pears, such as Asian pears, Asian brown pears, Korean pears, and Korea Shinko pears, are also from the trees of *Pyrus* spp., such as *Pyrus pyrifolia* Nakai., *Pyrus ussuriensis* Maxim., *Pyrus sinkiangensis* Yu, or others, their varieties, and cultivars (*4, 5*).

Previous studies reported the existence of arbutin, chlorogenic acid, *p*-coumaroylquinic acid, *p*-coumaroylmalic acid, dicaffeoylquinic acids, vanillic acid derivatives, catechin, epicatechin, proanthocyanidins, cyanidin 3-*O*-galactoside, the 3-*O*-glycosides of quercetin, isorhamnetin, and kaempferol, and some nonglycosylated flavones and flavonols in pear skins, pear flowers, and other parts of pear trees (**Figure 1**) (*1*−*6, 8*−*16*). The pear skins have much higher and more varied phenolic contents than the flesh of the fruit. Studies using liquid chromatography (LC) or liquid chromatography with mass spectrometric detection (LC-MS) have also reported such polyphenols in pear materials (*1, 2, 9, 10, 12, 16*). However, there have not been any comprehensive studies on the phenolic compounds of the skins of most pears found in the U.S. marketplace.

The flavonoids and other phenolic compounds of plant-derived foods have been reported to offer health-promoting benefits to humans, including reduced risk of cancer and cardiovascular disease (*1, 6, 7*). As part of a project to systematically identify glycosylated flavonoids and other phenolic compounds in food plants, we analyzed the skin extracts of 16 different commercial pears using a standardized profiling method (*17*) based on liquid chromatography with diode array and electrospray ionization mass spectrometric detection (LC-DAD-ESI/MS). We present chromatographic profiles and identify phenolic compounds.

MATERIALS AND METHODS

Standards and Chemicals. Quercetin dihydrate (minimum 98%), rutin (quercetin 3-*O*-rutinoside) trihydrate (minimum 95%), kaempferol (minimum 90%), apigenin (minimum 95%), arbutin (>98%), chlorogenic acid, kaempferol, (+)-catechin, and (−)-epicatechin were purchased from Sigma Chemical Co. (St. Louis, MO). Kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside, quercetin 3-*O*-glucoside, quercetin

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10.1021/jf8013487
Published on Web 09/09/2008

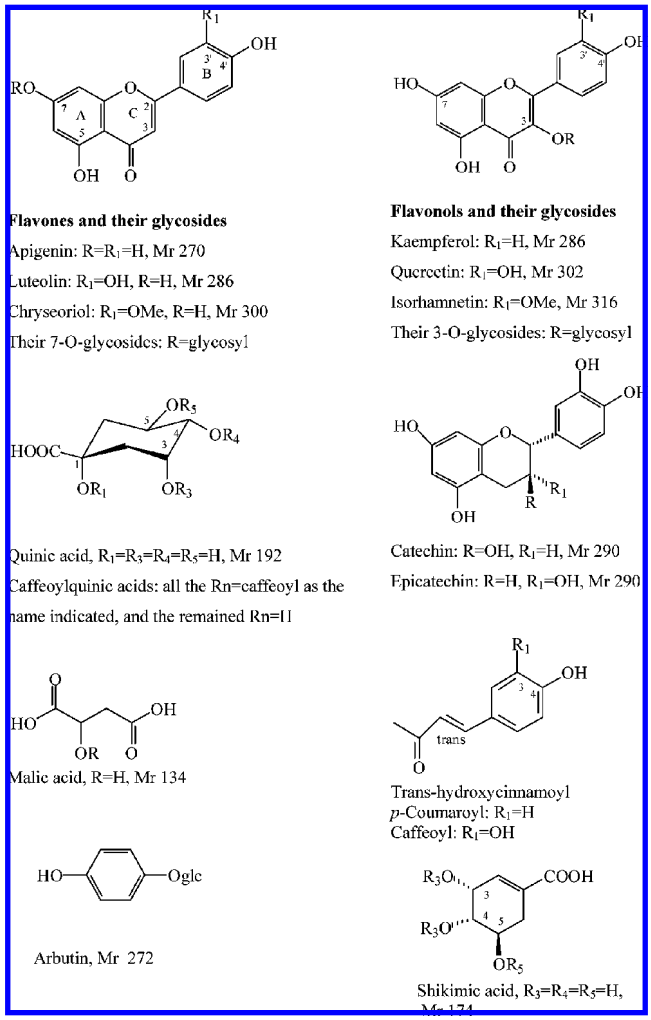

Figure 1. Structures of the phenolic compounds of pears.

3-*O*-galactoside, quercetin 3-*O*-rhamnoside, isorhamnetin, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-rutinoside, luteolin 7-*O*-glucoside, luteolin 4′-*O*-glucoside, luteolin, chrysoeriol, peltatoside, and diosmetin [all high-performance liquid chromatography (HPLC) grade] were purchased from Extrasynthese (Genay, Cedex, France). Cyanidin chloride and cyanidin 3-*O*-glucoside chloride, peonidin chloride, and peonidin 3-*O*-glucoside chloride were from Indofine Chemical Co. (Somerville, NJ).

Five compounds, 3- and 4-caffeoylquinic acids and 3,5-, 3,4-, and 4,5-dicaffeoylquinic acids, were isolated in this laboratory (*17*). Formic acid, hydrochloric acid (37%) and HPLC solvents (acetonitrile, methanol) were purchased from VWR Scientific (Seattle, WA). HPLC grade water was prepared from distilled water using a Milli-Q system (Millipore Laboratory, Bedford, MA).

Plant Materials. Fresh Asian, Asian brown, Korean, Korean Shinko, fragrant, Yali, red, Packham, Beurre, Forelle, Bartlett, Seckel, Bosc, D'Anjou, red D'Anjou, and Comice pears were purchased from local food stores in Maryland. The skins of each pear were cut into small pieces and dried at room temperature in a fume hood. All of the dried materials were finely powdered and passed through a 20 mesh sieve prior to extraction.

Plant Extracts. Dried ground material (100 mg) was extracted with methanol−water (5.00 mL, 60:40, v/v) using an FS30 Ultrasonic sonicator (Fisher Scientific, Pittsburgh, PA) at 40 KHz and 100 W for 60 min at room temperature. The extract was filtered through a 0.45 μm Nylon Acrodisk 13 filter (Gelman, Ann Arbor, MI). A 50 μL sample of the extract was injected onto the analytical column for analysis.

Heat-Induced Hydrolysis of Samples. The filtered extract (1.00 mL, pH around 5.0−6.0) was heated in a covered glass tube at 85 °C for 16 h. The extract was allowed to cool by standing at room temperature for 30 min and then filtered and injected onto the analytical column (*17*).

Acid Hydrolyzed Samples. The filtered extract (0.50 mL) was mixed with concentrated HCl (37%, 0.10 mL) and heated in a covered tube at 85 °C for 2 h. Then, 0.40 mL of methanol was added to the mixture and the solution was sonicated for 10 min. The solution was refiltered prior to HPLC injection (*17*).

LC-DAD and ESI-MS Conditions. The LC-DAD-ESI/MS consisted of an Agilent 1100 HPLC coupled to a diode array detector and mass spectrometer (MSD, SL mode) (Agilent, Palo Alto, CA). A 250 mm × 4.6 mm i.d., 5 μm Symmetry C18 column with a 20 mm × 3.9 mm i.d., 5 μm Symmetry Sentry guard column (Waters Corp., Milford, MA) was used at flow rate of 1.0 mL/min. The column oven temperature was set at 25 °C. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient was varied linearly from 10 to 26% B (v/v) in 40 min, to 65% B at 70 min, and finally to 100% B at 71 min and held at 100% B to 75 min. The DAD was set at 350, 310, 270, and 520 nm to record the peak intensity, and UV/visible spectra were recorded from 190−650 nm for plant component identification. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization (PI and NI) modes at low and high fragmentation voltages (100 and 250 V) over the range of *m/z* 100−2000. A drying gas flow of 13 L/min, a drying gas temperature of 350 °C, a nebulizer pressure of 50 psi, and capillary voltages of 4000 V for PI and 3500 V for NI were used. The LC system was directly coupled to the MSD without stream splitting.

To detect less intense peaks of aglycones in the hydrolyzed pear extracts, positive and negative selective ion monitoring (PI/NI SIM) detection was employed at *m/z* 271/269 (for trihydroxyflavones), 287/285 (for tetrahydroxyflavone), 303/301 (for pentahydroxyflavone), and 317/315 (for tetrahydroxy-methoxyflavone) (*17*).

Principal Component Analysis (PCA). Areas were recorded for 57 peaks found in the pear skin chromatograms. Data for each peak area were transformed to a unit vector. The resulting data array (16 samples by 57 normalized peak areas) was then submitted to PCA using SCAN (Software for Chemometrics Analysis, Minitab, Inc., State College, PA). A score plot was generated for the first two principal components.

RESULTS AND DISCUSSIONS

Identification of Pear Flavonoids. Chromatograms of the extracts from the skins of fragrant, red D'Anjou, Yali, Asian, and Comice pears are shown on **Figures 2** and **3**. Chromatograms of the acid-hydrolyzed pear skin extracts are shown in **Figures 3** and **4** for fragrant, red, red D'Anjou, and Comice pears. A summary of the peaks detected for each type of pear skin are presented in **Table 1**. The structures of pear phenolics are shown in **Figure 1**, and the retention times (t_R), wavelength of maximum absorbance (λ_{max}), protonated/deprotonated molecules ($[M + H]^+/[M - H]^-$), and major fragment ions (including the PI/NI of the aglycones, $[A + H]^+/[A - H]^-$) are listed in **Table 2**. Compounds identified by comparison to standards and from previous reports are noted in **Table 2**.

On the basis of the fact that quercetin (peak Ag-3, $t_R = 47.47$ min, **Figure 4**) and isorhamnetin (peak Ag-7, $t_R = 54.03$ min) were the only pentahydroxy- and tetrahydroxy-monomethoxy-flavonols, respectively, detected in the hydrolyzed pear skin extracts, the compounds with PI/NI fragments at *m/z* 303/301 and 317/315 (aglycone ions) should be the glycosides of quercetin and isorhamnetin. Thus, the UV data (λ_{max} 256, 266sh, and 354 nm) and MS data ($[M + H]^+$ at *m/z* 743, $[M +$ H-pentosyl$]^+$ at *m/z* 611, $[M +$ H-pentosyl-rhamnosyl$]^+$ at *m/z* 465, and $[A + H]^+$ at *m/z* 303) of peak 13 (**Figure 2**) suggested this was a quercetin 3-*O*-pentosyl-rhamnosylhexoside (*17, 18*). This quercetin 3-*O*-triglycoside was further identified as quercetin 3-*O*-2″-xylosyl-6″-rhamnosylglucoside by direct com-

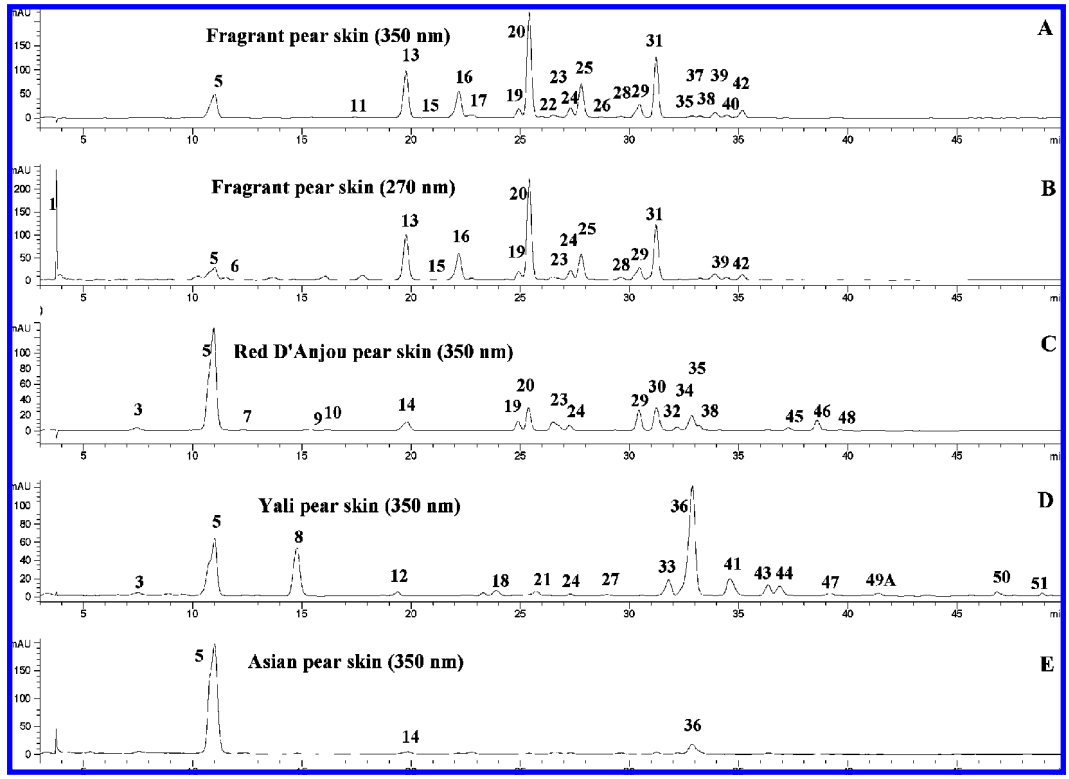

Figure 2. LC chromatograms of the skin extracts of (**A**) fragrant pear (350 nm), (**B**) fragrant pear (270 nm), (**C**) D'Anjou pear (350 nm), (**D**) Yali pear (350 nm), and (**E**) Asian brown pear (350 nm).

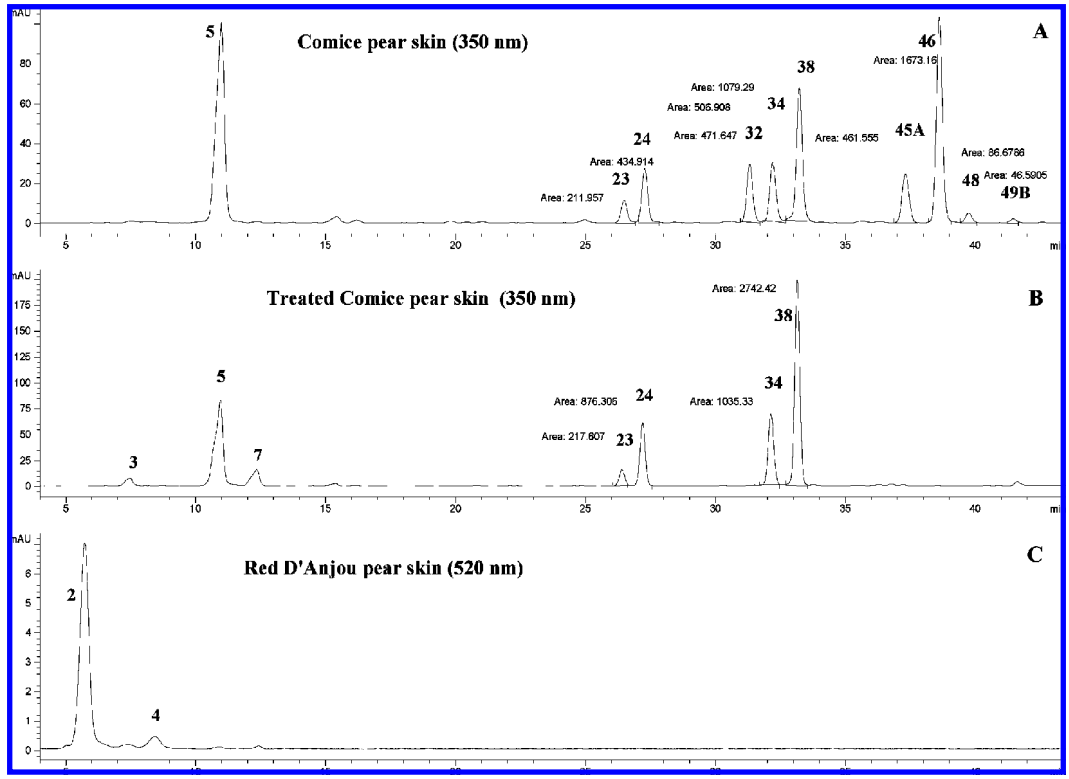

Figure 3. LC chromatograms of (**A**) extract of Comice pear (350 nm), (**B**) hydrolyzed extract of Comice pear (350 nm), and (**C**) and extract of red D'Anjou pear (520 nm).

parison with the positively identified quercetin 3-*O*-2″-xylosyl-6″-rhamnosylglucoside in green bean (*19*).

In the same way, peak 16 {t_R = 22.21 min, λ_{max} 254, 266sh, and 354nm, $[M + H]^+$/ $[M - H]^-$ at *m/z* 597/595, PI fragment at *m/z* 465 ($M + H$-pentosyl-hexosyl]$^+$) and $[A + H]^+$/$[A - H]^-$ at *m/z* 303/301} was identified as a quercetin 3-*O*-pentosylglucoside. It was determined, by direct comparison, that peak 16 was not quercetin 3-*O*-xylosylglucoside, which is found in pink beans (*20*). This suggests that peak 16 may be quercetin 3-*O*-arabinosylglucoside. Peaks 12, 15, 17, 19, 20, 23, 24, 32, and 47 were identified as the 3-*O*-glucosylgalactoside, 3-*O*-galactosylglucoside, 3-pentaside, 3-*O*-robinobioside [i.e., rhamnosyl-(1→6)-galactoside], 3-*O*-

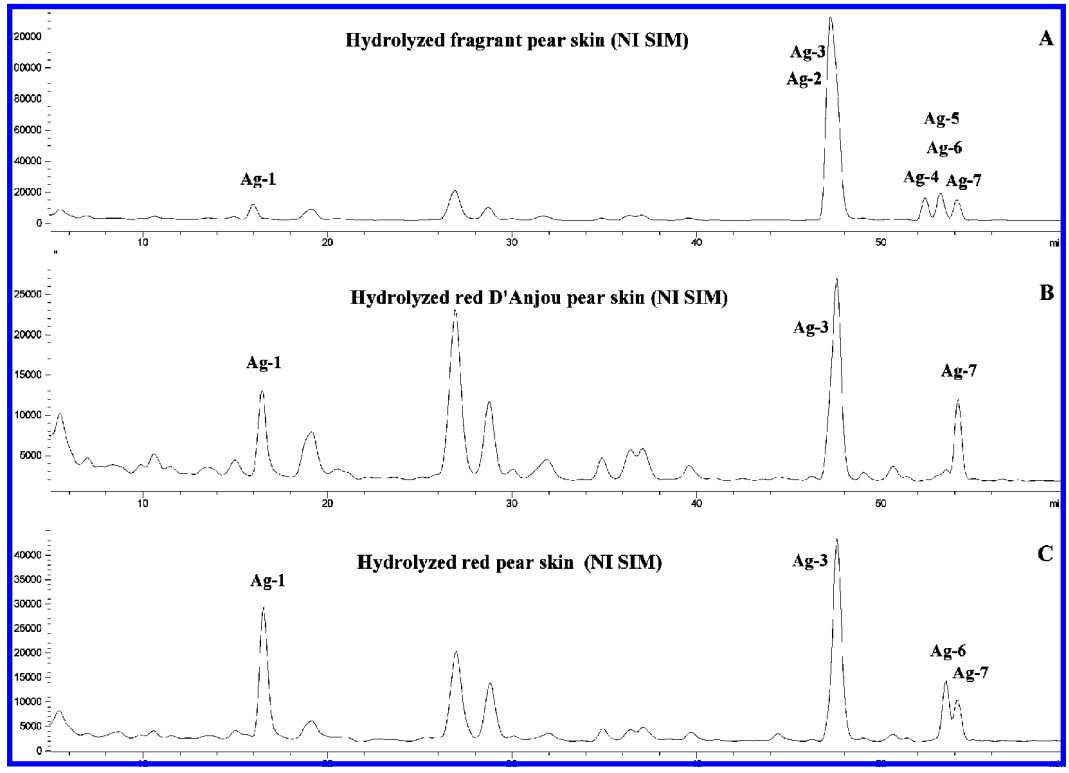

Figure 4. SIM MS chromatograms (negative ionization, selected ion monitoring) of the hydrolyzed skin extracts of (**A**) fragrant pear, (**B**) red D'Anjou pear, and (**C**) red pear. Peaks: Ag-1, cyanidin; Ag-2, luteolin; Ag-3, quercetin; Ag-4, apigenin; Ag-5, chrysoeriol; Ag-6, kaempferol; and Ag-7, isorhamnetin.

rutinoside, 3-*O*-galactoside, 3-*O*-glucoside, 3-*O*-6″-malonylglucoside, 3-*O*-6″-*p*-coumaroylglucoside, and a glycoside of quercetin (the positions for sugars were not determined), respectively. Some of these identifications were confirmed by direct comparison with standards (see notations in **Table 2**).

Peaks 30, 31, 34, 38, 45A, 46, and 48 have aglycone ions at *m*/*z* 317/315 and UV λ_{max} at 254, 266sh, and 354 nm, typical for 3-*O*-glucosides (*17*). The peaks were identified as isorhamnetin 3-*O*-glycosides. On the basis of their molecular ions (*7*), elution order, comparison with standards, and the reported isorhamnetin glycosides in pears (*1, 6*−*8*), these peaks were further identified as the 3-*O*-robinioside, 3-*O*-rutinoside, 3-*O*-galactoside, 3-*O*-glucoside, 3-*O*-6″-malonygalactoside, 3-*O*-6″-malonyglucoside, and 3-*O*-malonylglucoside of isorhamnetin, respectively.

The identification of the malonylated glycosides was made based on the peak enhancement of the parent glycosides after the malonyl groups were removed by heat-induced hydrolysis. For example, as shown in **Figure 3A**, Comice pears contained quercetin 3-*O*-galactoside (peak 23, peak area 212), quercetin 3-*O*-glucoside (peak 24, peak area 435), and one malonylhexoside (peak 32, peak area 472). After hydrolysis, the malonated compound (peak 32) disappeared, the intensity of peak 24 increased to 877 (roughly equal to the total area for peak 24 and 32), and the area of peak 23 did not change. Thus, peak 32 can be identified as quercetin 3-*O*-6″-malonylglucoside.

Peaks 45A and 48 were identified as isorhamnetin 3-*O*-6″-malonylgalactoside and isorhamnetin 3-*O*-malonylgalactoside based on their disappearance upon hydrolysis and the increase in the area of peak 34 from 507 to 1035, roughly equivalent to the sum of the intensities of peaks 45A (452) and 48 (87). Similarly, the increased intensity of peak 38 (isorhamnetin 3-*O*-glucoside) upon hydrolysis of the malonyl group by heating suggested that peaks 46 and 48 were isorhamnetin 3-*O*-6″-malonylglucoside and isorhamnetin 3-*O*-malonylglucoside, respectively. The identification of two isomeric quercetin 3-*O*-malonylgalactosides and quercetin 3-*O*-malonylglucosides was based on the fact that among coexisting isomers, the predominant isomer always has its malonyl at the 6″-postion of the sugar (*17, 21, 22*).

It is worth noting that the ions produced by negative ionization (at a high fragmentation energy) of the malonylated glycosides, such as quercettin 3-*O*-6″-malonylglucoside (**Figure 5A**) and isorhamnetin 3-*O*-6″-malonylglucoside (**Figure 5B**), always have a much stronger $[M - H - CO_2]^-$ (or $M - COOH]^-$) ion than the molecular ion $[M - H]^-$ since the malonyl function has a free COOH that easily loses a CO_2.

Kaempferol (peak Ag-6, t_R = 53.18 min, **Figure 4**) was detected in the hydrolyzed extracts of the pears from cultivars or varieties of *P. communis* L. (**Figure 3C**), indicating that kaempferol glycosides were present. In fragrant pears, both kaempferol (**Figure 4A**, peak Ag-6) and luteolin (peak Ag-2, t_R = 47.14 min) were detected in the hydrolyzed extract, indicating that the glycosides of these flavonoids would be prevalent. Because kampferol and luteolin are isomers, kaempferol 3-*O*-glycoside and luteolin 7-*O*-glycoside have the same mass and UV λ_{max} for band I around 346−348 nm. However, they have significant differences in the λ_{max} for UV band II. The different oxygenation of their B ring (3′- and 4′-hydroxyls for luteolin as opposed to the 4′-hydroxyl for kaempferol) gives luteolin and its 7-*O*-glycosides two peaks in the UV band II position while kaempferol and its 3-*O*-glycosides have only one peak as shown in **Figure 6** (*23*). Peaks 22, 25, 26, and 40 showed two peaks in UV band II and were easily identified as luteolin 7-*O*-rutinoside, luteolin 7-*O*-glucoside, luteolin 7-*O*-acetoylmelonylglucoside, and luteolin 4′-*O*-glucoside, respectively (**Table 1**). Similarly, peaks 29, 37, and 45B showed only one peak in their UV band II and were identified as kaempferol 3-*O*-rutinoside, kaempferol 3-*O*-glucoside, and kaempferol 3-*O*-6″-malonyglucoside, respectively. These identifications were

Table 1. Peak Areas for Pear Skins

peak no.	Fragrant	Asian	Asian brown	Korean	Korean Shinkon	Yali	Bartlett	Seckel	Bosc	D'Anjou	red	Comice	red D'Anjou	Peckham	Beurre	Forelle
1	1825	988	1013	1582	844	431	402	1119	744	887	398	779	676	495	290	332
2	0	0	0	0	0	0	0	177	0	240	52	30	241	0	0	0
3	39	20	35	0	0	20	70	85	134	40	44	58	70	63	73	20
4	0	0	0	0	0	0	0	36	0	16	0	0	22	0	0	0
5	2747	3371	5027	1058	960	1615	3031	10853	2336	6045	3123	5620	7713	1587	1286	1671
6	100	0	0	0	0	0	20	120	100	50	100	250	100	0	20	0
7	15	23	41	0	0	25	12	46	0	21	9	10	18	15	9	20
8	0	0	0	0	0	1127	0	0	0	0	36	0	0	0	9	17
9	61	0	0	0	0	0	0	0	0	0	0	0	110	0	0	0
10A	130	0	0	100	0	0	30	331	516	136	134	495	123	30	0	0
10B	30	0	0	0	0	0	0	100	0	64	70	0	60	0	0	0
11	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	91	0	0	0	0	0	0	0	0	0	0
13	4266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	228	100	0	694	323	9	727	215	0	27
15	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	2770	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	328	0	0	0	0	0	0	0	29	0	0	0	0	0	0	0
18	0	0	0	0	0	59	0	0	75	0	0	0	0	0	0	0
19	721	0	0	0	0	0	25	48	0	707	165	0	35	197	16	24
20	8523	105	0	57	0	0	153	74	0	2550	469	0	1171	662	35	127
21	0	0	0	45	0	169	0	0	0	0	0	0	0	0	0	0
22	112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
23	300	0	0	0	0	0	187	401	137	438	216	461	488	90	87	302
24	891	0	0	0	0	36	293	527	395	586	712	1028	262	220	154	971
25A	3112	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0
25B	0	0	0	0	0	0	44	0	0	10	38	0	20	0	0	0
26A	0	0	0	0	0	0	0	0	0	10	38	0	20	0	0	0
26B	84	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	56	0	0	0	0	0	0	0	0	0	0
28A	0	0	0	0	35	0	28	0	0	0	0	0	0	0	12	0
28B	141	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0
29	460	0	0	0	0	0	0	0	0	115	0	0	21	0	0	0
30	920	0	0	101	0	0	210	0	110	1405	110	0	1114	381	74	419
31	4876	0	0	101	0	0	473	820	120	3000	300	0	986	883	136	771
32	0	0	0	0	0	0	0	0	220	438	264	0	589	0	0	0
33	0	0	0	122	0	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	43	0	0	203	1443	440	1172	87	1208	202	200	372	867
36	57	0	276	0	0	2725	248	0	0	0	229	0	0	30	0	0
37	185	0	0	0	0	0	0	0	0	0	0	0	0	88	348	0
38	152	0	0	0	0	0	677	1258	612	656	266	2583	30	266	258	2959
39	513	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40A	255	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
40B	0	0	0	0	0	0	0	52	38	96	0	0	202	0	21	0
41	0	0	0	0	0	474	0	0	0	0	0	0	0	0	0	0
42	712	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	242	14	71	13	72	0	0	64	16	0	0
44	0	0	0	0	0	253	0	0	0	0	0	0	0	0	0	0
45A	0	0	0	0	0	0	0	473	73	278	0	1344	227	47	30	190
45B	0	0	0	0	0	0	0	0	0	0	159	0	0	10	0	0
46	0	0	0	0	0	0	456	1576	410	1775	207	4866	705	372	120	1352
47	0	0	0	0	0	97	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	28	98	0	99	14	177	58	39	0	65
49A	0	0	0	0	0	114	0	0	0	0	0	0	0	0	0	0
49B	0	0	0	0	0	0	0	0	0	0	0	0	0	39	0	0
50	0	0	0	0	0	90	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	39	0	0	0	0	0	0	0	0	0	0

also supported by previous reports of their presence in pears (*1, 8*−*10*).

Apigenin (**Figure 4**, peak Ag-4, t_R = 52.33 min) and chrysoeriol (peak Ag-5, t_R = 52.98 min) were also detected in the hydrolyzed extract of fragrant pear skins. On the basis of the UV and mass data and previous reports of glycosides of apigenin and chrysoeriol in pears (*8*), peaks 35, 39, 42, and 49B were identified as chrysoeriol 7-*O*-rutinoside, apigenin 7-*O*-glucoside, chrysoeriol 7-*O*-glucoside, and apigenin 7-*O*-malonlyglycoside, respectively.

Peak 41 (t_R = 34.61 min) of Yali pear skin chromatograms (**Figure 2D**) was identified as trihydroxymethoxyflavone sulfate based on its UV data (λ_{max} 266, 336 nm), mass data ($[M + H]^+/[M - H]^-$ at *m/z* 381/379 and $[A + H]^+/[A - H]^-$ at *m/z* 301/299), and the generation of a flavone aglycone with three OH functions and one OMe group (MW = 300) following hydrolysis of the extract. This compound has not been reported in pear previously.

Additional flavonoids detected in the pears were catechin (**Figure 2B**, peak 6) and epicatechin (**Figure 2C**, peak 10A). Cyanidin 3-*O*-galactoside (**Figure 3C**, peak 2) (*8*) and peonidin 3-*O*-galactoside (peak 4) were detected in red, red D'Anjou, Seckel, and D'Anjou pears.

Identification of Arbutin and Hydroxycinnamic Acid Conjugates of Pears. Arbutin and chlorogenic acid appeared as major phenolic compounds in all of the pears tested in this

Table 2. Peak Identification for Aqueous Methanol Extracts of Pear Skins (*Pyrus* spp.)[a]

peak no.	t_R (min)	$[M + H]^+/[M - H]^-$ (*m/z*)	$[A + H]^+/[A - H]^-$ other ion (*m/z*)	UV λ_{max} (nm)	identification
1	3.75	291/271	−/109	226, 284	arbutin[b,c]
2	5.73	449/447	287/285	280, 516	cyanidin 3-*O*-galactoside[b,c]
3	7.52	−/353	−/191, 179, 135	240, 300sh, 326	3-caffeoylquinic acid[c]
4	8.47	463/−	301/−	ND	peonidin 3-*O*-galactoside[c]
5	10.91	−/353	−/191, 179, 135	240, 300sh, 326	chlorogenic acid[b,c]
6	11.38	291/289		ND	catechin[b,c]
7	12.26	−/353	−/191, 179, 135	240, 300sh, 326	4-caffeoylquinic acid[c]
8	14.67		−/179	242, 300sh, 324	caffeic acid[b,c]
9	15.44	−/353	−/191, 179, 135	ND	*Z*-caffeoylquinic acid
10A	16.23	291/289		ND	epicatechin[b,c]
10B	16.43	−/337	−/191, 163	ND	*p*-coumaoylquinic acid[b]
11	17.48	−/335	−/179	ND	caffeoylshikimic acid
12	19.13	627/−	465, 303/−	254, 266sh, 354	quercetin 3-*O*-glucosylgalacoside
13	19.78	743/−	611, 465, 303/−	254, 266sh, 354	quercetin 3-*O*-2′′-xylosyl-6′′-rhamnosylglucoside
14	19.82	−/295		240, 300sh, 326	caffeoylmalic acid[b]
15	22.04	627/−	465, 303/−	254, 266sh, 354	quercetin 3-*O*-galactosylglucoside
16	22.21	597/595	465, 303/301	254, 266sh, 354	quercetin 3-*O*- arabinosylglucoside
17	22.83	435/433	303/−	ND	quercetin *O*-pentoside
18	23.22	611/609	287/285	ND	kaempferol *O*-dihexoside
19	24.97	611/609	465, 303/−	254, 266sh, 354	quercetin 3-*O*-rhanosylgalactoside
20	25.43	611/609	465, 303/301	254, 266sh, 354	quercetin 3-*O*-rutinoside[b,c]
21	25.76	−/677	−/515, 335, 269	240, 300sh, 326	ND
22	25.97	595/−	449, 287/−	ND	luteolin 7-*O*-rutinoside
23	26.55	465/463	303/301	254, 266sh, 354	quercetin 3-*O*-galactoside[b,c]
24	27.33	465/463	303/301	254, 266sh, 354	quercetin 3-*O*-glucoside[b,c]
25A	27.79	449/447	287/285	254, 266sh, 348	luteolin 7-*O*-glucoside[c]
25B	27.94	−/279	/163, 119	316	*p*-coumaroylmalic acid
26A	28.30	595/593	449, 287/285	254, 266sh, 348	kaempferol-3-*O*-rhanosylgalactoside
26B	28.66	577/575	287/285	254, 266sh, 348	luteolin 7-*O*-acetoylmalonylglucoside
27	28.87	523/521	ND	ND	ND
28A	29.67	−/309	−	314	feruloylmalic acid
28B	29.67	419/417	−	314	ND
29	30.05	595/593	287/285	266, 348	kaempferol 3-*O*-rutinoside[c]
30	30.48	647/645	479, 317/315	254, 266sh, 354	isorhamnetin 3-*O*-robinioside[b]
31	31.23	647/645	479,317/315	254, 266sh, 354	isorhamnetin 3-*O*-rutinoside[b,c]
32	31.40	551/549	303/505, 301	254, 266sh, 354	quercetin 3-*O*-6′′-malonylglucoside[b]
33	31.47	−/515	−/353, 191, 179, 135	240, 300sh, 326	3,4-dicaffeoylquinic acid
34	32.32	479/477	317/315	254, 266sh, 354	isorhamnetin 3-*O*-galactoside[b]
35	32.40	609/−	463, 301/−	ND	chrysoeriol 7-*O*-rutinoside[b]
36	32.56	−/515	−/353, 191, 179, 135	240, 300sh, 326	3,5-dicaffoylquinic acid[b]
37	32.88	449/447	287/285	266, 348	kaempferol 3-O-glucoside[c]
38	33.26	479/477	317/315	ND	isorhamnetin 3-*O*-glucoside[b,c]
39	33.96	433/−	271/−	270, 336	apigenin 7-*O*-glucoside[c]
40A	34.40	449/−	287/−	254, 268, 348	luteolin 4′-*O*-glucoside
40B	34.50	/515	/353, 191, 179, 135	ND	dicaffeoylquinic acid
41	34.61	381/379	301/299	266, 336	trihydroxymethoxyflavone sulfate
42	35.18	463/−	301/−	254, 268, 348	chrysoeriol 7-*O*-glucoside[b]
43	36.40	−/515	−/353, 191, 179, 163	240, 300sh, 326	4,5-dicaffeoylquinic acid
44	36.88	−/193	−/−	240, 300sh, 326	caffeic acid methyl ester
45A	37.28	565/563	317/519, 315	254, 266sh, 354	isorhamnetin 3-*O*-6′′-malonylgalactoside
45B	37.38	535/533	287/489, 285	266, 350	kaempferol 3-*O*-6′′-malonylglucoside
46	38.60	565/563	317/519, 315	254, 266sh, 354	isorhamnetin 3-*O*-6′′-malonylglucoside[b]
47	39.23	627/625	303/301	ND	quercetin glycoside
48	39.64	565/563	317/519, 315	254, 266sh, 354	isorhamnetin 3-*O*-malonylgalactoside
49A	41.41	−/677	−/515, 353, 191, 179	ND	1,3,5-tricaffeoylquinic acid
49B	41.48	651/649	271/605, 269	268, 336	apigenin 7-*O*-malonylglycoside
50	46.84	−/691	−/529, 515, 353, 191, 179	ND	feruloydicaffeoylquinic acid
51	49.87	−/677	−/515, 353, 191, 179	ND	3,4,5-tricaffeoylquinic acid

[a] ND, not determined or not identified. [b] Previously reported compounds in pear. [c] Identified by comparison to a standard.

study. They were identified by direct comparison with standards. Arbutin (**Figure 2B**, peak 1) had a retention time of 3.75 min, UV λ_{max} at 282 nm, $[M - H]^-$ at *m/z* 271, and a very weak $[M + H]^+$ at *m/z* 273. Instead of the normal molecular ion, a $[M + H]^+$, its strongest ions (with a high fragmentation energy) were $[M + H + H_2O]^+$ at *m/z* 291 and $[M + Na]^+$ at *m/z* 295.

Typical UV absorption spectra of hydroxycinnamic acid and their derivatives (consisting of quinic or malic acid for pears) have a λ_{max} between 305 and 330 nm (band I) and a shoulder between 290 and 300 nm (band II) (*17*). With MS detection, negative ionization offers much stronger ion peaks and many more fragments than positive ionization. The deprotonated molecular ion $[M - H]^-$ and fragments formed by the loss of the acyl indicate the number of the acyl groups. Monocaffeoylquinic acids had deprotonated molecular ions at *m/z* 353, while dicaffeoylquinic and tricaffeoylquinic acids had deprotonated molecular ions at *m/z* 515 and 677, respectively. In addition, as shown in **Table 2**, they have other important ions at *m/z* 191 (the quinic acid ion after the loss of the last caffeoyl), *m/z* 179 (the caffeic acid ion), *m/z* 173 (the fragment of quinic acid formed by loss of H_2O), *m/z* 161 (the caffeoyl), and *m/z* 135 (the fragment of caffeic acid ion by loss of CO_2) (*17, 24*).

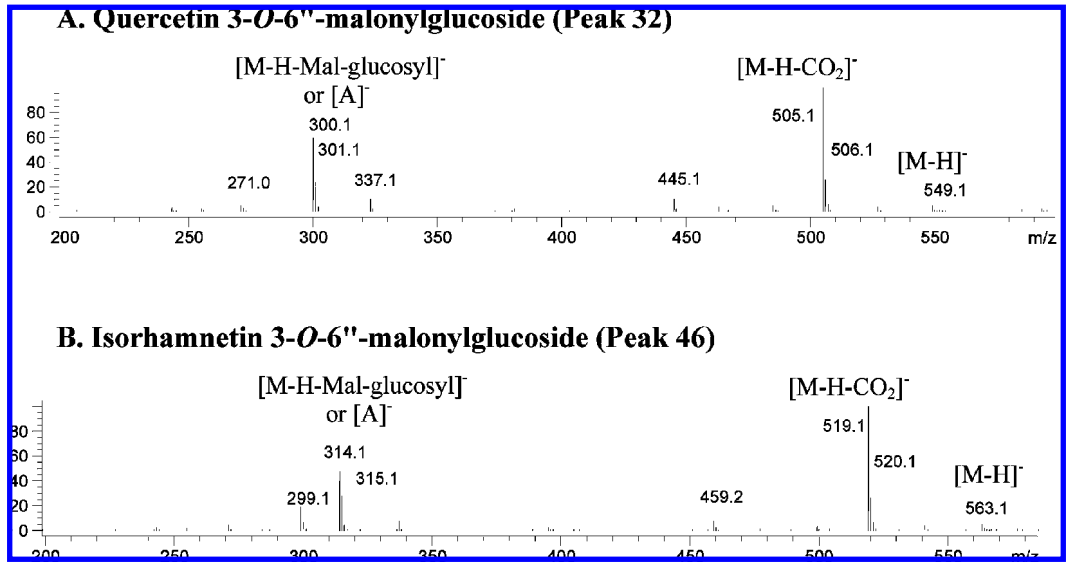

Figure 5. Mass spectra (NI 250 V) of (**A**) quercetin 3-*O*-6′′-maolonylglucoside (peak 32, **Figure 3A**), (**B**) isorhamnetin 3-*O*-6′′-maolonylglucoside (peak 46, **Figure 3A**). Mal, malonyl; and A, aglycon.

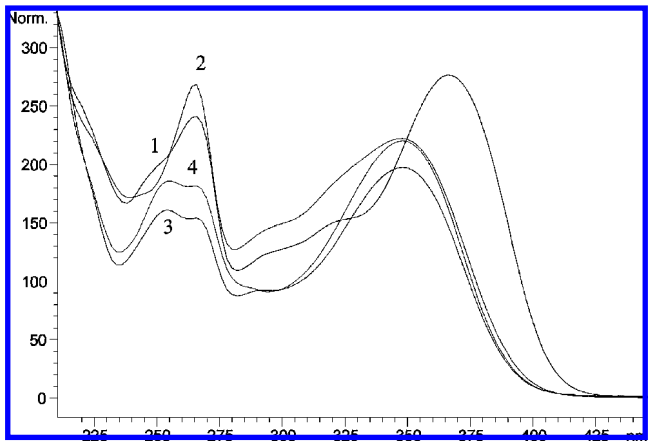

Figure 6. UV spectra of (1) kaempferol, (2) kaempferol 3-*O*-glucoside, (3) luteolin, and (4) luteolin 7-*O*-glucoside.

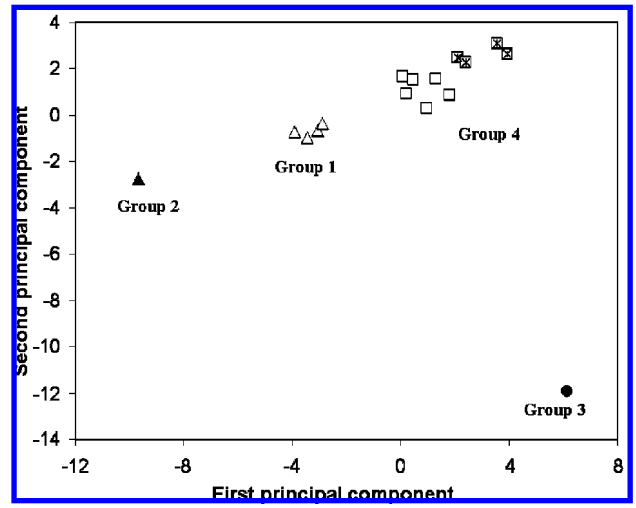

Figure 7. PCA of pear skin peak data (**Table 2**). Group 1 (△): Asian, Asian brown, Korean, and Korean Shinko pears. Group 2 (▲): Yali pear. Group 3 (●): fragrant pear. Group 4 (□): Bartlett, Beurre, Bosc, Comice, D'Anjou, Forelle, Peckham, Red, Red D'Anjou, and Seckel pears. The open squares with the asterisk inserted are D'Anjou, Red, Red D'Anjou, and Seckel pears.

These fragments suggested that these compounds had caffeoyl and quinic acid substitutions (*17, 24*). Because the isomers showed the same UV and mass data, they were identified by a direct comparison with standards as 3-caffeoylquinic acid (peak 3), chlorogenic acid (peak 5), and 4-caffeoylquinic acid (peak 7) in **Figure 2C** and 3,4-dicaffeoylquinic acid (peak 33), 3,5-dicaffeoylquinic acid (peak 36), and 4,5-dicaffeoylquinic acid (peak 43) in **Figure 2D**. Similarly, peaks 8, 9, 10B, 40B, 44, 49A, 50, and 51 in **Figure 2C**,**D** were identified as caffeic acid, *Z* (or *cis*)-caffeoylquinic acid, dicaffeoylquinic acid (one or more caffeoyl in cis form), 5-*p*-coumroylquinic acid, caffeic acid methyl ester, 1,3,5- and 3,4,5-caffeoylquinic acids ($[M - H]^-$ at *m/z* 677 and fragments at *m/z* 515 for the loss of one caffeoyl, *m/z* 353 for the loss of two caffeoyls, and so on), and feruloydicaffeoylquinic acid ($[M - H]^-$ at *m/z* 691 and the fragments at *m/z* 529 for the loss of one caffeoyl, *m/z* 515 for the loss of feruloyl, and *m/z* 353 for the loss of one caffeoyl and one feruloyl), respectively. The assignments of two tricaffeoylquinic acids were made with the recorded retention times of the reference compounds in arnica flower, a spice and herb, and the leaves of *Vernonia amygdalina* L., an African herb and vegetables.

Similarly, peaks 14, 25B, and 28A were identified as caffeoylmalic acid ($[M - H]^-$ *m/z* 295 with fragments at *m/z* 179 and 135 for caffeic acid and *m/z* 133 for malic acid), *p*-coumaroylmalic acid ($[M - H]^-$ *m/z* 279), and feruloylmalic acids ($[M - H]^-$ *m/z* 309), respectively.

Grouping of Pears. All of the pears tested in this study had the common feature that arbutin and chlorogenic acid were the main phenolic compounds (based on relative peak areas). The pattern of distribution of the remaining phenolic compounds suggested that the tested pears could be divided into four groups. This was confirmed by application of PCA to the data in **Table 1**. The score plots in **Figure 7** show four distinct groups. Although the data in **Table 1** do not represent a statistically valid sampling of the pears, the clusters support the general observation that there is a pattern to the distribution of the phenolic compounds.

The Asian (**Figure 2E**), Asian brown, Korean, and Korean Shinko (*Pyrus* spp.) pears formed group 1 (**Figure 7**). They contained only arbutin and chloregenic acid with only trace amounts of the other flavonoids. Yali pears (*P. bretschneideri*) (**Figure 2D**) formed group 2. They contained greater quantities

of 3,5-dicaffeoylquinic acid than chlorogenic acid, and they also contained significant quantities of caffeic acid. Fragrant pear (*P. serotina*) (**Figure 2A**,**B**) formed group 3 and contained significant amounts of rutin (peak 19), quercetin 3-*O*-2″-xylosyl-6″-rhamnosylglucoside (peak 13), and isorhamnetin 3-*O*-rutinoside (peak 28) as the main flavonoids. This pear also contained detectable amounts of the glycosides of luteolin, apigenin, and chrysoeriol.

The remaining 10 pears formed group 4, and all belong to the cultivars or varieties of *P. communis* L. They contained significant quantities of isorhamnetin glycosides, including the malonyl forms, and lesser quantities of quercetin glycosides (**Figure 2C**, red D'Anjou pears, and **Figure 4A**,**B**, Comice pears). The pears of this group still have some differences as can be seen by their distribution of their cluster in **Figure 7**. Red pear contained higher amounts of quercetin glycosides and lesser amounts of kaempferol glycosides, and both were present at higher quantities than the isorahmnetin glycosides (**Figure 3C**). Peckham pear contained trace amounts of apigenin glycoside. The colored pears, such as the red, D'Anjou, red D'Anjou, and Seckel pears, also contained detectable amounts of cyanidin 3-*O*-galactoside and lesser amounts of peonidin 3-*O*-galactoside. The concentration differences of the anthocyanins were not sufficient to justify further division of the group, although, as seen in **Figure 7** (open squares with asterisks inside), they have a systematic bias as compared to the other pears in the group.

The groupings shown in **Figure 7** follow taxonomic lines. The data presented here suggest that the phenolic compound profiles are generally useful for the identification of phenolic compounds in pears grown worldwide and could allow confirmation of the taxonomy of pears tested in the future. A more rigorous study should include pears from several seasons, locations, growing conditions, and of different ripeness.

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Received for review April 30, 2008. Revised manuscript received July 28, 2008. Accepted July 31, 2008. This research was supported by the Agriculture Research Service of the U.S. Department of Agriculture and the Office of Dietary Supplements at the National Institutes of Health.

JF8013487