ARTICLES

Identification of Some Phenolics in Pear Fruit

Wieslaw Oleszek,† Marie J. Amiot,*,‡ and Serge Y. Aubert‡

Department of Biochemistry, Institute of Soil Science and Plant Cultivation, Osada Palacowa, 24-100 Pulawy, Poland, and Station de Technologie des Produits Végétaux, INRA, Domaine Saint-Paul, B.P. 91, 84143 Montfavet Cedex, France

Four hydroxycinnamic acid esters and eight flavonol glycosides were isolated and identified in pear fruit. By chemical and spectral methods the cinnamics were identified as 5′-caffeoylquinic, *p*-coumaroylquinic, *p*-coumaroylmalic, and dicaffeoylquinic esters. The mixture of flavonols included three quercetin and five isorhamnetin glycosides. The quercetin 3-*O*-glycosides were identified as rutinoside, glucoside, and malonyl glucoside, and the isorhamnetin 3-*O*-glycosides included rutinoside, galactorhamnoside, glucoside, malonyl galactoside, and malonyl glucoside.

INTRODUCTION

Phenolics have been shown to have a role in tissue browning, flavor, and color characteristics of fruits and derived products (Ho et al., 1992). An understanding of phenolic composition in fresh fruit and the factors that affect phenolics is critical in the design of products and storage conditions.

Pear phenolics have been characterized by several investigators. The hydroxycinnamic ester composition was shown to be a mixture of chlorogenic, *p*-coumarylquinic, and dicaffeoylquinic acids (Cartwright et al., 1955; Hulme, 1958; Sioud and Luh, 1966; Challice and Williams, 1972; Billot et al., 1978; Wald et al., 1989; Amiot et al., 1992). The presence of (+)-catechin and (−)-epicatechin has also been reported (Sioud and Luh, 1966; Ranadive and Haard, 1971; Mosel and Herrmann, 1974). Glycosides of quercetin, isorhamnetin, and kaempferol and their malonyl glycosides make up the flavonol composition of pears (Duggan, 1969a,b; Nortje and Koppen, 1965; Wald et al., 1989). Procyanidins were also reported, but the data on specific structures are very limited (Sioud and Luh, 1966). All studies of phenolics in pears and their changes with processing and storage have been reviewed by Herrmann (1976), Macheix et al. (1990), and Spanos and Wrolstad (1992).

A close look at the literature, however, showed that early methodologies of phenolic determination were limited to nonspecific methods for total phenolic or semiquantitative assessments. Modern HPLC techniques have been applied for catechin and cinnamic determination (Blankenship and Richardson, 1985). Recent work by Spanos and Wrolstad (1990) identified some phenolics in the pear juice HPLC profile using diode array detection. However, the characterization of the flavonol region of the profile was limited to two glycosides, as these were the only standards that were available commercially. The total HPLC profile of flavonols remained uncharacterized. Since these compounds seemed to play an important role in the stabilization of color development (Oleszek et al., 1989; Goupy et al., 1993) and this effect may be structure dependent, we deemed it of interest to obtain appropriate standards for full characterization. Thus, the aim of the present work was to isolate, identify, and purify some phenolics in pear using HPLC and mass spectrometry.

MATERIALS AND METHODS

Pears. Guyot pears were grown at the Station d'Expérimentation Arboricole de la région Provence Alpes Côte d'Azur (Mallemort, France). Green fruits were collected in July 1993 (3 weeks before commercial maturity), cut into small pieces into liquid nitrogen, and freeze-dried.

Extraction. The dried material was finely powdered, and a 300-g portion was blended with 3 × 2 L of MeOH for 5 min. The mixture was filtered and the filtrate concentrated in vacuo (40 °C) to a volume of 0.1 L, and then distilled water (1 L) was added. The extract was washed three times with hexane to remove lipids, carotenoids, and chlorophyll and then passed through a LiChroprep RP18 column (3 cm × 5 cm, 25–40 μm, Merck) to remove sugars (Oleszek et al., 1988). The column was washed with water (100 mL); phenolic acids and catechins were eluted with 0.5 L of 25% MeOH (fraction 1; 1.2 g). Finally, the flavonols were washed out with 0.5 L of 60% MeOH (fraction 2; 3.5 g).

Isolation of Phenolic Acid Esters. Fraction 1 was evaporated until the MeOH was removed. The pH was adjusted to 7.0 with NaOH, and then fraction 1 was passed through the C_{18} column as above. Acids and esters passed through the column, and after the pH was adjusted to 2.6 with HOAc, these were extracted into ethyl acetate and the solvent was removed by evaporation in vacuo (fraction 1a; 0.5 g). Catechins remaining on the column were washed out with MeOH, and the solvent was evaporated as for fraction 1a (fraction 1b; 0.6 g).

Fraction 1a was loaded onto a glass column (30 cm × 1.5 cm), filled with RP18 (25–40 μm, Merck). The column was eluted with 25% MeOH in water (pH 2.6 made with H_3PO_4), and 10-mL fractions were collected with a fraction collector. Each fraction was monitored with analytical HPLC, and those showing the presence of a single compound were combined. Hydroxycinnamic acids were extracted into ethyl acetate and dried.

Separation of Flavonol Glycosides. Fraction 2 was loaded onto a glass RP18 column (30 cm × 3 cm, 25–40 μm, Merck). The column was washed with a linear gradient (GM1 gradient mixer, Pharmacia Fine Chemicals) of 20–60% MeOH in water (pH 2.6 made with H_3PO_4). Ten-milliliter fractions were collected and monitored with analytical HPLC. The fractions showing similar characteristics were combined and concentrated. They were further purified on a steel Lichrosorb column (25 cm × 1 cm, 10 μm, Interchrom). Samples were introduced to the column with

* Author to whom correspondence should be addressed.
† Institute of Soil Science and Plant Cultivation.
‡ INRA.

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a sample injector (500-μL loop). The column was washed isocratically with 45% solvent B (acetonitrile–MeOH–water 1:3:1) in solvent A (H_2O, pH 2.6 made with H_3PO_4), delivered with a HP1050 series pump at a flow rate of 2 mL/min. Separations were monitored with a HP1050 series diode array detector, and fractions showing the presence of a single compound were collected.

Analysis of Glycosides by Analytical HPLC. The purity of the separated compounds, their Rt's, and their general phenolic composition in pear extracts were measured with an analytical HPLC unit (Hewlett-Packard 1050 series) with a diode array detector, using an Adsorbosphere C_{18} 3U (150 mm × 4.6 mm i.d., Alltech) cartridge. The solvent system used was the gradient of A (water, pH 2.6 made with H_3PO_4) and B (acetonitrile–MeOH–water 1:3:1). The following gradient was applied: 0–10 min, 12% B isocratic; 10–16 min, 15% B linear; 16–26 min, 15% B isocratic; 26–55 min, 50% B linear; 55–65 min, 50% B isocratic; 65–70 min, 12% B linear. The solvent flow rate was 0.8 mL/min.

Analysis of Hydrolysis Products. Individual esters were hydrolyzed with 2 N NaOH under N_2 at ambient temperature for 120 min. The mixture was then acidified with HCl and passed through a C_{18} Sep-Pak (Waters); phenolic acids were washed out with MeOH and analyzed by TLC and HPLC. The aqueous solution was evaporated in vacuo (40 °C), and the dry residue dissolved in 0.5 mL of MeOH, and the organic acids were analyzed by TLC. Acylated flavonol glycosides were hydrolyzed with 0.2 N NaOH under N_2, and hydrolysis products were analyzed as above.

Glycosides were hydrolyzed in 2N HCl at 100 °C for 30 min and the mixture was passed through a C_{18} Sep-Pak. Eluants were collected, evaporated in vacuo (40 °C) and analyzed for sugars with TLC. Aglycons retained on the Sep-Pak were washed out with methanol and analyzed by TLC.

Thin-Layer Chromatography. Phenolic acids were chromatographed on cellulose (DC-Fertigplatten, Merck). Plates were developed with a chloroform–acetic acid–water (4:1:4, lower phase) solvent system. Phenolic acids were monitored under UV light before and after developing with Benedikt reagent (Reznik and Egger, 1961). Caffeic and 5′-caffeoylquinic acids became nonfluorescent, while coumaric acid and its derivatives became blue fluorescent with this spray.

Flavonol aglycons were analyzed on cellulose together with appropriate standards, using 50% acetic acid as a solvent system. Compounds were monitored under UV (at 365 nm) after exposure to ammonia vapor.

Organic acids were chromatographed on cellulose with 1-propanol–methyl benzoate–formic acid–water (7:3:2:1). Plates were dried and developed by spraying with bromocresol green/bromophenol blue (375/125 mg/0.5 L of EtOH) followed by $KMnO_4/Na_2CO_3$ (250/500 mg/0.5 L of H_2O) solutions (Morton-Gaudry et al., 1972).

Sugars were chromatographed on cellulose using a toluene–butanol–pyridine–water (1:5:3:3) solvent system. Plates were developed twice in the system, and after drying, the sugars were made visible by spraying the plates with aniline–phthalate followed by heating at 105 °C. Standards of flavonols were purchased from Extrasynthese (Genay, France) and sugars from Sigma (Saint-Quentin-Fallavier, France); malonic acid was from Prolabo (Vaulx-En-Velin, France).

Spectral Analyses. The spectra and bathochromic shifts were recorded with a spectrophotometer (Hewlett-Packard 8452A) equipped with a diode array detector and a computer. Spectra were assigned according to the method of Mabry et al. (1970).

Fast Atom Bombardment Mass Spectrometry (FAB-MS). Positive mode spectra were recorded with a MR-JEOL-DX300 3-keV spectrometer using NBA as a matrix.

RESULTS

Separation of fraction 1a on a RP18 column applying isocratically 25% MeOH provided three compounds with the following characteristics.

Compound F1a-1: Rt 33 min; UV λ_{max}, nm, (MeOH) 328; FAB-MS m/z 376 $(M + Na)^+$, 354 $(M + H)^+$, 181 (caffeic acid + H)$^+$. Mild alkaline hydrolysis yielded caffeic acid (TLC R_f 0.90, HPLC) and quinic acid (TLC R_f 0.15).

Compound F1a-2: Rt 41.5 min; UV λ_{max}, nm, (MeOH) 328; FAB-MS m/z 361 $(M + Na)^+$, 339 $(M + H)^+$, 165 (coumaric + H)$^+$. Alkaline hydrolysis afforded *p*-coumaric acid (TLC R_f 0.92, HPLC) and quinic acid (TLC).

Compound F1a-3: Rt 48.2 min; UV λ_{max}, nm, (MeOH) 310; FAB-MS m/z 281 $(M + H)^+$, 165 (coumaric + H)$^+$. Alkaline hydrolysis yielded *p*-coumaric acid (TLC, HPLC) and malic acid (TLC R_f 0.37).

Fraction 1b was analyzed with TLC and HPLC, and these tests revealed the presence of several compounds, with epicatechin being predominant. Procyanidins were not purified and identified in this study.

Fraction 2 was chromatographed on the RP18 column in a gradient of MeOH (20–60%) in acidified water and yielded five fractions, each containing one or two major components. These fractions were further purified (or separated) on a micropreparative column with the isocratic system. This procedure yielded nine individual compounds with the following characteristics.

Compound F2-0: Rt 55.8 min; UV λ_{max}, nm, 332; FAB-MS m/z 539 $(M + Na)^+$, 517 $(M + H)^+$, 163 (caffeic acid $- H_2O + H)^+$. Alkaline hydrolysis yielded caffeic acid (TLC, HPLC) and quinic acid (TLC).

Compound F2-1: Rt 56.4 min; UV λ_{max}, nm, (MeOH) 258, 302 sh, 358, (NaOMe) 272, 328 sh, 408, ($AlCl_3$) 274, 306 sh, 434, ($AlCl_3$/HCl) 268, 302 sh, 366 sh, 402, (NaOAc) 268, 378, (NaOAc/H_3BO_3) 262, 380; FAB-MS m/z 643 $(M + Na)^+$, 611 $(M + H)^+$. Acid hydrolysis yielded quercetin (TLC R_f 0.26), rhamnose (TLC R_f 0.74) and glucose (TLC R_f 0.44).

Compound F2-2: Rt 56.9 min; UV λ_{max}, nm, (MeOH) 256, 356, (NaOMe) 272, 326 sh, 404, ($AlCl_3$) 274, 302 sh, 434, ($AlCl_3$/HCl) 268, 302 sh, 356 sh, 402, (NaOAc) 266, 364, (NaOAc/H_3BO_3) 260, 378; FAB-MS m/z 487 $(M + Na)^+$. Acid hydrolysis revealed quercetin and glucose.

Compound F2-3: Rt 57.9 min; UV λ_{max}, nm, (MeOH) 256, 296 sh, 358, (NaOMe) 272, 324 sh, 406, ($AlCl_3$) 272, 302 sh, 432, ($AlCl_3$/HCl) 266, 302 sh, 366 sh, 400, (NaOAc) 262, 324 sh, 388, (NaOAc/H_3BO_3) 260, 284 sh, 386. FAB-MS m/z 573 $(M + Na)^+$, 487 $(M - 86 + Na)^+$, 465 $(M - 86 + H)^+$. Acid hydrolysis yielded quercetin and glucose. Mild basic hydrolysis yielded compound F2-2 and malonic acid (TLC R_f 0.60).

Compound F2-4: Rt 61.5 min; UV λ_{max}, nm, 254, 268 sh, 356, (NaOMe) 272, 326 sh, 410, ($AlCl_3$) 268, 302 sh, 362 sh, 406, ($AlCl_3$/HCl) 268, 304 sh, 358 sh, 402, (NaOAc) 272, 326 sh, 362, (NaOAc/H_3BO_3) 254, 268 sh, 356; FAB-MS m/z 647 $(M + Na)^+$, 625 $(M + H)^+$. Acid hydrolysis provided isorhamnetin (TLC R_f 0.14), galactose (TLC R_f 0.36), and rhamnose.

Compound F2-5: Rt 62.3 min; UV λ_{max}, nm, (MeOH) 254, 354, (NaOMe) 272, 322 sh, 406, ($AlCl_3$) 266, 302 sh, 360 sh, 398, ($AlCl_3$/HCl) 264, 302 sh, 360 sh, 398, (NaOAc) 254, 270 sh, 326 sh, 362, (NaOAc/HB_3O_3) 254, 354; FAB-MS m/z 501 $(M + Na)^+$, 479 $(M + H)^+$. Acid hydrolysis provided isorhamnetin and glucose.

Compound F2-6: Rt 62.3 min; UV λ_{max}, nm, (MeOH) 254, 270 sh, 356, (NaOMe) 270, 324 sh, 414, ($AlCl_3$) 268, 302 sh, 360 sh, 404, ($AlCl_3$/HCl) 268, 304 sh, 360 sh, 398, (NaOAc) 272, 322 sh, 372, (NaOAc/H_3BO_3) 254, 270 sh, 356; FAB-MS m/z 646 $(M + Na)^+$, 462 $(M + H - hexose)^+$. Acid hydrolysis yielded isorhamnetin, glucose, and rhamnose.

Compound F2-7: Rt 64.3 min; UV λ_{max}, nm, (MeOH) 256, 354, (NaOMe) 274, 318 sh, 402, ($AlCl_3$) 270, 304 sh, 354 sh, 396, ($AlCl_3$/HCl) 270, 304 sh, 354 sh, 396, (NaOAc)

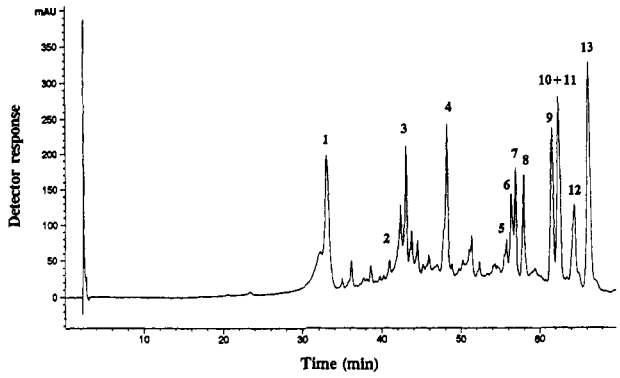

Figure 1. HLPC profile of pear skin phenolics: 1, 5′-caffeoylquinic acid; 2, *p*-coumaroylquinic acid; 3, epicatechin; 4, *p*-coumaroylmalic acid; 5, dicaffeoylquinic acid; 6, quercetin rutinoside; 7, quercetin glucoside; 8, quercetin glucomalate; 9, isorhamnetin rhamnogalactoside; 10+11, isorhamnetin glucoside + isorhamnetin rutinoside; 12, isorhamnetin galactomalonate; 13, isorhamnetin glucomalonate.

272, 320 sh, 366, ($NaOAc/H_3BO_3$) 256, 354; FAB-MS *m/z* 587 $(M + Na)^+$, 565 $(M + H)^+$. Acid hydrolysis provided isorhamnetin and galactose. Mild alkaline hydrolysis yielded glycoside, with Rt similar to that of compound F2-4, and malonic acid.

Compound F2-8: Rt 66.1 min; UV λ_{max}, nm, (MeOH) 254, 292 sh, 354, (NaOMe) 274, 322 sh, 402, ($AlCl_3$) 266, 302 sh, 370 sh, 402, ($AlCl_3$/HCl) 264, 302 sh, 360 sh, 398, (NaOAc) 270, 322 sh, 366, ($NaOAc/H_3BO_3$) 254, 356; FAB-MS *m/z* 587 $(M + Na)^+$, 565 $(M + H)^+$. Acid hydrolysis revealed isorhamnetin and glucose. Mild alkaline hydrolysis provided isoquercetin glucoside and malonic acid.

DISCUSSION

A typical HPLC chromatogram of pear fruit skin is shown in Figure 1. Two peaks in this chromatogram can be readily identified with a diode array detector as 5′-caffeoylquinic acid and epicatechin (peaks 1 and 3 in Figure 1), respectively. These two compounds are widely distributed in the plant kingdom, and the appropriate standards are commercially available. The other components of the HPLC profile had to be characterized. Preliminary analysis of the spectrum of extract, taken with the diode array detector, indicated the presence of three other hydroxycinnamoyl derivatives (Rt 41.5, min, peak 3; 48.2, min, peak 4; and 55.8 min, peak 5) and at least seven flavonols that remained to be identified.

To achieve this aim, the pear methanolic extract was partitioned into three major fractions using a protocol similar to that previously applied to the identification of apple phenolics (Oleszek et al., 1988).

The fraction containing catechins and procyanidins was analyzed with TLC, and this showed the presence of epicatechin and several minor components. These were not analyzed further.

The fraction containing hydroxycinnamoyl derivatives was successfully separated on the C_{18} column with isocratic system. This yielded three compounds, which were identified with chemical, chromatographic, and spectral techniques. Compound F1a-1 (peak 1 in Figure 1) on FAB-MS produced a molecular ion at 352 corresponding to chlorogenic caid. An additional peak present in the spectrum at 181 mu was identified as a protonated caffeic acid, in agreement with the work of Sakushima et al. (1985), establishing that in the positive-ion FAB technique protonated ion is always generated on the caffeoyl moiety. On the basis of these findings and the similarity of the Rt to that of the original standard, compound **F1a-1** was identified as 5′-caffeoylquinic acid.

Compound **F1a-2** (peak 2 in Figure 1) yielded *p*-coumaric and quinic acids on alkaline hydrolysis. The mass spectrum showed that the molecular weight was 338 mu, which corresponded to a coumarylquinic molecule cleaved in a 1:1 ratio. On the basis of these findings, compound **F1a-2** was recognized as a *p*-coumarylquinic ester.

Compound **F1a-3** (peak 4 in Figure 1), yielded *p*-coumaric and malic acids on alkaline hydrolysis. The molecular ion of 281 mu corresponded to a coumarylmalic molecule connected in a 1:1 ratio. Hence, this was identified as a *p*-coumarylmalic ester, in agreement with the findings of Risch and Herrmann (1988).

The fourth hydroxycinnamic ester, compound **F2-0** (peak 5 in Figure 1), was identified in fraction 2. This compound had much lower polarity than the other cinnamics, and its chemical behavior indicated the lack of a free carboxylic group. However, on alkaline hydrolysis this yielded caffeic and quinic acids, components similar to 5′-caffeoylquinic acid. However, a mass spectrum showed a molecular ion of 516, which corresponded to one molecule of quinic acid and two molecules of caffeic acid. On the basis of these findings compound **F2-0** was identified as a dicaffeoylquinic ester, in agreement with the findings of Wald et al. (1989). This compound has recently been identified in carrots (Babic et al., 1993).

The presence of the four cinnamics in pear fruits identified in this work has been reported previously (Macheix et al., 1990). Relatively less information is available on dicaffeoylquinic and particularly on *p*-coumarylmalic esters. All of these esters are of interest to food technologists as they may contribute to enzymatic browning. The *p*-coumarylmalic ester may be of particular interest, as, due to its low oxidation level (Amiot, unpublished data), this may serve as an indicator of the oxidative processes occurring during fruit storage. In the variety studied, this compound was found to be the dominant ester present.

Low-pressure column chromatography on the C_{18} support with MeOH–H_2O gradient, followed by high-pressure semipreparative chromatography, was successful in the separation of individual flavonols present in fraction 2. Several individual flavonols were separated and their structures confirmed by chemical, chromatographic, and spectroscopic analysis. Thus, bathochromic shifts of compounds **F2-1**, **F2-2**, and **F2-3** showed similar patterns indicating the presence of 3,5,7,3′,4′-OH groups in the molecules, with glycosidic bonds at the 3-*O*-position. All three compounds yielded the same aglycon on acid hydrolysis, which was shown by TLC to be identical to the quercetin standard.

FAB-MS provided rather weak but clear complementary spectra showing protonated and sodium adduct ions. Thus, compound **F2-1** (peak 6 in Figure 1), which on acid hydrolysis yielded quercetin, glucose, and rhamnose and produced a protonated molecular ion at *m/z* 611 and a sodium adduct ion at *m/z* 643, was identified as quercetin rutinoside.

Compound **F2-2** (peak 7 in Figure 1) yielded quercetin and glucose when hydrolyzed. Mass spectrometry showed a strong sodium adduct ion at *m/z* 487, indicating the presence of one hexose. Hence, compound **F2-2** was identified as quercetin glucoside. Compound **F2-3** (peak 8 in Figure 1) afforded the same components as quercetin glucoside on acid hydrolysis. However, mass spectrometry revealed a sodium adduct ion at *m/z* 573, which was 86 mu

higher than that found for quercetin glucoside, suggesting acylation with malonic acid. The presence of malonic acid was confirmed by TLC after mild alkaline hydrolysis. In this way, compound **F2-3** was identified as a quercetin malonyl glucoside.

Compounds **F2-4**, **F2-5**, **F2-6**, **F2-7**, and **F2-8** produced bathochromic shifts similar to those of the three quercetin glycosides, with the only difference being obtained with $NaOAc/H_3BO_3$ reagent. The lack of shift for $NaOAc/H_3BO_3$ indicated the absence of a 3',4'-*o*-diOH configuration (Markham, 1989). Thin-layer chromatography showed the identity of the aglycon to be isorhamnetin (3'-CH_3-quercetin) by comparison with an authentic standard. All five compounds showed bathochromic shifts of band I (in MeOH) to band Ia ($AlCl_3/HCl$) ranging from 40 to 48 nm, a feature characteristic of 5-hydroxy-3-*O*-substituted flavonols (Mabry et al., 1970). On the basis of the analysis of the hydrolysis products and mass spectra, compounds **F2-4**, **F2-5**, and **F2-6** were identified as the galactorhamnoside, the glucoside, and the rutinoside of isorhamnetin, respectively (peaks 9, 10, and 11 in Figure 1).

Compounds **F2-7** (peak 12 in Figure 1) and F2-8 (peak 13 in Figure 1) yielded malonic acid on mild alkaline hydrolysis. Their mass spectra were identical, showing sodium adduct ions at *m/z* 587 and protonated ion at *m/z* 565, which correspond to the molecule containing isorhamnetin–hexose–malonic acid. They also produced very similar bathochromic shifts. On acid hydrolysis both compounds released isorhamnetin but differed in sugar components: compound **F2-7** yielded galactose and compound **F2-8** glucose as shown by TLC. Considering these findings, compounds **F2-7** and **F2-8** were identified as isorhamnetin malonyl galactoside and malonyl glucoside, respectively.

To our knowledge, isorhamnetin malonyl galactoside has not been reported in pear fruit. All other flavonol glycosides documented here have been identified previously in pears as reviewed by Macheix et al. (1990). The present research allowed clear location of the particular compounds in the HPLC profile for the first time. Compounds **F2-1**, **F2-2**, **F2-3**, **F2-6**, and **F2-7** had retention times distinctly different from those of the other components under the present separation conditions, and they may be easily quantified. Some difficulties, however, may be faced in the quantifying compounds **F2-4**, **F2-5**, and **F2-6**. Their retention times were very similar and peaks could easily overlap. Thus, it was very difficult to distinguish between rhamnogalactoside and galactoside and between glucoside and rutinoside of isorhamnetin. In the present study isorhamnetin galactoside was not identified as a genuine compound. However, thin-layer chromatography of compound **F2-4** showed traces of an accompanying flavonol, with R_f values identical to that of isorhamnetin galactoside and produced by mild alkaline hydrolysis of the isorhamnetin malonyl galactoside. Under the present isolation protocol isorhamnetin galactoside was not separated from isorhamnetin galactorhamnoside. This could be a reason why isorhamnetin galactoside has so far been mentioned in only four cultivars (Duggan, 1969b).

Another issue is the presence of monoglycosides acylated with malonic acid. Such acylated compounds have been found in many plants (Harborne, 1989). The malonyl group is usually linked at the 6-position of the glucose, and this linkage is very labile under unfavorable extraction/purification conditions (temperature, pH). Thus, the extraction conditions are critical in obtaining the genuine composition of pear flavonols. The acylation process appears to be important since this may facilitate the transport of flavonoid glycosides through the tonoplast to the vacuole (Teusch and Forkman, 1987). However, the function of acylated flavonols in oxidation or color development/stabilization processes remains unknown. The function of acylated pear flavonols in this respect should not be neglected and should be closely researched. The present study allowed full characterization and determination of these compounds.

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