JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

MARCH 2000
VOLUME 48, NUMBER 3

REVIEWS

Measurement of Food Flavonoids by High-Performance Liquid Chromatography: A Review

Howard M. Merken* and Gary R. Beecher

Food Composition Laboratory, Beltsville Human Nutrition Research Center Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705

The flavonoids are plant polyphenols found frequently in fruits, vegetables, and grains. Divided into several subclasses, they include the anthocyanidins, pigments chiefly responsible for the red and blue colors in fruits, fruit juices, wines, and flowers; the catechins, concentrated in tea; the flavanones and flavanone glycosides, found in citrus and honey; and the flavones, flavonols, and flavonol glycosides, found in tea, fruits, vegetables, and honey. Known for their hydrogen-donating antioxidant activity as well as their ability to complex divalent transition metal cations, flavonoids are propitious to human health. Computer-controlled high-performance liquid chromatography (HPLC) has become the analytical method of choice. Many systems have been developed for the detection and quantification of flavonoids across one, two, or three subclasses. A summary of the various HPLC and sample preparation methods that have been employed to quantify individual flavonoids within a subclass or across several subclasses are tabulated in this review.

Keywords: *Antioxidant; flavonoids; HPLC; mass spectrometry; polyphenols*

INTRODUCTION

Three of the most important natural pigments are carotenoids, tetrapyrrole derivatives, and flavonoids. Flavonoids, derived biosynthetically from phenylalanine, are pigments found widespread in plants (Ooghe et al., 1994). Three moles of malonyl-coenzyme A (CoA) from glucose metabolism condense to form ring A, catalyzed by chalcone synthetase (Figure 1). Rings B and C also come from glucose metabolism, but via the shikimate pathway through phenylalanine, which is converted to cinnamic acid and then to coumaric acid. Coumaric acid CoA and three malonyl CoAs are condensed in a single enzymatic step to form naringenin chalcone. The C-ring closes and becomes hydrated to form 3-hydroxyflavonoids (e.g., catechins), 3,4-diol flavonoids (e.g., quercetin), and procyanidins (Formica and Regelson, 1995; Heller and Forkmann, 1994).

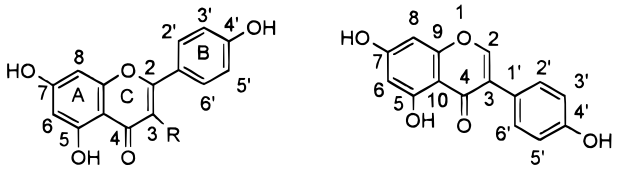


Figure 1. General structure of flavonoids (left: R = OH in flavonols, R = H in flavones) and isoflavones (right).

There are >4000 known flavonoids comprising 12 subclasses (Strack and Wray, 1994). The orange, red,

* Author to whom correspondence should be addressed [telephone (301) 504-9370; fax (301) 504-8314; e-mail merken@bhnrc.usda.gov].

10.1021/jf990872o
Published on Web 02/29/2000

and blue colors in vegetables, fruits, flowers, and plant storage tissue are due to water-soluble anthocyanins, which are reduced from the yellow flavonoids due to loss of oxygen (Chandra et al., 1992; Constant, 1997; Versari et al., 1997). Anthocyanins help attract animals, resulting in seed dispersal and pollination (Strack and Wray, 1994). Flavonoids have two aromatic rings enclosing a heterocyclic six-membered ring with oxygen (Figure 1) (Hertog et al., 1992; Ooghe et al., 1994). As such, they could be considered derivatives of diphenylpropanes (Hertog et al., 1992). Isoflavones, found in soy and soy products, have a similar structure (Wang and Murphy, 1994) but with a different linkage to the propane bridge (Figure 1).

Dietary flavonoids are usually glycosylated and can be classified as anthocyanins, flavanols (catechins), flavones, flavanones, and flavonols; the last three are the anthoxanthins (McDonald et al., 1998; Robards and Antolovich, 1997). Flavonoids are found in nearly every plant. Flavanones and flavones are often present in the same plant (often citrus), yet flavones and flavonols are generally not found together, nor are flavanones and anthocyanins (Rice-Evans et al., 1996). Isoflavones are usually treated separately from the former five subclasses, as they are found in significant concentrations in legumes, chiefly in foods containing soybeans (Franke et al., 1994). A comprehensive text edited by Harborne includes minor classes of flavonoids (Bohm, 1994), flavans, proanthocyanidins (Porter, 1994), neoflavonoids (Donnelly and Boland, 1994), bi- and triflavonoids (Geiger, 1994), and the biological implications of flavonoids (Harborne and Grayer, 1994; Middleton and Kandaswami, 1994). Detailed ^{1}H NMR spectral information, and 72 ^{1}H NMR spectra, can also be found in Harborne's text (Markham and Geiger, 1994).

Health Benefits. Flavonoids are hydrogen-donating radical scavengers (antioxidants). By complexing iron ions, flavonoids suppress the superoxide-driven Fenton reaction (Rice-Evans et al., 1996). Copper complexation is also an important activity of certain flavonoids, especially those with the catechol structure in the B-ring (Brown et al., 1998).

By reducing the α-tocopheroxyl radical, flavonoids regenerate α-tocopherol. Flavonoids also quench singlet oxygen (Rice-Evans et al., 1996).

Rice-Evans et al. (1996) reviewed the structure−antioxidant activity relationships, and Formica and Regelson (1995) reviewed the biology of flavonoids. Flavonoids have shown activity against allergies, inflammation, viruses, hypertension, arthritis, mutations, and carcinogens, cancer, and AIDS (Hertog et al., 1992; Middleton, 1996; Plessi et al., 1998; Robards and Antolovich, 1997). Polyphenols inhibit cGMP and cAMP phosphodiesterase, xanthine oxidase, and elastase (Plessi et al., 1998).

The antioxidative activity of catechins is thought to be due to radical scavenging. The catechins are oxidized by donating hydrogens from the hyroxyl groups on the phenyl rings, preventing the oxidation of linoleic acid (Kumamoto and Sonda, 1998). Catechin-rich persimmon extract induces programmed cell death (apoptosis) in human lymphoid leukemia cells (Hibasami et al., 1996).

Flavonoids regenerate ascorbic acid (vitamin C), which in turn regenerates vitamin E (Cossins et al., 1998). However, despite the number of in vitro studies published, there is little information available from actual human feeding studies that could help to answer the question of what antioxidant mechanisms are taking place in humans (Robards and Antolovich, 1997).

Isoflavones are structurally isomeric to flavonoids. Found chiefly in soy, they may have health effects similar to those of flavonoids (Hendrich et al., 1999). Isoflavones and their metabolites have structures similar to that of mammalian estradiol. As phytoestrogens, they are believed to block estrogen reception by competitive inhibition, at the estrogen receptor, and to inhibit estrogen synthesis (Bingham et al., 1998; Kurzer, 1992). They may reduce the risk of hormone-dependent cancers such as prostate and breast cancers (Dwyer et al., 1994). Messina included the estrogenic effects of isoflavones in his review of the nutrition and health effects of legumes and soybeans (Messina, 1999).

Health implications require detailed knowledge of the flavonoid content of the food supply, hence, this review of methods on the measurement of flavonoids in foods.

HPLC OF FLAVONOIDS

HPLC was first used for the determination of flavonoids in 1976 by Fisher and Wheaton (Hasegawa et al., 1996). Daigle and Conkerton reviewed the HPLC analysis of flavonoids in 1983 (Daigle and Conkerton, 1983), and updated their review in 1988 (Daigle and Conkerton, 1988). Reviews of chromatographic methods of detection include those by Robards and Antolovitch (1997). This review concentrates on the HPLC methods of detection published from 1989 to early 1999. The methodology is divided into anthocyanins, catechins, flavanones, flavones and flavonols together, and isoflavones, with a sixth section dedicated to methods that have determined flavonoids across two or more subclasses (excluding flavones and flavonols together).

Chromatographic Conditions. Columns are almost exclusively reversed-phase (RP), ranging from 100 to 300 mm in length and usually with a 4.6 mm internal diameter. Stereochemistry is rarely an issue in the recent literature. However, Cyclobond I, a β-cyclodextrin-bonded stationary phase, was used in the reversed-phase mode and in the normal phase mode to separate the 2*R* and 2*S* diastereomers of flavanone glycosides and benzoylated flavanone glycosides, respectively (Krause and Galensa, 1991). Work on the enantiomeric separation of flavanones and the diastereomeric separation of flavanone glycosides, without food extractions, has been reported (Ficarra et al., 1995; Krause and Galensa, 1990). In earlier work, Saenger (1980) reviewed cyclodextrins, and Croft and Bartsch (1983) reviewed cyclodextrin synthesis.

Elution systems are usually binary, with an aqueous acidified polar solvent such as aqueous acetic acid, perchloric acid, phosphoric acid, or formic acid (solvent A) and a less polar organic solvent such as methanol or acetonitrile, possibly acidified (solvent B). Recent work at the National Institute of Standards and Technology (NIST) showed that trifluoroacetic acid in both solvents enhances the resolution of catechins and eliminates their peak tailing (Dalluge et al., 1998). Less frequently, runs are isocratic or tertiary, and even quaternary systems have been reported (de Pascual-Teresa et al., 1998; Tamura et al., 1994).

Runs are generally an hour maximum, with equilibration between runs. A striking exception is found in the 340-min run used for the HPLC of isoflavones in soy sauces for pattern recognition analysis (Kinoshita et al., 1997, 1998). Flow rates are usually 1.0 or 1.5 mL/

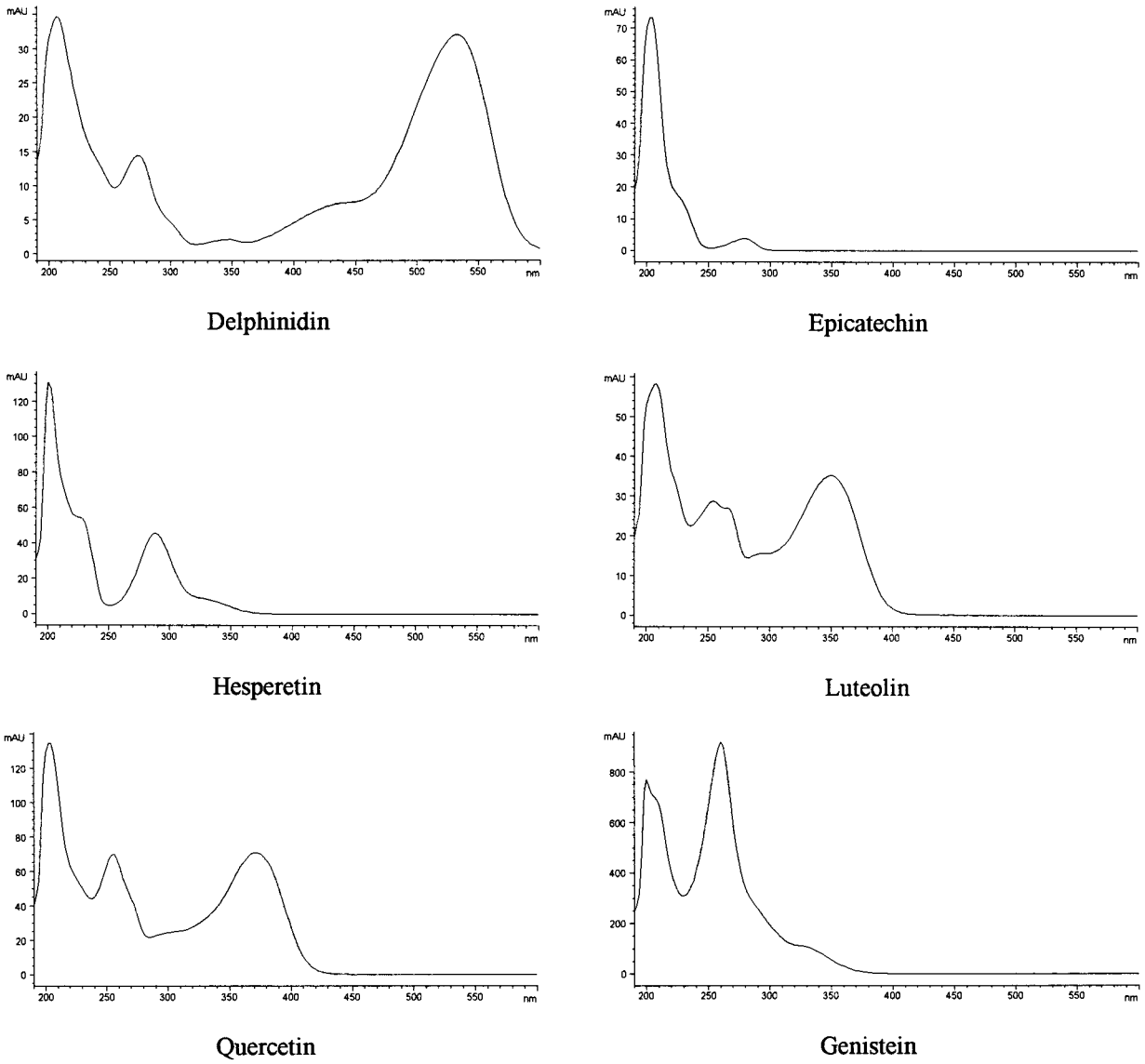


Figure 2. UV−vis spectra of the anthocyanidin delphinidin, the catechin epicatechin, the flavanone hesperetin, the flavone luteolin, the flavonol quercetin, and the isoflavone genistein.

min. Thermostatically controlled columns are normally kept at ambient or slightly above ambient temperatures. Injections generally range from 1 to 100 μL.

Detection. Phenols absorb in the ultraviolet (UV) region. Two absorption bands are characteristic of flavonoids. Band II, with a maximum in the 240−285 nm range, is believed to arise from the A-ring. Band I, with a maximum in the 300−550 nm range, presumably arises from the B-ring (Mabry et al., 1970; Robards and Antolovich, 1997). Spectra of 175 flavonoids, their molecular extinction coefficients, and their UV spectral data in several solvents were published in a 1970 work that includes nuclear magnetic resonance (NMR) spectra of 128 flavonoids (Mabry et al., 1970).

Anthocyanins show band II and band I absorption maxima in the 265−275 and 465−560 nm regions, respectively (Robards and Antolovich, 1997). Because there is little or no conjugation between the A- and B-rings, UV spectra of flavanones and isoflavones usually have an intense band II peak but a small band I peak (Mabry et al., 1970). This lack of conjugation also results in small band I peaks for the catechins (Figure 2). UV spectra of flavones and flavonols have a band II peak at around 240−280 nm and a band I peak around 300−380 nm (Mabry et al., 1970).

Figure 2 shows UV−vis spectra representative of each of the major subclasses of monomeric flavonoid aglycons (unpublished).

Detection of (iso)flavonoids in food analysis is usually by UV−vis with diode array detection (DAD). Typical wavelengths for analysis and quantification of anthocyanins are 502 nm (Bridle and Garciá-Viguera, 1997), 510 nm (Bakker et al., 1992), 520 nm (Boyles and Wrolstad, 1993), and 525 nm (Donner et al., 1997). Catechins were generally quantified at 210 nm (Bronner and Beecher, 1998; Dalluge et al., 1998), 278 nm (Khokhar et al., 1977), and 280 nm (Kumamoto and Sonda, 1998). Arts and Hollman used a fluorescence detector (280 nm for excitation, 310 nm for emission) connected in series to a UV detector (270 nm) for catechins (Arts and Hollman, 1998). Chemical reaction detection using *p*-dimethylaminocinnamaldehyde, which yields colored adducts with catechins, was used by de Pascual-Teresa et al. (1998), allowing for detection at 640 nm. Ogawa et al. (1999) used chemiluminescence for higher sensitivity in the detection of green tea extracts at 280 nm.

Flavanones and their glycosides were generally detected at 280 nm (Krause and Galensa, 1991) and 290 nm (Bogdanov, 1989). Flavones, flavonols, and flavonol glycosides were usually detected at wavelengths such as 270 nm (Brolis et al., 1998), 365 nm (Crozier et al., 1997), and 370 nm (Ewald et al., 1999), although detection at 280 and 350 nm was used (De Cooman et al., 1998).

Isoflavones were generally detected at 236 nm (Graham, 1991a,b), 260 nm (Garrett et al., 1999), 262 nm (Barnes et al., 1994), and 280 nm (Kinoshita et al., 1997, 1998). Wang used UV detection at 254 nm and fluorescence detection at 365 nm for excitation with a 418 nm emission filter (Wang et al., 1990). DAD and coulometric detection were used simultaneously for detection of isoflavones in soy foods (Franke et al., 1998).

Sample Preparation. Grenadine syrup required no sample preparation (Cherif and Ayed, 1997). Teas were boiled. Sometimes, liquid−liquid extraction (LLE) and/or solid-phase extraction (SPE) was then used (Table 2). Countercurrent chromatography and solid-phase columns such as Sephadex LH-20 and Sep-Pak were also used (Amarowicz and Shahidi, 1996; Liang et al., 1990).

Wines were also quite easy to prepare, requiring no preparation (Goldberg et al., 1998), filtering (Archier et al., 1992), or solid-phase extractions (Revilla et al., 1989).

Extractions to remove lipids, carotenoids, and chlorophyll were used with French apple cider (Guyot et al., 1998). Citrus extractions were slightly more complicated, including hand-squeezing, dilution, centrifugation, and filtration (Mouley et al., 1998) or extractions to remove carotenoids and methoxylated flavones (Marini and Balestrieri, 1995). Honey usually required solid-phase extractions (Bogdanov, 1989; Table 5).

Solid food samples were more complicated to extract. Examples include homogenization of blueberries and sweet cherries (Gao and Mazza, 1995a,b), crushing of pomegranate seeds (Gil et al., 1995a,b), breaking black beans by mortar and pestle (Takeoka et al., 1997), and LLEs and SPEs of potatoes and onions (Donner et al., 1997; Rodriguez-Saona et al., 1998). Soy was often ground, extracted, and hydrolyzed with acid (Hutabarat et al., 1998).

Hydrolysis. Hydrolyses, used frequently but not exclusively to remove the sugar moieties from glycosides, were acidic, basic, or enzymatic.

Hydrolysis of anthocyanins to anthocyanidins is often necessary due to the difficulty of obtaining anthocyanin standards. Hydrolysis of dry anthocyanin was typically done in refluxing HCl solutions, such as 50% MeOH/2 N HCl (aq) (v/v) (Gao and Mazza, 1994a) and 2 M HCl (Goiffon et al., 1991; Lee and Wicker, 1991).

Alkaline hydrolysis cleaved the acylated portions of acylated anthocyanins (Hong and Wrolstad, 1990). The phenolic extract of sunflower honey was hydrolyzed in 2 N NaOH (Sabattier, 1992).

Technical enzyme (EL-1−77; Röhm, Darmstadt, Germany) was used to hydrolyze flavonol glycosides (Finger et al., 1991a; Siewek and Galensa, 1984). The glycosides of flavones and flavonols were hydrolyzed in refluxing 1.2 M HCl in 50% MeOH/H_2O (v/v) (Hertog et al., 1992; Crozier et al., 1997; McDonald et al., 1998). Sodium diethyldithiocarbamate (20 mM) was used as an antioxidant in this hydrolytic solution (McDonald et al., 1998). However, neither anthocyanins nor catechins can be analyzed using this extraction method (Häkkinen et al., 1999), due to destruction and/or rearrangement of the compounds (Merken and Beecher, unpublished results).

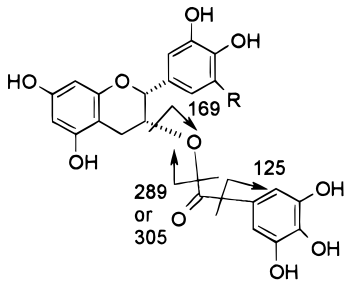


Figure 3. Cleavage of epicatechin gallate (ECG; R = H, MW = 442) and epigallocatechin gallate (EGCG; R = OH, MW = 458).

Structural Characterizations with Mass Spectrometry. After HPLC, samples can be further purified for mass spectrometry (MS) using column chromatography, as was done for Zambian munkoyo beverage (Zulu et al., 1994). An HPLC can be connected to a mass spectrometer interface, as was done in the analysis of fruits, vegetables, and beverages (Justesen et al., 1998).

Fast atom bombardment mass spectrometry (FABMS) of pelargonidin 3-glycoside in strawberry purée collected after HPLC confirmed its identity (Bakker et al., 1992). HPLC-quadrupole MS was used for detecting flavones in olive oil (Rovellini et al., 1997).

Lin et al. (1993) found the expected (−)-epicatechin, (−)-epicatechin 3-*O*-gallate, (−)-epigallocatechin, and (−)-epigallocatechin 3-*O*-gallate in tea using tandem MS following HPLC or LC-MS-MS. Poon (1998) used two MS systems for tea extracts. One was tandem MS using a triple-quadrupole mass spectrometer with an electrospray ionization source. The other was a quadrupole mass spectrometer. He found that catechin gallate esters cleave as shown in Figure 3, yielding fragments with *m*/*z* 169 for the gallate portion attached to C3, *m*/*z* 125 for the same portion minus CO_2, and *m*/*z* 289 or 305 for

Scheme 1. Retro-Diels−Alder Reactions of the Molecular Ion from Apigenin

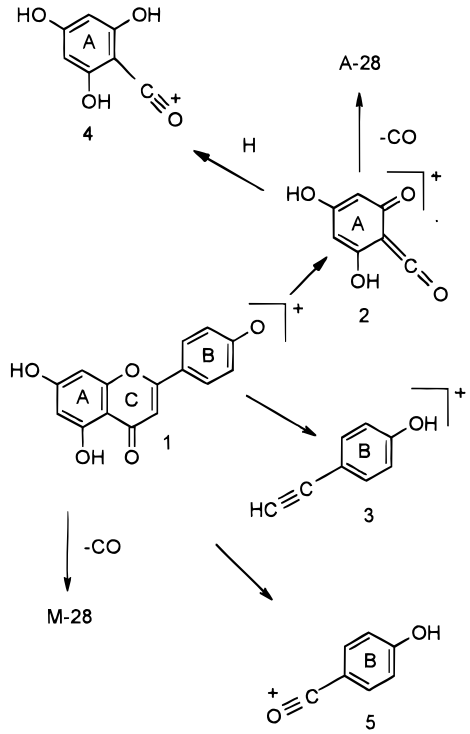


Table 1. HPLC of Anthocyanins and Anthocyanidins

food	sample preparation	guard	stationary phase	mobile phase[a]	references
beans, black	broken by mortar and pestle, extracted with 0.5% HCl/MeOH, LLE, SPE	*b*	ODS/B (250 × 4.6 mm, 5 μm, 100 Å)	A: 10% CH_2O_2/H_2O; B: CH_2O_2/H_2O/MeOH (10:40:50 v/v); gradient: 40−80% B, 0−50 min	Dao et al., 1998 Takeoka et al., 1997
blackberry and red raspberry juices and wines	filtration	*c*	Supelcosil LC-1 (250 × 4.6 mm, 5 μm)	A: 15% CH_3COOH; B: CH_3CN; gradient: 100% A, 0−5 min; 0−5% B, 5−15 min; or 100% A, 0−10 min; 0−15% B, 10−20 min	Rommel et al., 1990, 1992
blueberries, highbush	skins extracted with methanol	X	Lichrosorb 100 RP-18 (250 × 4 mm, 5 μm)	A: H_2O/CH_2O_2 (90:10 v/v); B: H_2O/CH_3CN/MeOH/CH_2O_2 (40:22.5:22.5:10 v/v); gradient: 20% B, 0−2 min; 20−25% B, 2−15 min; 25−40% B, 15−60 min; 40−40% B, 60−80 min	Kader et al., 1996
blueberries and sweet cherries	homogenized; SPE reported in 1995 papers	X	SuperPac Pep-S (250 × 4 mm, 5 μm)	A: 5% CH_2O_2/H_2O (5:95 v/v); B: MeOH; gradient for blueberries: 10−12% B, 0−4 min; 12−15% B, 4−10 min; 15−20% B, 10−20 min; 20% B, 20−23 min; 20−30% B, 23−32 min; 30−35% B, 32−40 min; 35−37% B, 40−48 min; 37−70% B, 48−50 min; 70% B, 50−53 min; 70−10% B, 53−55 min gradient for cherries: 30% B initially; 35% B, 8 min; 40% B, 8.5 min; 46% B, 20 min; 60% B, 30 min; 85% B, 30.5−34.5 min; 30% B, 35 min	Gao and Mazza, 1994a, 1995a,b
cherries, black	LLE; SPE; saponification and SPE; acid hydrolysis and SPE to anthocyanidins	*d*	PolyLC ODS C-18 (250 × 4.6 mm, 5 μm)	A: CH_3CN; B: 1% H_3PO_4, 10% CH_3COOH, 5% CH_3CN (v/v) in H_2O; gradient, anthocyanins: 0−12% A, 0−13 min; 12−20% A, 13−28 min; gradient, saponified anthocyanins and anthocyanidins: 0−30% A, 0−30 min	Ordaz-Galindo et al., 1999
cherries, tart	SPE	X	Chemcopak and Capellpak C-18 (250 × 10 mm, 5 μm)	isocratic: 4% aq H_3PO_4/CH_3CN (80:20 v/v)	Chandra et al., 1992, 1993 Wang et al., 1997
cranberry juice	LLE, SPE	*e*	Supelcosil ODS (250 × 5 mm, 5 μm); Polymer Labs PLRP-S (250 × 4.6 mm, 5 μm)	Supelcosil (anthocyanidins), A: CH_3COOH/H_2O, 15:85 (v/v) B: CH_3CN isocratic, 85% A, 15% B PLRP-S (anthocyanins), A: H_3PO_4/H_2O, 4:96 (v/v); B: CH_3CN; gradient: 6% B, 0−10 min; 6−20% B, 10−50 min; 20% B, 50−65 min	Hong and Wrolstad, 1990
elderberries and strawberries	SPE	X	ODS-Hypersil (200 × 2.1 mm, 5 μm)	A: 0.6% $HClO_4/H_2O$; B: CH_3OH; C: THF;gradient, strawberry: 15−55% B in A, 10−40 min; gradient, elderberry: 2−30% C in A, 0−28 min	Bridle and Garciá-Viguera, 1997
elderberry, pomegranate, and strawberry (juices, jams, extracts)	fruit: LLE, SPE; jam: LLE; pomegranate juice injected directly	X	Lichrochart 100 RP-18 (125 × 4 mm, 5 μm)	A: 5% CH_2O_2/H_2O (v/v); B: MeOH; gradient: 15−30% B, 0−15 min; 30% B, 15−20 min; 30−95% B, 20−25 min	Zafrilla et al., 1998; Garciá-Viguera et al., 1999
fruits, red	centrifuged, filtered	X	RP-18 LiChrospher (250 × 10 mm, 7 μm)	four isocratic phases: $H_2O/CH_3CN/CH_2O_2$ (84:6:10, 81:9:10, 80:10:10, 75:10:10, v/v); also gradient of 87:3:10−84:6:10, v/v	Goiffon et al., 1991

Table 1 (Continued)

food	sample preparation	guard	stationary phase	mobile phase[a]	references
fruit juices, red; concentrated juices, and syrups (eight fruits)	SPE	X	Lichrospher 100 CH 18/2 (250 × 4.5 mm, 5 μm)	isocratic; red currant and raspberry, $H_2O/CH_3CN/CH_2O_2$ (84:6:10, v/v); six other fruits, $H_2O/CH_3CN/CH_2O_2$ (81:9:10, v/v) to elute anthocyanidins (degradation products) or acylated anthocyanins (small amounts in grape and blueberry), A: H_2O; B: CH_3CN; C: CH_2O_2; gradient: 84% A, 6% B, 10% C, 0−25 min; to 65% A, 25% B, 10% C, 25−35 min; same until 45 min	Goiffon et al., 1999
grapes	extraction to remove fat-soluble compounds; SPE	X	Nucleosil C_{18} (250 × 4.6 mm, 5 μm)	A: 10% CH_2O_2/H_2O; B: CH_3CN; gradient: 5−9% B, 0−5 min; 9−11% B, 5−15 min; 11−15% B, 15−40 min; 15−20% B, 40−50 min; 20−30% B, 50−65 min	Hebrero et al., 1989
grapes	extraction with HCl/MeOH; SPE; hydrolysis to anthocyanins	X	RP-18 Spherisorb (150 × 4.6 mm, 5 μm)	A: 10% CH_2O_2/H_2O; B: MeOH; gradient, anthocyanins: 95−72% A, 0−5 min; gradient, anthocyanidins: 95−91% A, 0−5 min; 91−89% A, 5−10 min; 98−85% A, 10−15 min; 89−75% A, 15−25 min	Gao and Cahoon, 1995
grapes	extraction to remove cutin, extracted in 0.1% HCl/MeOH	X	LiChrosorb RP 18 (250 × 4 mm)	A: CH_3COOH/H_2O, 15:85 (v/v); B: $H_2O/CH_3COOH/MeOH$, 65:15:10 (v/v); C: MeOH; gradient: 1% B, 99% A, 0−2 min; to 3, 5, 11, 25, 48, and 100% B after 10, 15, 20, 25, 30, and 40 min; C increased to 8% after 20 min, then back to 0% after 25 min	Lamikanra, 1989
grapes	SPEs	X	Develosil C_{18} (250 × 4.6 mm, 5 μm)	A: $CH_3COOH/CH_3CN/H_2O/H_3PO_4$ (8:10:80.5:1.5); B: $CH_3COOH/CH_3CN/H_2O:H_3PO_4$ (20:25:53.5:1.5); C: 30% MeOH/H_2O, 0.5% CF_3COOH; D: 70% MeOH/H_2O, 0.5% CF_3COOH; gradient: A to B in 30 min; also C to D in 30 min	Tamura et al., 1994
grapes, red	extracted with acidified MeOH	X	C18 Hypersil ODS (5 μm)	A: 0.3% $HClO_4/H_2O$; B: MeOH; gradient: 27−36% B, 0−11 min; 36−45.2% B, 11−18 min; 45.2−51.2% B, 18−21 min; 51.2−64% B, 21−26 min	Castia et al., 1992
grapes and wine	SPE	X	Ultrabase (150 × 4.6 mm, 5 μm), C-8 RP	A: CH_3COOH/H_2O (10:90 v/v); B: H_2O; gradient: 10−82% A, 0−47 min; 82−100% A, 47−15 min	Ricardo da Silva, 1990
grenadine syrups	none	X	Lichrochart 100 RP 18 (125 × 4 mm, 5 μm)	A: 5% H^+/MeOH; B: CH_3OH; gradient: 15−35% B, 0−15 min; 35% B 15−35 min	Cherif and Ayed, 1997
huckleberry juice	SPE	*f*	(1) Supelcosil C18 (250 × 4.6 mm); (2) Spherisorb ODS-2 (250 × 4.6 mm); (3) PLRP-S polymer (250 × 4.6 mm, 5 μm)	column 1, A: 15% CH_3COOH/H_2O; B: CH_3CN; isocratic: 85% A columns 2 and 3, A: 4% H_3PO_4/H_2O; B: CH_3CN; gradient: 12−16% B, 0−40 min; 16−25% B, 40−45 min	Price and Wrolstad, 1995
lychee fruit	SPE; acid and alkaline hydrolysis to yield aglycons	*g*	PLRP-S (250 × 4.6 mm) for glycosides Aminex HPX-87H (300 × 7.8 mm) for aglycons	glycosides, A: 3.5% H_3PO_4/H_2O; B: CH_3CN; gradient: 6% B, 0−5 min; 6−20% B to 45 min; 20% B for 10 min; 20−100% B in 1 min; 100% B for 10 min; aglycons, isocratic: 0.0045 N H_2SO_4	Lee and Wicker, 1991
onions, red	LLE, SPE, and semipreparative column	X	analytical: Zorbax SB-C_{18}; Stablebound (250 × 4.6 mm, 5 μm)	A: CH_2O_2/H_2O (5:95, v/v); B: MeOH; gradient: 10−24% B, 0−20 min; 24−32% B, 20−32 min; 32−45% B, 32−47 min; 45−50% B, 47−53 min; 50−80% B, 53−54 min; 80−10% B, 58−60 min; rechromatographed, A: CH_2O_2/H_2O (5:95, v/v); B: CH_3CN; gradient: 8−20% B, 0−55 min; 20−80% B, 55−56 min; 80% B, 56−59 min; 80−8% B, 59−60 min	Donner et al., 1997

Table 1 (Continued)

food	sample preparation	guard	stationary phase	mobile phase[a]	references
pomegranate (1995); pomegranate juice (1998)	seeds crushed; peels extracted with MeOH; juice centrifuged and filtered (1998)	X	LiChrochart 100 RP-18 (125 × 4 mm, 5 μm)	A: 5% CH_2O_2; B: MeOH; gradient: 15−35% B, 0−15 min; 35% B, 15−20 min (1995) (15−17 min, 1998)	Gil et al., 1995a,b; Artés et al., 1998
potatoes	extracted with CH_3COOH/MeOH	*h*	Applied Biosystems Brownlee Aquapore RP-18 (220 × 4.6 mm)	A: 10% CH_2O_2/H_2O (v/v); B: CH_3CN; gradient: 0−30% B, 0−30 min; 30% B, 30−35 min; washed with 50% B, 35−40 min	Lewis et al., 1998
potatoes, red-fleshed	LLE, SPE	*i*	PLRP-S (250 × 4.6 mm, 5 μm)	A: CH_3CN; B: 4% H_3PO_4/H_2O; gradient: 10−20% A, 0−25 min; 20% A, 25−30 min	Rogdriguez-Saona et al., 1998
purple passion fruit and fruit of *P. suberosa*	LLE, two SPEs	X	ODS Hypersil (200 × 5 mm, 5 μm)	CH_2O_2/H_2O (1:9, v/v); MeOH/CH_2O_2/H_2O (5:1/4, v/v); gradient: 10% B, 0−4 min; 10−100% B, 4−21 min	Kidøy et al., 1997
raspberry juice, red	filtration and SPE	*j*	Supelcosil LC-18 (250 × 5 mm, 5 μm); Polymer Labs PLRP-5 (250 × 5 mm, 5 μm)	anthocyanidins, Supelcosil A: 15% CH_3COOH/H_2O; B: CH_3CN; isocratic: 85% A and 15% B anthocyanins, two gradients: (1) Supelcosil A: 15% CH_3COOH/H_2O; B: CH_3CN; gradient: 100% A, 0−5 min; 0−5% B, 5−15 min; (2) PLRP-5 A: 4% H_3PO_4/H_2O; B: CH_3CN; gradient: 6% B, 0−10 min; 6−20% B, 10−55 min;20% B, 55−65 min	Boyles and Wrolstad, 1993
rice seeds[k]	LLE	X	Bakerbond standard octadecyl (C_{18}) (250 × 4.6 mm, 5 μm)	A: MeOH/CH_2O_2/H_2O (5:1:4) B: H_2O/CH_2O_2 (9:1); gradient: 0−25 min, 15−90% B; 25−35 min, 90% B	Lee et al., 1998
rice seeds, pigmented[l]	LLE, SPE	X	μBondapak C-18 (300 × 19 mm)	A: MeOH/CH_2O_2/H_2O (5:4:1); B: H_2O/CH_2O_2 (9:1); gradient: 15−90% B, 0−25 min	Lee et al., 1998
strawberry juice	pectolytic enzyme to improve clarification	*m*	Shandon with Spherisorb ODS-2 (100 × 5 mm, 5 μm)	$HClO_4$ (6 g/L); CH_3OH; gradient: 200−300 mL of MeOH/L $HClO_4$ solution, 10 min; 300 mL of MeOH/L, 5 min; 300−400 mL of MeOH/L, 15 min; 400−500 mL of MeOH/L, 15 min; 550−650 mL of MeOH/L, 5 min: 650−950 mL of MeOH/L, 1 min; 950 mL of MeOH/L, 3 min; 950−200 mL of MeOH/L, 1 min	Bakker et al., 1992
wines	detection of anthocyanidins: filtered detection of anthocyanins: hydrolysis (Pinot) or saponification followed by hydrolysis (Cabernet Savignon)	*n*	PLRP-S (250 × 4.6 mm, 5 μm)	A: 4% H_3PO_4/H_2O; B: CH_3CN; gradient, Pinot noir: 10% B, 0−10 min; 10−15% B, 10−12 min; 35% B, 12−17 min; 35−50% B, 17−27 min; gradient, Cabernet Savignon: 10% B, 0−10 min; 10−20% B, 10−35 min; 20−30% A, 35−55 min; 30−100% B, 55−62 min; 100% B, 62−67 min	Wightman et al., 1997
wines, red	none	X	μBondapak C_{18} (250 × 4.6 mm, 10 μm)	A: 4.5% CH_2O_2/H_2O (v/v); B: CH_3CN; gradient: 10−15% B, 0−10 min; 15−20% B, 10−20 min; 20−30% B, 20−37.5 min	Rivas-Gonzalo et al., 1992; Santos et al., 1991; Hebrero et al., 1988

[a] All gradients are linear. [b] Supelguard LC-18-DB. [c] ODS-10 Micro-Guard (40 × 4.6 mm). [d] ODS-10 Micro-Guard (40 × 4.6 mm). [e] Bio-Rad ODS for Supelcosil; Polymer Labs guard for PLRP-S. [f] Bio-Rad ODS-10 guard column for (1) and (2); Polymer Labs guard column for (3). [g] PLRP-S for glycosides. [h] RP-18 (15 × 3.2 mm). [i] Polymer Labs (15 × 4.6 mm). [j] Bio-Rad ODS-10 for Supelcosil; Polymer Labs PLRP for PLRP-5. [k] Different cultivars from those of entry below. [l] Different cultivars from those of entry above. [m] Cartridges SC1 and SC6. [n] PLRP-S (30 × 5 mm).

the A- and C-rings, depending on the substituent attached to C5′. Catechin and epicatechin without the B-ring have masses of 181 Da (Bailey and Nursten, 1994).

Flavones and flavonols have no site of easy bond rupture, so the molecular ion is the most intense peak in the spectrum. Having fewer than four hydroxyl groups leads to a cleavage pattern in which retro-Diels−Alder reactions (RDA) occur, as shown for apigenin in Scheme 1 (Kingston, 1971).

The molecular ion **1** can lose CO to yield a radical cation 28 Da lower in molecular weight, or it can

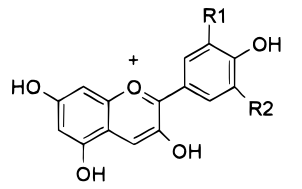
Table 2. HPLC of Catechins

food	sample preparation	guard	column	mobile phase[a]	references
apple skins	freeze-dried, ground, LLE	X	Hypersil ODS (250 × 4.6 mm, 3 μm)	A: 5% CH_3COOH/H_2O (5:95, v/v); B: MeOH; gradient: 9% B, 0−10 min; 9−17% B, 10−25 min; 17% B, 25−30 min; 17−24% B, 30−40 min; 24−40% B, 40−55 min; 40−90% B, 55−95 min; 95% B, 95−110 min	Treutter, 1988; Treutter and Feucht, 1990
apples, black grapes, and canned kidney beans	apples and grapes cut and freeze-dried; beans allowed to leak out; all samples freeze-dried, then ground to powder, LLE	*b*	Inertsil ODS-2 (150 × 4.6 mm, 5 μm)	A: 5% CH_3CN; B: 25% CH_3CN; both in 25 mM PO_4^{3-}, pH 2.4 gradient: 10% B, 0−5 min; 10−80% B, 5−20 min; 80−90% B, 20−22 min; 90% B, 22−25 min; 90−10% B, 25−28 min; 10% B, 28−37 min	Arts and Hollman, 1998
grapes and wines	SPE	X	Superspher 100 RP18 (250 × 4 mm, 4 μm)	A: H_2O; B: 10% CH_3COOH/H_2O (v/v); gradient: 10−80% B, 0−5 min; 80−100% B, 5−29 min; 100% B, 29−45 min	Sun et al., 1998
red wine, beers, apple cider, sour cherry, and blackthorn fruit liqueurs	direct injection after filtration	X	Spherisorb ODS2 (150 × 4.6 mm, 3 μm)	A: H_2O; B: MeOH; C: 4.5% aq CH_2O_2; D: 4.5% aq CH_2O_2/MeOH (90:10, v/v); gradient: 100%A-100% C, 0−10 min; 0−15% D in C, 10−20 min; 15% D in C, 20−30 min; 15−35% D in C, 30−40 min; 35% D in C, 40−45 min; 35−45% D in C, 45−60 min; 45−100% D in C, 60−75 min; 0−50% B in D, 75−175 min; 50−80% B in D, 175−180 min	de Pascual-Teresa et al., 1998
red wines	none	*c*	ODS Hypersil	A: CH_3COOH; B: MeOH; C: H_2O; gradient: 5% A, 15% B, 80% C 5 min; 5% A, 20% B, 75% C, 5−30 min; 5% A, 45% B, 50% C, 30−40 min	Goldberg et al., 1998
red wine	SPE		Spheri-5 RP-18 (220 × 4.6 mm)	A: 10% CH_3COOH; B: H_2O; gradient: 10−82% A, 0−79 min; 82−100% A, 89 min; 100% A, 89−97 min	Revilla et al., 1989
white wine	SPE	*d*	Altech Si C18 (250 × 4.0 mm)	A: 10% CH_3COOH/ H_2O; B: H_2O; gradient: 10% A, 0−79 min; 82% A, 79−89 min; 100% A, 89−95 min	Kovác et al., 1989
very young wines	filter	X	Merck 16056, 250-4, 100 RP-18	A: 1% CH_3COOH/H_2O (v/v); B: 6% CH_3COOH/H_2O (v/v); C: $CH_3COOH/CH_3CN/H_2O$, 5:30:65 (v/v); gradient: 100% A, 0 min; 100% B, 15, 30 min; 90% B, 10% C, 50 min; 80% B, 20% C, 60 min; 70% B, 30% C, 80 min; 100% C, 120 min	Archier et al., 1992
Chinese tea	boiled, countercurrent chromatography Sephadex LH-20 column; Lichrosorb RP-18 (semipreparative)	X	Spherisorb ODS-2 (250 × 4.5 mm, 10 μm)	isocratic: H_2O/CH_3CN/MeOH/ CH_3COOH (79.5:18:2:0.5, v/v)	Amarowicz and Shahidi, 1996
commercial green tea	boiled, extraction to remove caffeine, LLE	X	Hypersil ODS (100 × 4.6 mm, 3 μm)	A: 0.5% CH_3COOH/H_2O; B: 30% CH_3CN in 0.5% CH_3COOH; gradient: 0−100% B, 0−25 min	Copeland et al., 1998
green tea	boiled	*e*	Zorbax Eclipse XDB-C_{18}f	two systems: system 1, A: H_2O + 0.05% CF_3COOH; B: CH3CN + 0.05% CF_3COOH; gradient: 12−21% B, 0−25 min; 21−25% B, 25−30 min; 25−100% B, 30−35 min; system 2, A: H_2O + 0.05% CF_3COOH B: 60:40 MeOH/CH_3CN + 0.05% CF_3COOH; gradient: 10−15% B, 0−5 min; 15−40% B, 5−50 min	Dalluge et al., 1998
green tea	LLE, filtered	*g*	Develosil ODS-HG-5 (150 × 4.6 mm)	A: H_2O/CH_3CN/85% H_3PO_4, 95.45/4.5/ 0.05 (v/v); B: H_2O/CH_3CN/85% H_3PO_4, 49.95/50.0/0.05 (v/v); gradient: 10% B, 0−5 min; 10−30% B, 5−8 min; 30% B, 8−10 min; 30−80% B, 10−15 min; 80% B, 15−20 min	Goto and Yoshida, 1999

Table 2 (Continued)

food	sample preparation	guard	column	mobile phase[a]	references
green tea	none	X	Capcellpak C18 AG120 (250 × 4.6 mm)	isocratic: 0.05% H_3PO_4/H_2O, CH_3CN/EtOAc, 90:12:0.6 (v/v)	Kumamoto and Sonda, 1998
green Chinese tea	extracted with hot water; SPE, LLE	X	Waters RP C_{18} (250 × 2.0 mm, 5 μm)	isocratic: 30% MeOH/H_2O with 0.05% CF_3COOH	Lin et al., 1993
longjing (green) tea	LLE	X	Hypersil ODS (250 × 4.6 mm, 5 μm)	isocratic: H_2O with 0.05% H_2SO_4, CH_3CN, $CH_3CO_2C_2H_5$ (86:12:2, v/v)	Zhu et al., 1999
green, oolong, and black teas	boiled, filtered, LLE, freeze-dried	X	Whatman Partisphere ODS-2	A: 0.5% CH_3COOH/H_2O; B: CH_3CN; gradient from 100% A to 65% A over 60 min	Xie et al., 1993
green, oolong, and black teas	boiled with shaking, pH adjusted to 3.2 with citric acid, diluted, filtered	*h*	Intertsil ODS-2 (150 × 4.0 mm, 5 μm)	A: 5% CH_3CN; B: 25% CH_3CN, both in phosphate buffer, 0.025 M, pH 2.4; gradient: 15% B, 0−15 min; 15−80% B, 5−20 min; 80% B, 20−23 min; 15% B, 23−25 min	Khokhar et al., 1997
green, oolong, pu-erh, and black teas	dried, boiled, filtered, diluted	X	Cosmosil C18-MS (250 × 4.6 mm, 5 μm)	two systems: (a) isocratic: MeOH/H_2O/CH_2O_2 (19.5:80.2:0.3, v/v); (b) A: MeOH/CH_2O_2/H_2O (20:0.3:79.7, v/v); B: MeOH/CH_2O_2 (99.7:0.3, v/v); gradient: 100% A, 0−10 min; 0−10% B, 10−25 min; 10−30% B, 25−60 min; 30% B, 60−75 min	Lin et al., 1998
green, black, and jasmine tea	boiled	*i*	Alltima C_{18} modified silica (250 × 4.6 mm, 5 μm)	three gradients: (1) A: 1.0 mM CH_3COOH, 1.0 mM CH_3COONa/H_2O, pH 4.5; B: CH_3CN; gradient: from 12 to 21% A, 0−18 min; 21−65% B, 18−40 min (2) A: 1.0 mM CH_3COOH, 1.0 mM CH_3COONa in H_2O; B: MeOH; 30−50% A over 40 min (3) A: 1.0 mM CH_3COOH, 1.0 mM CH_3COONa, 0.10 mM ascorbic acid in H_2O; B: CH_3CN; 15−19% B, 0−16 min; 19−31% B, 16−40 min	Bronner and Beecher, 1998
tea	boiled in water or extracted with MeOH, filtered, SPE	*j*	Hypersil ODS (250 × 4.6 mm, 5 μm)	A: 2% CH_3COOH; B: CH_3CN gradient: 88% A, 0−6 min; to 75% A, 6−11 min; 75% A, 11−26 min	Kuhr and Engelhardt, 1991

[a] All gradients are linear. [b] Opti-Guard PR C18 Violet A. [c] LiChrospher 100 RP-18. [d] Co Pelle ODS 30−38 μm. [e] No guard column mentioned in chart. [f] This was the best of seven columns tested. Zorbax Rx-C_{18} and SMT OD-5-100 columns also worked for system 1; Zorbax Rx-C_{18} worked for system 2. [g] Develosil ODS-HG-5 (10 × 4 mm). [h] Opti-Guard PR C18 Violet A. [i] Brownlee ODS-GU (30 × 4.6 mm). [j] Nucleosil C-18 (10 × 4.6 mm).



Anthocyanidin	R1	R2
Cyanidin	H	OH
Delphinidin	OH	OH
Malvidin	OMe	OMe
Pelargonidin	H	H
Petunidin	OMe	OH
Peonidin	OMe	H

Figure 4. Anthocyanidin skeleton.

undergo an RDA to **2** and **3**, one of which will be a radical cation. Radical cation **2** can then lose CO or abstract a hydrogen to become cation **4**. Another pathway is an RDA in which **1** leads to cation **5**.

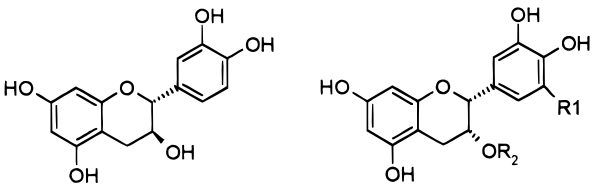
Loss of hydrogen leads to an $(M - H)^+$ peak. This can be from a hydroxyl or possibly a methoxyl hydrogen. Methoxyflavones often show an $(M - CH_3)^+$ peak. Doubly charged ions resulting from aromatic stabilization are common for flavones and flavonols (Berahia et al., 1994; Kingston, 1971).

Anthocyanins. Anthocyanins are acylglycosides and glycosides of anthocyanidins. They are usually C3 monosides, biosides, and triosides, although there are also 3,5- and 3,7-diglycosides (Strack and Wray, 1994).

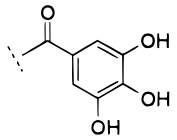
There are six anthocyanidins commonly found in fruit (Figure 4) (Goiffon et al., 1991). The most common is cyanidin. Blueberries contain all but pelargonidin (Robards and Antolovich, 1997). Pelargonidin 3-glucoside gives strawberry most of its red color, although it also contains cyanidin 3-glucoside (Bakker et al., 1992). Pomegranate juices with delphinidin as the main anthocyanidin are violet, whereas those with mainly pelargonidin are scarlet (Gil et al., 1995a).

Analysis of anthocyanins was not carried out after hydrolysis procedures based on Hertog's extraction method (Ewald et al., 1999; Häkkinen et al., 1998; Hertog et al., 1992; Justesen at al., 1998; McDonald et al., 1998; Patil et al., 1995b), despite the analysis of cranberry by Hertog et al. and of strawberry and blackcurrant by Häkkinen et al. Rather, other extraction methods were used, often including SPE, and sometimes followed by MS to aid in the identification of the glycosides.

Table 1 summarizes many of the HPLC systems used for the detection of anthocyanins. The entries are arranged in alphabetical order of the foods.



Catechin	R1	R2
(-)-Epicatechin (EC)	H	H
(-)-Epigallocatechin (EGC)	OH	H
(-)-Epicatechin-3-gallate (ECG)	H	Gall[a]
(-)-Epigallocatechin-3-gallate (EGCG)	OH	Gall



[a]Gallate

Figure 5. (+)-Catechin (left); (−)-epicatechin skeleton (right).

Flavanols (Catechins). Catechins are found mainly in brewed tea (Bronner and Beecher, 1998) and in red wine (Goldberg et al., 1998). The concentrations of catechins are higher in green tea than in black or oolong tea (Khorkhar et al., 1997) as green tea is made from fresh leaf and black tea leaves have dark compounds such as theaflavins and thearubigins due to enzymatic oxidation of polyphenols. Oolong tea, which is partially oxidized, contains much of the original quantity of catechins (Xie et al., 1993).

Figure 5 shows the common catechins (Guyot et al., 1998; Kuhr and Engelhardt, 1991).

Analysis of teas is often done by boiling and filtering, although LLE and even SPE has been used, for example, by Lin et al. (1993). Catechins in wine have been analyzed without sample preparation (Goldberg et al., 1998), although SPE was also used (Kovác et al., 1989; Revilla et al., 1989). Catechins in red wine, beers, apple cider, and fruit liqueurs were measured after filtration (de Pascual-Teresa et al., 1998).

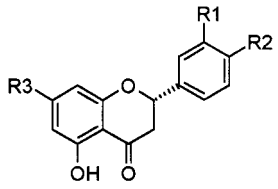
Table 2 lists some of the HPLC systems recently used for the detection of catechins, listing fruits, then wines, and then teas, starting with green teas.

Flavanones. Flavanones are predominant in citrus, where they are usually found as mono- and diglycosides. HPLC of fruit juices shows peaks for flavanone glycosides that vary from fruit to fruit. One of the primary uses of HPLC technology has been to identify adulterated juices (Ooghe et al., 1994; Robards et al., 1997).

Naringin and neohesperidin have been found in grapefruit juices and are important for quality control and bitterness. Hesperidin and neohesperidin have been found in common sweet oranges. High concentrations of eriocitrin and neoeriocitrin have been found in lemon juices and sour oranges, respectively (Mouly et al., 1993, 1994).

The flavanone glycoside naringin and the stilbenes limonin and nomilin also cause juice bitterness (Hasegawa et al., 1996). Figure 6 shows the flavanone skeleton, with the structures of the common flavanones and flavanone glycosides listed.

There is less work apparent in the literature on the HPLC of flavanones and their glycosides than on that of anthocyanins or catechins. Due to their presence in citrus fruits, flavanones and their glycosides are extracted with more difficulty than are catechins. SPE and



Flavanone	R1	R2	R3
Didymin	H	OMe	ORut[a]
Eriocitrin	OH	OH	ORut
Eriodictyol	OH	OH	OH
Hesperetin	OH	OMe	OH
Hesperidin	OH	OMe	ORut
Isosakuranetin	H	OMe	OH
Naringenin	H	OH	OH
Naringin	H	OH	ONeo[b]
Narirutin	H	OH	ORut
Neoeriocitrin	OH	OH	ONeo
Neohesperidin	OH	OMe	ONeo
Pinocembrin	H	H	OH
Poncirin	H	OMe	ONeo
Prunin	H	OH	OGlu[c]

[a]rutinoside; [b]neohesperidose; [c]glucose

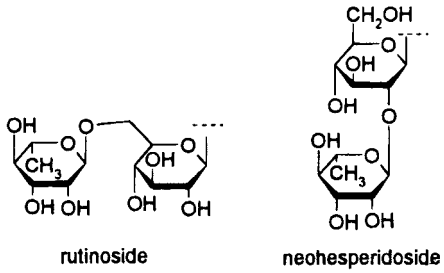


Figure 6. Flavanone skeleton.

even multiple extractions were used (Krause and Galensa, 1991; Perfetti et al., 1988).

Table 3 lists some of the more recent HPLC systems used for the detection of flavanones and flavanone glycosides in foods, listing honey and propolis and then various citrus juices.

Flavones and Flavonols. Flavones and flavonols are usually found in plants as *O*-glycosides. The flavonols have a hydroxyl at C_3, where the flavones have a hydrogen. Glycosides of the flavonol quercetin predominate in vegetables, whereas glycosides of the flavonol kaempferol and of the flavones apigenin and luteolin also exist. In fruits, glycosides of quercetin are usually the only flavonols found, with glycosides of myricetin and kaempferol existing in trace amounts (Hertog et al., 1992). Common flavones and flavonols are listed in Figures 7 and 8, respectively.

The vegetables, herbs, and teas containing flavones, flavonols, and flavonol glycosides were often extracted using LLE and even SPE, after lyophilization. Teas and wines were easier to extract. Semipreparative HPLC was used for green beans (Price et al., 1998c).

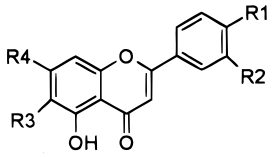
Table 4 lists several modern HPLC methods for the detection of flavones and flavonols in foods, including buckwheat, hops, vegetables, fruits, tea, and wine. Two botanicals are also included.

Isoflavones. About 20 of the 13000 species of legumes are eaten by people. Soy and its products are the most widely studied for their isoflavone content (Mazur et al., 1998). At least 15 isoflavones are found in food, usually as glycosides, although aglycons are found in fermented soy products. Low levels of isoflavone are found in other legumes (Bingham et al., 1998).

Table 3. HPLC of Flavanones and Flavanone Glycosides

food	sample preparation	guard	stationary phase	mobile phase[a]	references
honey and propolis	honey: SPE; propolis: homogenized in EtOH	X	Lichrochart RP 18 (250 mm, 5 μm)	isocratic: H_2O/ MeOH/CH_3COOH (60:75:5, v/v).	Bogdanov, 1989
citrus juices	hand-squeezed, diluted in DMF, diluted in water, centrifuged, filtered	*b*	Alltima (250 × 4.6 mm, 5 μm)	A: H_2O/CH_3COOH (96:4, v/v) B: CH_3CN; gradient: concave, 0% A, 100% B, 0 min; 8% A, 92% B, 12 min; 34% A, 66% B, 43 min; 70% A, 30% B, 44 min linear, 30% B, 44−49 min convex: 30−100% B, 49−50 min	Mouly et al., 1998
citrus juice	SPE	X	Cyclobond I (β-cyclodextrin, 250 × 4.6 mm)	A: H_2O/MeOH/CH_3COOH (90:10:0.5) B: MeOH/H_2O (95:5); gradient: A, 0−1 min; 5−50% B in 25 min	Krause and Galensa, 1991
juices: grapefruit, lemon, lime; sweet, sour orange	diluted in DMF and ammonium oxalate solution, heated, centrifuged	*c*	RP-18 UHS (250 × 4.6 mm, 5 μm)	isocratic: H_2O/CH_3CN/THF/CH_3COOH (80:16:3:1, v/v)	Mouly et al., 1993, 1994
orange and grapefruit concentrates	extracted with MeOH	X	Alltima RP C_{18} modified silica (250 × 4.6 mm, 5 μm)	isocratic: H_2O/CH_3CN/2-propanol/ CH_2O_2, 158:23:19:0.2, (v/v); H_2O/THF (18:7, v/v), qualitative analysis only	Bronner and Beecher, 1995
orange and grapefruit juices	two extractions to remove carotenoids and methoxylated flavones	*d*	Zorbax ODS C18 (250 × 4.6 mm)	A: 1% CH_3COOH/ H_2O B: 1% CH_3COOH/CH_3CN gradient: 20−50% B in 10 min	Marini and Balestrieri, 1995
orange juice	centrifuged, filtered	X	M.S. Gel C_{18} (150 × 4.6 mm, 5 μm)	A: 0.1 M NaH_2PO_4, 10 mg/L SDS, H_3PO_4 to pH 3.35; B: CH_3CN, 0.1 M NaH_2PO_4 with 50 mg/L SDS, MeOH (60:30:10, v/v/v), pH 3.45 with H_3PO_4 gradient: 6% B, 0−10 min; +1.2% B/min, 10−30 min; +7% B/min, 30−40 min; 100% B, 40−45 min	Gamache et al., 1993
orange juice	hot water bath, centrifuged, filtered	X	Novapak RP-18 (150 × 3.9 mm, 4 μm)	A: KH_2PO_4, H_3PO_4, H_2O; B: KH_2PO_4, H_3PO_4, CH_3CN, H_2O; gradient: 100% A, 0−3 min; 58% A, 42% B, 38 min; 100% B, 40−43 min; 100% A, 46−58 min	Ooghe et al., 1994
orange juice	comprehensive recovery scheme	X	Zorbax ODS (250 × 4.6 mm)	A: 1% CH_3COOH/H_2O; B: 1% CH_3COOH/CH_3CN gradient: 20−50% B in 10 min	Perfetti et al., 1988
Brazilian orange juice	fruits hand-squeezed; steamed with DMF and ammonium oxalate	*b*	C18 Nucleosil (250 × 4.6 mm, 5 μm)	isocratic: H_2O/CH_3CN/THF/CH_3COOH, 80:16:3:1 (v/v)	Pupin et al., 1998

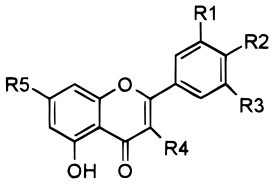
[a] All gradients are linear unless noted. [b] Alltima C18 (7.5 × 4.6 mm, 5 μm). [c] RP-18 UHS (30 × 4.6 mm). [d] Spheri 5 (C18) (50 × 4.6 mm).



Compound	R1	R2	R3	R4
Apigenin	OH	H	H	OH
Baicalein	H	H	OH	OH
Chrysin	H	H	H	OH
Diosmin	OMe	OH	H	ORut[a]
Genkwanin	OH	H	H	OMe
Isorhoifolin	OH	H	H	ORut
Luteolin	OH	OH	H	OH
Rhoifolin	OH	H	H	ONeo[b]
Techtochrysin	H	H	H	OMe

[a]rutinose; [b]neohesperidose (see flavanone section for structures)

Figure 7. Flavone skeleton.



Compound	R1	R2	R3	R4	R5
Astragalin	H	OH	H	OGlu[a]	OH
Hyperoside	OH	OH	H	OGal[b]	OH
Isoquercitrin	OH	OH	H	OGlu	OH
Isorhamnetin	OMe	OH	H	OH	OH
Kaempferide	H	OMe	H	OH	OH
Kaempferol	H	OH	H	OH	OH
Myricetin	OH	OH	OH	OH	OH
Quercetin	OH	OH	H	OH	OH
Quercitrin	OH	OH	H	ORham[c]	OH
Rhamnetin	OH	OH	H	OH	OMe
Rutin	OH	OH	H	ORut[d]	OH

[a]glucose; [b]galactose; [c]rhamnose; [d]rutinose

Figure 8. Flavonol skeleton.

Figure 9 shows the structures of common isoflavones.

Isoflavones are analyzed from soybeans and soy-based foods generally after LLE, which may be preceded by grinding, although direct injection was used for soy sauces by Kinoshita et al. (1997, 1998). Acidic hydrolysis is common (Table 5). Enzymatic hydrolysis was also used (Franke et al., 1994, 1995).

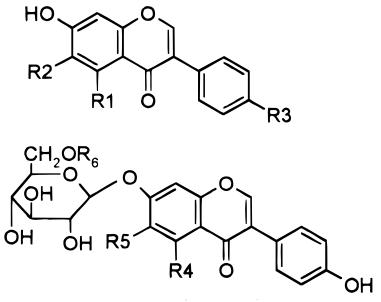
Table 4. HPLC of Flavones and Flavonols

food or herb	sample preparation	guard	stationary phase	mobile phase[a]	references
buckwheat	dried, ground, extracted with MeOH, Soxhlet	X	Nucleosil 7C_{18} (250 × 6 mm)	isocratic: 2.5% CH_3COOH/MeOH/CH_3CN (35:5:10)	Minami et al., 1998
hops	extraction to remove nonpolar components; LLE	*b*	Spherical LiChrospher 100 CH-18/2 (250 × 4 mm, 5 μm)	A: CH_2O_2/H_2O (1:19; v/v); B: CH_3CN/MeOH (1:19, v/v); gradient: 15% B, 0−3 min; 15−24% B, 3−8 min; 24% B, 8−11 min; 24−34% B, 11−18 min; 34−44% B, 18−28 min; 44−81% B, 28−36 min; 81−95% B, 36−42 min; 95% B, 42−50 min; 95−15% B, 50−57 min; 15% B, 57−60 min	De Cooman et al., 1998
bean, seed coat	seed coat removed, then lyophilized	X	Shiseido Capcell Pak RP C_{18} (250 × 4.6 mm, 5 μm)	isocratic: CH_3CN/H_2O (30:70, v/v), 0−20 min	Beninger et al., 1998
beans, green	LLE, SPE, then semi-preparative HPLC	X	Prodigy 5u ODS3 (250 × 4.6 mm)	A: H_2O/THF/TFA, 98:2:0.1 (v/v); B: CH_3CN; gradient: 17% B, 0−2 min; 17−25% B, 2−7 min; 25−35% B, 7−15 min; 35−50% B, 15−20 min; 50−90% B, 20−25 min; 17% B, 25−40 min for reequilibration	Price et al., 1998c
beans, green and yellow French	lyophilized, LLE, SPE	*c*	LiChrospher 100 RP-18 endcapped (250 × 4 mm, 5 μm)	A: CH_3CN; B: 2% CH_3COOH; gradient: 10−30% A, 0−35 min; 30−45% A, 35−37 min; 45% A, 37−42 min; 45−10% A, 42−44 min; 10% A until done	Hempel and Böhm, 1996
green beans, onions, and peas	processed, then prepared according to commercially available instructions; hydrolysis in HCl/H_2O/MeOH with TBHQ	X	Inertsil ODS-2 (150 × 4.6 mm, 7 μm)	isocratic: 30% CH_3CN in 0.025 M KH_2PO_4 (pH 2.4)	Ewald et al., 1999
celery, lettuce, onions, and tomatoes	tomatoes and onions cooked; lyophilized, powdered with mortar and pestle	*d*	Symmetry C_{18} (150 × 3.9 mm, 5 μm)	gradient: 15−35% CH_3CN/H_2O, pH 2.5 with CF_3COOH, 20 min	Crozier et al., 1997
vegetables and fruits: cranberry, endive, leek, lettuce, amd onion	lyophilized, ground, LLE, hydrolyzed with 1.2 M HCl/50% MeOH, refluxed, sonicated, filtered	*e*	Nova-Pak C_{18} (150 × 3.9 mm, 4 μm)	two isocratic mobile phases: 25% CH_3CN in 0.025 M KH_2PO_4 (pH 2.4) and 45% MeOH in 0.025 M KH_2PO_4 (pH 2.4)	Hertog et al., 1992
onion bulbs	LLE	X	Shim-pack CLC-ODS (150 × 6 mm)	isocratic: MeOH/25 mM KH_2PO_4 (1:1, v/v)	Hirota et al., 1998
onions	extraction to remove fat-soluble materials, two SPEs, hydrolysis in HCl, LLE	X	C_{18} Radial-Pak (100 × 8 mm)	A: 5% CH_3COOH; B: MeOH; gradient: 20−100% B, 0−25 min	Park and Lee, 1996
onions	blended with EtOH and filtered, twice; hydrolyzed in 2 N HCl	*f*	Bondapak C-18 (250 × 4.6 mm, 10 μm)	A: 0.5% H_3PO_4/H_2O; B: 0.5% H_3PO_4/MeOH; gradient: 40−90% B, 0−10 min; 90% B, 3.5 min	Patil et al., 1995a,b
fruits and vegetables	LLE, hydrolysis with HCl to remove glycosides, SPE	X	Lichrosorb RP 18	isocratic: 40% THF containing 1% CH_3COOH.	Mizuno et al., 1992
broccoli florets; tea	tea: boiled, LLE, semi-preparative HPLC; broccoli: some raw, some cooked, all freeze-dried and LLE	X	Prodigy 5u ODS3 RP silica gel (250 × 4.6 mm)	A: H_2O/THF/CF_3COOH (98:2:0.1, v/v); B: CH_3CN; gradient: 17% B, 0−2 min; 17−25% B, 2−7 min; 25−35% B, 7−15 min; 35−50% B, 15−20 min	Price et al., 1998a,b
tea liquors, black	boiled and filtered	X	Hypersil ODS C_{18} (250 × 4.9 mm, 5 μm, pore size 12 nm)	A: 1% (w/v) citric acid with NaOH; to pH 2.8; B: CH_3CN; gradient: 8−31% B, 0−50 min	Bailey et al., 1991 McDowell et al., 1995
tea	SPE	*g*	Hypersil-ODS, (250 × 4.6 mm, 5 μm)	flavonol diglycosides, isocratic: 2% CH_3COOH/CH_3CN (85:15, v/v) flavonol triglycosides, gradient: 2% CH_3COOH/CH_3CN (17:3); 2% CH_3COOH/1,4-dioxane/MeOH (77:13:10)	Finger et al., 1991a,b

Table 4 (Continued)

food or herb	sample preparation	guard	stationary phase	mobile phase[g]	references
teas	boiled	X	Hypersil ODS (250 × 4.6 mm, 5 μm)	A: 2% CH_3COOH/H_2O; B: CH_3CN; gradient: 8−31% B, 0−50 min	McDowell et al., 1991; Bailey et al., 1990
health tea and green tea	extracted with boiling water, acidified with 6 N HCl, LLE	*h*	Inertsil ODS-3 (C_{18}, 4.5 × 250 mm, 5 μm)	isocratic: 0.5% H_3PO_4/MeOH (1:1, v/v)	Toyoda et al., 1997
vegetables and red wine	powdered or minced; homogenized and centrifuged; phenylboric acid cartridges used before HPLC for all samples	X	Shim-pack CLC-C8 (M) (250 × 4.6 mm, 5 μm)	isocratic: H_2O/CH_3CN/TFA, 71:28:1 (v/v)	Tsuchiya, 1998
red wines	hydrolysis, LLE	*i*	Genesis C18 cartridge (150 × 3.0 mm, 4 μm)	gradient: 20−40% of CH_3CN in H_2O, CF_3COOH to pH 2.5, 20 min	McDonald et al., 1998
white wine	LLE	X	Novapak C18 (150 mm × 3.9 mm, 5 μm)	flavonols: isocratic with H_2O/MeOH/CH_3COOH (55:40:5, v/v) flavonol glycosides: isocratic with H_2O/THF/CH_3COOH (80:17.5:2.5, v/v)	Kovác et al., 1989
roots for munkoyo beverage	extracted with aq MeOH	X	μ-Bondapak C-18 phenyl (300 × 3.9 mm)	A: H_2O/CH_3COOH (19:1, v/v); B: MeOH/CH_3COOH/H_2O (18:1:1, v/v); gradient: 25−100% B, 0−23 min	Zulu et al., 1994
Gingko biloba	extracted with 60% aqueous acetone	X	C_8 Aquapore RP 300 (250 × 4 mm, 7 μm)	A: H_2O/2-propanol (95:5); B: 2-propanol/THF/H_2O (40:10:50); gradient: 20−60% B, 0−40 min	Pietta et al., 1991
St. John's wort	extracted with hot MeOH	*j*	201 TP 54 RP-18 (250 × 4 mm, 5 μm, 300 Å)	A: H_2O/85% H_3PO_4 (99.7:0.3, v/v); B: CH_3CN; C: MeOH; gradient: 100% A-85% A, 15% B, 0−10 min; to 70% A, 20% B, 10% C, 10−30 min; to 10A %, 75% B, 15% C, 30−40 min; to 5% A, 80% B, 15% C, 40−55 min; to 100% A, 55−56 min; 100% A, 56−65 min	Brolis et al., 1998

[a] All gradients are linear. [b] Irregular LiChrosorb RP-18 (30 × 4 mm, 7 μm). [c] LiChrospher 100 RP-18 (4 × 4 mm). [d] C_{18} Symmetry (20 × 3.9 mm, 5 μm). [e] Perisorb RP-18 (40 × 3.9 mm, 330−40 μm). [f] Bondapak C-18. [g] Nucleosil C18 (10 mm × 4.6 mm, 5 μm). [h] Inertsil ODS-3 (C_{18}, 10 × 4.0 mm, 5 μm). [i] C18 Genesis cartridge (10 × 4.0 mm, 4 μm). [j] Alltech direct-connect universal column prefilter of 2 μm porosity.



Compound	R1	R2	R3
Biochanin A	OH	H	OMe
Daidzein	H	H	OH
Formononetin	H	H	OMe
Genistein	OH	H	OH
Glycitein	H	OMe	OH

Compound	R4	R5	R6
Daidzin	H	H	H
Genistin	OH	H	H
Glycitin	H	OMe	H
6"-O-Acetyldaidzin	H	H	$COCH_3$
6"-O-Acetylgenistin	OH	H	$COCH_3$
6"-O-Acetylglycitin	H	OMe	$COCH_3$
6"-O-Malonyldaidzin	H	H	$COCH_2COOH$
6"-O-Malonylgenistin	OH	H	$COCH_2COOH$
6"-O-Malonylglycitin	H	OMe	$COCH_2COOH$

Figure 9. Twelve isoflavone isomers (Liggins et al., 1998; Wang and Murphy, 1994).

Table 5 lists several modern HPLC methods for the detection of isoflavones in soy and soy products. The first entry is infant formula. Entries that employed similar columns are listed sequentially.

There are many foods with more than one subclass of flavonoids. Table 6 shows methods of modern HPLC analysis for foods containing two or more subclasses, including (in order) buckwheat, honey, vegetables, fruits, berries, jams, wines, and teas.

DISCUSSION

Knowledge of the flavonoid content of plant-based foods is paramount to understanding their role in plant physiology and human health. In addition, such knowledge has been employed as the bases of chemotaxanomic systems (Robards and Antolovich, 1997), which have been extended to the identification of adulteration of beverages as well as several other uses. Although other modern separation systems have been used to a limited extent for the measurement of flavonoids in foods, that is, capillary zone electrophoresis (Andrade et al., 1998; Arce et al., 1998; Bridle et al., 1997; Costa et al., 1998; Pietta et al., 1994; Prasongsidh and Skurray, 1998) and micellar electrokinetic capillary chromatography (Ferreres et al., 1994a; Hilhorst et al., 1998), by far the most widely employed technique has been HPLC. The most often used columns have been packed with reversed-phase C_{18} column material. Packings of the C_8 type have

Table 5. HPLC of Isoflavones

food	sample preparation	guard	stationary phase	mobile phase[a]	references
American ground-nut tubers	sliced, LLE, SPE	X	C_{18} reversed phase[b]	MeOH, 25−100%[c]	Krishnan, 1998
infant formula	LLE	*d*	YMC-Pack ODS-AM 303 (250 × 4.6 mm, S-5 μm 120 Å)	A: 0.1% CH_3COOH/H_2O; B: CH_3COOH/CH_3CN[e]	Garrett et al., 1999
commercial soybean foods	ground, LLE	X	YMC-Pack ODS-AM 303 (250 × 4.6 mm)	A: 0.1% CH_3COOH/H_2O; B: CH_3COOH/CH_3CN; gradient: 15−35% B, 0−50 min; 35% B, 50−60 min	Wang and Murphy, 1994a,b
soy sauces	none (direct injection)	*f*	Wakosil-II 5C18 HG (250 × 4.6 mm)	A: 0.05% CF_3COOH/H_2O; B: 0.05% $CF_3COOH/CH_3CN/H_2O$ gradient: 100% A, 0−20 min; 0−25% B, 20−290 min; 25−50% B,290−340 min	Kinoshita et al., 1997, 1998
soybean foods	extracted with 80% MeOH (aq), LLE	X	Brownlee Aquapore C_8 reversed-phase (300 × 4.5 mm)	A: 0.1% (v/v) CF_3COOH/H_2O; B: CH_3CN; gradient: 0−46.4% B, increasing by 2.25%/min	Coward et al., 1993
soy foods	extracted with either 80% $MeOH/H_2O$ or 80% CH_3CN/ 0.1% HCl	X	Aquapore C_8 (250 × 4.6 mm)	A: either 0.1% CF_3COOH or 2 or 10 mM NH_4OAc; B: CH_3CN; gradient: 0−50% B, periods ranging from 0−10 to 0−30 min; 100% B for 5 min	Barnes et al., 1994
soy foods	LLE	X	Aquapore C_8 reversed-phase (100 × 4.6 mm, 300 Å)	A: 10 mM NH_4OAc, pH 6.5; B: CH_3CN; gradient: 0−50% B, 0−10 min	Barnes et al., 1998
soybean cotyledons; soy; soybean seedling tissues	ground; LLE	*g*	Hibar Ec containing Merck Lichrosorb RP 18 10 μm C18 reverse phase packing (250 × 4.6 mm)	A: H_2O, pH 3;[h] B: CH_3CN; gradient: 0−55% B, 0−25 min; step increase to 100% CH_3CN, held for 2 min; step return to 100% A	Graham et al., 1990; Graham, 1991a,b
soybean	ground, LLE/ acidic hydrolysis	X	NovaPak C_{18} reversed-phase (150 × 3.9 mm, 4 μm)	A: CH_3CN; B: 1% CH_3COOH/H_2O (v/v); isocratic: 33% A, 67% B.	Hutabarat et al., 1998
>40 food items, mostly legumes	powdered or lyophilized; acidic or enzymatic hydrolysis	*i*	NovaPak C_{18} reversed-phase (150 × 3.9 mm, 4 μm)	A: CH_3COOH/H_2O (10:90, v/v); B: CH_3CN; gradient: 23−70% B, 0−8 min; 23% B, 8−20 min for equilibration	Franke et al., 1994, 1995
soy foods	acidic hydrolysis or extraction with $MeOH/H_2O$	*i*	NovaPak C_{18} reversed-phase (150 × 3.9 mm, 4 μm)	A: CH_3COOH/H_2O (10:90, v/v); B: $MeOH/CH_3CN/CH_2Cl_2$ (10:5:1, v/v); gradient: 5% B, 0−5 min; 5−45% B, 5−45 min; 45−70% B, 45−51 min; 70−5% B, 51−54 min; 5% B, 54−69 min for equilibration	Franke et al., 1998
soy products	lyophilized, ground, LLE	X	reversed-phase Spherisorb 5-μm ODS 2 (250 × 4.6 mm)	CH_3CN/H_2O to pH 7.5 with Kolthoff's borax−phosphate mixture; A: buffered to 10% CH_3CN/H_2O; B: buffered to 40% CH_3CN/H_2O; gradient: 100% A-100% B, 0−30 min; 100% B, 30−50 min	Jones et al., 1989
soybean and its processed products	ground or blended, LLE/acidic hydrolysis	*j*	μ-Bondapak C_{18} (300 × 3.9 mm, 10 μm)	isocratic: MeOH/1 mM NH_4OAc (6:4)	Wang et al., 1990

[a] All gradients are linear. [b] Brand not listed. [c] Times not listed. [d] Hichrom RPB (10 × 0.3 mm) [e] Gradient not listed, but article refers to Barnes et al. (1994). [f] Wakosil-II 5C18 HG (30 × 4.6 nm). [g] Merck Lichrosorb RP 18 10 μm C18 reverse phase packing. [h] 1990 paper does not mention pH 3. [i] Adsorbosphere C18 (10 × 4.6 mm, 5 μm). [j] C_{18}/Corasil (37−50 μm).

been employed to a limited extent and then only when the flavonoids that were separated were somewhat more polar, for example, aglycons and glycosides of isoflavones (Barnes et al., 1994, 1998; Coward et al., 1993).

It is interesting to note that separation systems for flavonoids in foods have been oriented toward the measurement of all (usually several subclasses) of the prominent flavonoids in a single food, that is, wine (Lamuela-Raventós and Waterhouse, 1994), tea (Liang et al., 1990; Powell et al., 1993; Shao et al., 1995), apples (Lister et al., 1994), etc., or procedures which quantify a single or a few subclasses in several foods (Hertog et al., 1992). Many of these analytical systems have been employed to investigate several aspects related to plant physiology including response to environmental changes, differences among species and/or cultivars, changes during ripening, etc. A few procedures were developed to specifically measure flavonoid concentrations in

Table 6. HPLC of Foods Containing Multiple Subclasses of Flavonoids

food	flavonoid	sample preparation	guard	stationary phase	mobile phase[a]	reference
buckwheat	catechins and rutin	ground, LLE, SPE, semi-preparative HPLC	X	Cosmosil 5C18 (250 × 4.6 mm)	A: 2.5% CH_3COOH/H_2O; B: MeOH; two gradients (different semi-preparative HPLC fractions): 23−50% B, 0−40 min; 5−95% B, 0−30 min	Watanabe, 1998
honey	flavanones, flavones, and flavonols	LLE	X	reverse-phase ChromSpher C18 (100 × 3 mm, 5 μm)	A: H_3PO_4/H_2O, pH 2.6; B: CH_3CN; gradient: 0−9% B, 0−12 min; 9−13% B, 12−20 min; 13−40% B, 20−40 min; 40−70% B, 40−50 min	Amiot et al., 1989
honey	hesperitin, flavanones, and apigenin	two SPEs	X	(1) Spherisorb ODS-2 (250 × 4 mm, 3 μm); (2) LiChro-CART RP-18 (125 × 5 mm, 5 μm)	(a) A: H_2O CH_2O_2 (95:5); B: MeOH gradient: 40−45% B, 0−10 min; 45−60% B, 10−35 min; 60% B, 35−50 min; (b) A: H_2O + 5% CH_2O_2; B: CH_3CN; gradient: 20% B, 0−5 min; 20−25% B, 5−15 min; 25−35% B, 15−30 min; 35% B, 30−50 min; (c) A: MeOH/THF/H_2O with 5% CH_2O_2 (25:15:60); B: MeOH; gradient: 100% A, 0−5 min; 0−10% B, 5−20 min; 10−25% B, 20−30 min; 25% B, 30−40 min	Tomás-Barberán et al., 1993
honey	pinocembrin, flavones, and flavonols	two SPEs	X	Lichrochart RP-18 (100 × 4 mm, 5 μm)	isocratic: MeOH/H_2O/CH_2O_2 (50:47:3, v/v)	Ferres et al., 1991
honey	flavanones, flavones, and flavonols	SPEs	X	LiCrochart RP-18 (125 × 4 mm, 5 μm)	A: H_2O/CH_2O_2 (19:1); B: MeOH; gradient: 30% B, 0−15 min; 30−40% B, 15−20 min; 40−45% B, 20−30 min; 45−60% B, 30−50 min; 60−80% B, 50−52 min; 80% B, 52−60 min	Ferreres et al., 1994b; Soler et al., 1995
citrus honey	flavanones, flavones, and flavonols	two SPEs	X	Lichrochart RP-18 (125 × 4 mm, 5 μm)	A: H_2O/CH_2O_2, 5%; B: MeOH; gradient: 40−45% B, 0−10 min; 45−60% B, 10−35 min	Ferres et al., 1993
sunflower honey	flavanones, flavones, and flavonols	LLE, hydrolyzed in 2 N NaOH	b	Lichrosorb RP 18 (200 × 3 mm, 7 μm)	A: CH_3CN to pH 2.6 with H_3PO_4; B: CH_3CN; gradient: 0−9% B, 0−12 min; 9−13% B, 12−20 min; 13−40% B, 20−40 min; 40−70% B, 40−50 min	Sabatier et al., 1992
apple, eggplant, onion, and tomato	all five subclasses	lyophilized; LLE	X	Nova-Pak C_{18} (250 × 4.6 mm, 4 μm)	A: 20% MeOH in 0.1% HCl; B: CH_3CN; gradient: 5% B, 0−10 min; 5−50% B, 10−50 min; 50−5% B, 50−55 min; 5% B, 55−60 min	Paganga et al., 1999
fruits, vegetables, and beverages	flavanones, flavones, and flavonols	lyophilized, extracted in MeOH/H_2O	*c*	RP C_{18} (250 × 4.6 mm, 5 μm)	A: MeOH/H_2O, 30:70 (v/v) with 1% CH_2O_2; B: MeOH; gradient: 25−86% B in 50 min	Justesen et al., 1998
fresh squeezed and concen-trated juices	flavanones, flavones, and flavonols	LLE	X	(1) Nova-Pak C18 (150 × 3.9 mm, 5 μm); (2) Nova-Pak C18 (100 × 3.9 mm 3 μm)	(1) A: PO_4^{3-} to pH 3.05; B: CH_3CN/H_2O, 7:3 (v/v); gradient: 100% A to 42% B, 0−38 min; 42−100% B, 42−54 min; (2) A: H_2O; B: CH_3CN/H_2O, 7:3 (v/v); gradient: 100% A, 0−3 min; to 21% CH_3CN, 3−38 min; to 100% B, 38−43 min; 100% B, 43−46 min; then to 100% A	Robards et al., 1997
apples	catechins and quercetin glycosides	extracted with MeOH	X	Waters Nova Pack C_{18} RP cartridge	A: THF; B: 1 g/L CF_3COOH/H_2O; concave gradient: 20−25% A, 0−5 min; 25−35% A, 5−10 min; 35−60% A, 10−15 min; 60−75% A, 15−17 min; 75−100% A, 17−20 min	McRae et al., 1990
apples	catechins and flavonols	LLE	X	NovaPak C_{18} (30 × 3.9 mm)	A: H_2O/CH_3COOH, 98:2 (v/v); B: $H_2O/CH_3CN/CH_3COOH$, 78:20:2, (v/v); gradient (curve 5): 100% A, 0−2 min, 0−40% B, 2−10 min; 40−50% B, 10−15 min; 50−60% B, 15−20 min; 60−70% B, 20−35 min; 70−75% B, 35−42 min; 75−85% B, 42−45 min; 85% B, 45−50 min; 85−90% B, 50−75 min; 90% B-100% A, 75−80 min	Pérez-Ilzarbe et al., 1991

Table 6 (Continued)

food	flavonoid	sample preparation	guard	stationary phase	mobile phase[a]	reference
two apple peel cultivars	quercetin glycosides and cyanidin glycosides	extracted with CH_3COOH/MeOH	*d*	Applied Biosystems Aquapore RP-18 (220 × 4.6 mm)	A: CH_3COOH/H_2O, 1:10 (v/v); B: CH_3CN; gradient: 0−20% B, 0−20 min	Lister et al., 1994
French cider apple	anthocyanins, catechins, and flavonols	extraction to remove lipids, carotenoids, and chlorophyll; two more LLEs; BuOH−HCl hydrolysis or thiolysis of some extract	X	Nova-Pak C_{18} (100 × 3.9 mm, 4 μm)	A: 2.5% CH_3COOH/H_2O; B: CH_3CN; gradient: 3% B, 0−3 min; 3−9% B, 3−13 min; 9−11% B, 13−18 min; 11−18% B, 18−25 min; 18% B, 25−30 min; 18−30% B, 30−45 min	Guyot et al., 1998
apple peels and pulp	catechins and flavonol glycosides	peels separated from pulp, peel homogenized, pulp cut into pieces; LLE	X	Nucleosil 120 C_{18} (250 × 4.6 mm, 5 μm)	A: 0.01 M H_3PO_4/H_2O; B: MeOH.; gradient: 5% B, 10 min; 50% B for 10 min; 70% B for 5 min; 80% B for 5 min; 100% B for 5 min	Escarpa and González, 1998
apple juice	catechins and flavonol glycosides	extracted with EtOAc	X	(1) Spherisorb ODS-2 (250 × 4.6 mm, 3 μm); (2) Nova-Pak C_{18} (300 × 3.9 mm, 4 μm)	A: H_3PO_4 to pH 2.80; B: MeOH; curved gradient: 2−42% B, 0−50 min, curve 6; 42−50% B, 50−60 min, curve 6; 50% B, 60−75 min; 50−2% B, 75−77 min (curve 6)	Suárez Vallés et al., 1994
citrus juices	flavanones, flavones, and flavonols	SPE	X	RP-18 (Hewlett-Packard) (125 × 4 mm, 5 μm)	A: 0.01 M MeOH; B: MeOH; gradient: 20% B, 0−2 min; 20−100% B, 2−55 min	Kawaii et al., 1999
unshiu, hirado-buntan (Japanese citrus)	flavanones, flavones, and flavonols	centrifuge and SPE	X	LiChrospher RP C_{18} (250 mm × 4.0 mm, 5 μm)	A: 0.01 M H_3PO_4; B: MeOH; gradient: 30−45% B, 0−55 min; 45−100% B, 55−95 min; 100% B, 95−100 min	Nogata et al., 1994
lemons	flavanones, flavones, and rutin	LLE	X	C18 RP (250 × 4 mm)	A: 0.01 M H_3PO_4; B: MeOH; gradient: 20−100% B, 0−55 min	Vandercook et al., 1989
peaches, Cresthaven	anthocyanidins, catechins, and flavonols	lyophilized, sonicated in MeOH	X	C_{18} RP Pecosphere (80 mm, 3 μm)	A: H_2O with 0.1% H_3PO_4; B: MeOH with 0.1% H_3PO_4; gradient: 5−95% B, 0−30 min	Senter et al., 1989
plums, Agen	anthocyanins and rutin	freeze-dried, extracted to remove carotenoids, LLE	*e*	anthocyanins: Bondapak $C_{18}W_3$ column (300 × 7.8 mm) polyphenols (rutin): Spherisorb ODS 2 (250 × 4.6 mm, 5 μm)	anthocyanidins: A: CH_2O_2/MeOH/H_2O, 10:50:40 (v/v); B: CH_2O_2/H_2O, 10:90 (v/v); gradient: 5−30% A, 0−20 min; 30−100% A, 20−40 min; rutin A: CH_3COOH/H_2O, 5:95 (v/v); B: $CH_3COOH/CH_3CN/H_2O$, 5:80:15 (v/v); gradient: 100% A to 22% B, 0−50 min	Raynal et al., 1989
strawberries	anthocyanins, catechins, and flavonols	LLE, SPE	X	Waters Spherisorb S5-ODS2 (250 × 4.6 mm)	A: 2.5% CH_3COOH/H_2O (v/v); B: CH_3CN; gradient: 0−10% B, 0−5 min; 10−30% B, 5−25 min; 30−50% B, 25−45 min	López-Serrano and Ros Barceló, 1999
berries	catechins and flavonols	LLE	*f*	ODS-Hypersil (100 × 4 mm, 3.5 μm)	A: 50 mM $(NH_4)H_2PO_4$, pH 2.6; B: 0.2 mM H_3PO_4, pH 1.5; C: 20% A in 80% CH_3CN; gradient: 100% A, 0−5 min; 96% A, 4% C, 5−15 min; to 92% A, 8% C, 15−25 min; to 92% B, 8% C, 25−25.01 min; to 80% B, 20% C, 25.01−45 min; to 70% B, 30% C, 45−50 min; to 60% B, 40% C, 50−55 min; to 20% B, 80% C, 55−60 min; to 20% B, 80% C, 60−65 min; to 100% A, 65−70 min	Häkkinen et al., 1998
seven types of jam	flavanones and flavonols	two LLEs, SPE	X	Lichrochart 100 RP-18 RP (125 × 4 mm, 5 μm)	A: H_2O/CH_2O_2 (95:5); B: MeOH; gradient: 5−30% B, 0−20 min; 30−50% B, 20−25 min; 50−80% B, 25−35 min	Tomás-Lorente et al., 1992

Table 6 (Continued)

food	flavonoid	sample preparation	guard	stationary phase	mobile phase[a]	reference
red raspberry jam	anthocyanins and flavonols	LLE, SPE	X	anthocyanins: Lichrochart 100 RP-18 (125 × 4 mm, 5 μm); flavonols: Lichrosorb RP-18 (250 × 4 mm, 5 μm)	A: 50 mL/L CH_2O_2; B: MeOH; anthocyanins, gradient: 15−30% B, 0−15 min; 30% B, 15−20 min; 30−95% B, 20−25 min; flavonols, gradient: 20−50% B, 0−20 min; 50−60% B, 20−30 min; 60−95% B, 30−35 min	García-Viguera et al., 1998
grapes and wine	anthocyanins and flavonols	LLE	X	PLRP-S (250 × 4.6 mm, 100 Å, 5 μm)	isocratic: 1.5% H_3PO_4, 19.7% CH_3CN, 78.8% H_2O, 100 min	Price et al., 1995
white juices and wine	procyanidins, catechins, and flavonols	juices centrifuged; wines concentrated to remove EtOH	X	Nucleosil 120 C_{18} (250 × 4 mm, 5 μm)	A: H_2O to pH 2.65 with CH_3COOH; B: 20% A/80% CH_3CNl; gradient: 100% A initially; 2% B, 5 min; 4% B, 10 min; 10% B, 15 min; 20% B, 30 min; 30% B, 35 min; 100% B, 40 min; 100% A, 45 min	Betés-Saura et al., 1996
wines	catechins and flavonols	none	*g*	ODS-Hypersil (250 × 4 mm, 5 μm)	A: CH_3COOH; B: MeOH; C: H_2O; gradient: 5% A, 15% B, 80% C, 0−5 min; 5% A, 20% B, 75% C, 5−30 min; 5% A, 45% B, 50% C, 30−40 min	Goldberg et al., 1996
wines	catechins and flavonols	none (direct injection)	X	Spherisorb S5 ODS2 (250 × 4.6 mm)	A: H_2O CH_2O_2 (98:2); B: MeOH/H_2O/CH_2O_2 (69:29:2); gradient: 100% A, 0−3 min; 0−10% B, 3−10 min; 10−40% B, 10−60 min; 40−60% B, 60−80 min; 60−100% B, 80−105 min; 100% B, 105−120 min; 100% B−100% A, 120−140 min	Ho et al., 1999
red wine	anthocyanidins, catechins, and rutin	filtered	X	Novapack C18 (150 × 3.9 mm, 4 μm)	A: 50 mM $(NH_4)H_2PO_4$ to pH 2.6 with H_3PO_4; B: 20% A with 80% CH_3CN; C: 0.2 M H_3PO_4 adjusted with ammonia to pH 1.5; gradient: 100% A, 0−5 min; to 96% A, 4% B, at 15 min; to 92% A, 8% B, at 25 min; to 8% B, 92% C, at 25.01 min; to 20% B, 80% C, at 45 min; to 30% B, 70% C, at 50 min; to 40% B, 60% C, at 55 min; to 80% B, 20% C, at 60 min; to 100% A, at 65 min	Lamuela-Raventós and Waterhouse, 1994
red Spanish wines	anthocyanins, catechins, and flavonols	diluted and filtered	*h*	two columns: Hypersil BDS-C18 (125 × 3 mm, 3 μm); Nucleosil 120 C-18 (250 × 4.6 mm, 5 μm)	A: H_2O to pH 2.65 with CH_3COOH; B: 20:80 CH_3COOH/CH_3CN; gradient: 25−100% B, 0−50 min	Larrauri et al., 1999
red raspberry juice	catechins, flavones, and flavonols		*i*	C_{18} Spherisorb ODS-1 (250 × 4.6 mm, 5 μm)	A: 1% CH_3COOH/H_2O; B: CH_3CN; gradient: 5 min at 16% B, to 19% B in 30 min; 5 min at 19% B, to 30% B in 7 min; to 50% B in 10 min; to 100% B in 5 min; 5 min at 100% A, to 16% B in 5 min	Rommel et al., 1993
two Chinese green teas	catechins and flavonols	catechins: EtOH/H_2O extraction; flavonols: LLE, SPE	X	catechins: μ-Bondapak C18 (300 × 3.9 mm); flavonols: μ-Bondapak fatty acid column (300 × 4 mm)	catechins: A: CH_3COOH/MeOH/H_2O, 1:1:98 (v/v); B: CH_3COOH/MeOH/Me_2NH/H_2O, 1:1:50:48 (v/v); gradient: 20% A to 100% B, 0−25 min; flavonols: isocratic, MeOH/H_2O, 0.555:1 (v/v), H_3PO_4 to pH 3.0	Liang et al., 1990
green, black, and Pu'er teas	catechins and flavonols	extraction to remove caffeine	X	Hypersil ODS (100 × 4.6 mm, 3 μm)	A: CH_3COOH/H_2O, 1:200 (v/v); B: A in 30% CH_3CN/H_2O; convex gradient: 100% A to 100% B, 35 min	Powell et al., 1993; Shao et al., 1995
tea, Malawi (black)	catechins and flavonol glycosides	boiled in water	X	Hypersil ODS (250 × 4.6 mm, 5 μm)	A: 2% CH_3COOH/H_2O; B: CH_3CN; gradient: 8−31% B, 0−50 min	Bailey et al., 1990

[a] Gradients are linear unless noted. [b] C_{18} (10 × 2.1 mm, 30−40 μm). [c] LC_{18}. [d] Applied Biosystems Aquapore RP-18 (15 × 3.2 mm). [e] Brownlee C18 (30 × 4.6 mm) guarded the Bondapak column. [f] RP-18 (10 × 4 mm, 5 μm). [g] LiChrospher 100 RP-18, 4 × 4 mm, 5 μm). [h] C-18 (10 × 3 mm) plus LiChrospher 100 RP-18 (4 × 3 mm, 5 μm). [i] C_{18} (10 mm, 5 μm).

several commonly consumed foods (Bronner and Beecher, 1998; Hertog et al., 1992). However, only one HPLC procedure (Paganga et al., 1999) has been developed that separates and measures prominent food flavonoids which are members of all five subclasses (isoflavones are usually analyzed with independent systems because they are found almost exclusively in soybeans and soy-based foods). Even this system separated only a limited number of flavonoids and other phenolics.

In general, the mobile phases that have been employed with reversed-phase HPLC columns have been acetonitrile and/or methanol in combination with water containing an acid. Occasionally tetrahydrofuran and 2-propanol also have been used as the nonpolar solvent. The greatest alteration observed in the mobile phases was the type of acid used as the modifier to minimize peak tailing. Most often acetic acid or formic acid was employed; however, phosphate buffer at low pH, ammonium acetate, citric acid, and trifluoroacetic acid (TFA) also have been the source of acid. Dalluge found that deactivated C_{18} columns and the use of TFA as the acidic modifier of the mobile phase greatly improved peak shape and reproducibility of retention times of catechins in tea (Dalluge et al., 1998). Preliminary work from the authors' laboratory corroborates these observations (Merken and Beecher, unpublished results).

Sample preparation procedures for the analysis of flavonoids range from "filter and inject" in the case of several beverages to hydrolysis of glycosides (digestion), sample preparation (SPE column), filtration, and analysis for solid foods. When the glycosylated forms of the flavonoids are of interest, digestion is not required. However, when data for a large number of flavonoids are required, usually the aglycon forms of the flavonoids are measured. Hydrolysis of flavonoid glycosides requires relatively high concentrations (1−2 M) of mineral acids under reflux conditions for methanol/water mixtures (50:50, v/v) (Hertog et al., 1992). These conditions also degrade anthocyanidins and catechins (Häkkinen et al., 1999; Merken, and Beecher, unpublished results) as well as partially destroy myricetin (70% recovery) (Merken and Beecher, unpublished results). These observations highlight the need to develop sample preparation procedures for the formation of aglycons of flavonoids without degrading the flavonoids themselves.

CONCLUSION

Flavonoids have certainly shown in vitro benefits to human health. More in vivo studies are needed to ascertain the propitious effects of flavonoids and to see if there are any dangers in possible overdoses. Several hundred papers on the HPLC of flavonoids have been published in the past 20 or so years, yet HPLC methods can detect flavonoids across one, two, or perhaps three subclasses in one run. Foods may contain several subclasses, and mixed diets contain all subclasses. A method is needed to simultaneously measure all prominent flavonoids in food and drink.

ABBREVIATIONS

aq	aqueous
BuOH	butanol
CF_3COOH	trifluoroacetic acid
CH_2Cl_2	dichloromethane
CH_3CN	acetonitrile
$CH_3CO_2C_2H_5$	ethyl acetate
CH_3COOH	acetic acid
CH_3COONa	sodium acetate
CHD	coronary heart disease
CH_2O_2	formic acid
CO	carbon monoxide
Da	Daltons
DMF	dimethylformamide
EtOAc	ethyl acetate
EtOH	ethanol
FABMS	fast atom bombardment mass spectrometry
gal	galactose
glu	glucose
$HClO_4$	perchloric acid
^1H NMR	proton nuclear magnetic resonance
HPLC	high-performance liquid chromatography
H_2O	water
H_3PO_4	phosphoric acid
H_2SO_4	sulfuric acid
LC	liquid chromatography
LDL	low-density lipoprotein
LLE	liquid−liquid extraction
M	molar
MeOH	methanol
Me_2NH	dimethylamine
min	minutes
MS	mass spectrometry
N	normal
NaH_2PO_4	monobasic sodium phosphate
NaOH	sodium hydroxide
NIST	National Institute of Standards and Technology
neo	neohesperidose
$(NH_4)H_2PO_4$	dihydrogen ammonium phosphate
NH_4OAc	ammonium acetate
NMR	nuclear magnetic resonance
PO_4^{3-}	phosphate
RDA	retro-Diels−Alder reaction
rham	rhamnose
RP	reverse phase
rut	rutinose
SDS	sodium dodecyl sulfate
SPE	solid-phase extraction
TBHQ	*tert*-butylhydroquinone
THF	tetrahydrofuran
UV	ultraviolet
v/v	proportions by wet measured volume
w/v	weight per volume

LITERATURE CITED

Amarowicz, R.; Shahidi, F. A rapid chromatographic method for separation of individual catechins from green tea. *Food Res. Int.* **1996**, *29*, 71−76.

Amiot, M. J.; Aubert, S.; Gonnet, M.; Tacchini, M. Les composés phénoliques des miels: étude préliminaire sur l'identification et la quantification par familles. *Apidologie* **1989**, *20*, 115−125.

Andrade, P.; Seabra, R.; Ferreira, M.; Ferreres, F.; Garcia-Viguera, C. Analysis of non-coloured phenolics in port wines bycapillary zone electrophoresis. Influence of grape variety and aging. *Z. Lebensm.-Unters.-Forsch. A* **1998**, *206*, 161−164.

Arce, L.; Rios, A.; Valcarcel, M. Determination of anticarcinogenic polyphenols present in green tea using capillary electrophoresis coupled to a flow injection system. *J. Chromatogr. A* **1998**, *827*, 113−120.

Archier, P.; Coen, S.; Roggero, J.-P. Composition phénolique de vins issues de monocépages. *Sci. Aliments* **1992**, *12*, 453−466.

Artés, F.; Tudela, J. A.; Gil, M. I. Improving the keeping quality of pomegranate fruit by intermittent warming. *Z. Lebensm.-Unters.-Forsch. A* **1998**, *207*, 316−321.

Arts, I. C. W.; Hollman, P. C. H. Optimization of a quantitative method for the determination of catechins in fruits and legumes. *J. Agric. Food Chem.* **1998**, *46*, 5156−5162.

Bailey, R. G.; McDowell, I.; Nursten, H. E. Use of an HPLC photodiode-array detector in a study of the nature of a black tea liquor. *J. Sci. Food Agric.* **1990**, *52*, 509−525.

Bailey, R. G.; Nursten, H. E.; McDowell, I. Comparative study of the reversed-phase high-performance liquid chromatography of black tea liquors with special reference to the thearubigins. *J. Chromatogr.* **1991**, *542*, 115−128

Bailey, R. G.; Nursten, H. E.; McDowell, I. A comparison of the HPLC, mass spectra, and acid degradation of theafulvins from black tea and proanthocyanidin polymers from wine and cider. *J. Sci. Food Agric.* **1994**, *64*, 231−238.

Bakker, J.; Bridle, P.; Koopman, A. Strawberry juice colour: the effect of some processing variables on the stability of anthocyanins. *J. Sci. Food Agric.* **1992**, *60*, 471−476.

Barnes, S.; Kirk, M.; Coward, L. Isoflavones and their conjugates in soy foods: extraction conditions and analysis by HPLC-mass spectrometry. *J. Agric. Food Chem.* **1994**, *42*, 2466−2474.

Barnes, S.; Coward, L.; Kirk, M.; Sfakianos, J. HPLC-mass spectrometry analysis of isoflavones. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 254−262.

Beninger, C. W.; Hosfield, G. L.; Nair, M. G. Flavonol glycosides from the seed coat of a new Manteca-type dry bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **1998**, *46*, 2906−2910.

Berahia, T.; Gaydou, E. M.; Cerrati, C.; Wallet, J.-C. Mass spectrometry of polymethoxylated flavones. *J. Agric. Food Chem.* **1994**, *42*, 1697−1700.

Betés-Saura, C.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M. Phenolics in white free run juices and wines from Penedès by high-performance liquid chromatography: changes during vinification. *J. Agric. Food Chem.* **1996**, *44*, 3040−3046.

Bingham, S. A.; Atkinson, C.; Liggins, J.; Bluck, L.; Coward, A. Phyto-oestrogens: where are we now? *Br. J. Nutr.* **1998**, *79*, 393−406.

Bogdanov, S. Determination of pinocembrin in honey using HPLC. *J. Apic. Res.* **1989**, *28*, 55−57.

Bohm, B. A. The minor flavonoids. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman & Hall: London, U.K., 1994.

Bombardelli, E.; Morazzoni, P. *Hypericum perforatum*. *Fitoterapia* **1995**, *66*, 43−68.

Boyles, M. J.; Wrolstad, R. E. Anthocyanin composition of red raspberry juice: influences of cultivar, processing, and environmental factors. *J. Food Sci.* **1993**, *58*, 1135−1141.

Bridle, P.; Garciá-Viguera, C. Analysis of anthocyanins in strawberries and elderberries. A comparison of capillary zone electrophoresis and HPLC. *Food Chem.* **1997**, *59*, 299−304.

Brolis, M.; Gabetta, B.; Fuzzati, N.; Pace, R.; Panzeri, F.; Peterlongo, F. Identification by high-performance liquid chromatography−diode array detection−mass spectrometry and quantification by high-performance liquid chromatography−UV absorbance detection of active constituents of *Hypericum perforatum*. *J. Chromatogr. A* **1998**, *825*, 9−16.

Bronner, W. E.; Beecher, G. R. Extraction and measurement of prominent flavonoids in orange and grapefruit juice concentrates. *J. Chromatogr. A* **1995**, *705*, 247−256.

Bronner, W. E.; Beecher, G. R. Method for determining the content of catechins in tea infusions by high-performance liquid chromatography. *J. Chromatogr. A* **1998**, *805*, 137−142.

Brown, J. E.; Khodr, H.; Hider, H.; Rice-Evans, C. A. Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. *Biochem. J.* **1998**, *330*, 1173−1178.

Castia, T.; Franco, M. A.; Mattivi, F.; Muggiolu, G.; Sferlazzo, G.; Versini, G. Characterization of grapes cultivated in Sardinia: chemometric methods applied to the anthocyanic fraction. *Sci. Aliments* **1992**, *12*, 239−255.

Chandra, A.; Nair, M. G.; Iezzoni, A. Evaluation and characterization of the anthocyanin pigments in tart cherries (*Prunus cerasus* L.). *J. Agric. Food Chem.* **1992**, *40*, 967−969.

Chandra, A.; Nair, M. G.; Iezzoni, A. Isolation and stabilization of anthocyanins from tart cherries (*Prunus cerasus* L.). *J. Agric. Food Chem.* **1993**, *41*, 1062−1065.

Cherif, J. K.; Ayed, N. La grenadine et les sirops de grenade: méthode de révélation de l'authencité des sirops naturels. *Fruits* **1997**, *52*, 99−109.

Constant, J. Alcohol, ischemic heart disease, and the French paradox. *Clin. Cardiol.* **1997**, *20*, 420−424.

Copeland, E. L.; Clifford, M. N.; Williams, C. M. Preparation of (−)-epigallocatechin gallate from commercial green tea by caffeine precipitation and solvent partition. *Food Chem.* **1998**, *61*, 81−87.

Cossins, E.; Lee, R.; Packer, L. ESR studies of Vitamin C regeneration, order of reactivity of natural source phytochemical preparations. *Biochem. Mol. Biol. Int.* **1998**, *45*, 583−597.

Costa, C. T. da; Nelson, B. C.; Margolis, S. A.; Horton, D. Separation of blackcurrant anthocyanins by capillary zone electrophoresis. *J. Chromatogr. A* **1998**, *799*, 321−327.

Coward, L.; Barnes, N. C.; Setchell, K. D. R.; Barnes, S. Genistein, daidzein, and their β-glycoside conjugates: antitumor isoflavones in soybean foods from American and Asian diets. *J. Agric. Food Chem.* **1993**, *43*, 1961−1967.

Croft, A. P.; Bartsch, R. A. Synthesis of chemically modified cyclodextrins. *Tetrahedron* **1983**, *39*, 1417−1474.

Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *J. Agric. Food Chem.* **1997**, *45*, 590−595.

Daigle, D. J.; Conkerton, E. J. Analysis of flavonoids by HPLC. *J. Liq. Chromatogr.* **1983**, *6*, 105−118.

Daigle, D. J.; Conkerton, E. J. Analysis of flavonoids by HPLC: an update. *J. Liq. Chromatogr.* **1988**, *11*, 309−325.

Dalluge, J. J.; Nelson, B. C.; Brown Thomas, J.; Sander, L. C. Selection of column and gradient elution system for the separation of catechins in green tea using high-performance liquid chromatography. *J. Chromatogr., A* **1998**, *793*, 265−274.

Dao, L. T.; Takeoka, G. R.; Edwards, R. H.; De Berrios, J. Improved method for the stabilization of anthocyanidins. *J. Agric. Food Chem.* **1998**, *46*, 3564−3569.

De Cooman, L.; Everaert, E.; De Keukeleire, D. Quantitative analysis of hop acids, essential oils and flavonoids as a clue to the identification of hop varieties. *Phytochem. Anal.* **1998**, *9*, 145−150.

de Pascual-Teresa, S.; Treutter, D.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Analysis of flavonols in beverages by high-performance liquid chromatography with chemical reaction detection. *J. Agric. Food Chem.* **1998**, *46*, 4209−4213.

Donnelly, D. M. X.; Boland, G. Neoflavonoids. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman & Hall: London, U.K., 1994.

Donner, H.; Gao, L.; Mazza, G. Separation and characterization of simple and malonylated anthocyanins in red onions, *Allium cepa* L. *Food Res. Int.* **1997**, *30*, 637−643.

Dwyer, J. T.; Goldin, B. R.; Saul, N.; Gualtieri, L.; Barakat, S.; Adlercreutz, H. Tofu and soy drinks contain phytoestrogens. *J. Am. Diet. Assoc.* **1994**, *94*, 739−743.

Escarpa, A.; González, M. C. High-performance liquid chromatography with diode-array detection for the determination of phenolic compounds in peel and pulp from different apple varieties. *J. Chromatogr., A* **1998**, *823*, 331−337.

Ewald, C.; Fjelkner-Modig, S.; Johansson, K.; Sjöholm, I.; Akesson, B. Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chem.* **1999**, *64*, 231−235.

Ferres, F.; Tomás-Barberán, F. A.; Gil, M. I.; Tomás-Lorente, F. An HPLC technique for flavonoid analysis in honey. *J. Sci. Food Agric.* **1991**, *56*, 49−56.

Ferres, F.; García-Viguera, C.; Tomás-Lorente, F.; Tomás-Barberán, F. A. Hesperetin: a marker of the floral origin of citrus honey. *J. Sci. Food Agric.* **1993**, *61*, 121−123.

Ferreres, F.; Blazquez, M. A.; Gil, M. I.; Tomás-Barberán, F. A. Separation of honey flavonoids by micellar electrokinetic capillary chromatography. *J. Chromatogr., A* **1994a**, *669*, 268−274.

Ferreres, F.; Tomás-Barberán, F. A.; Soler, C.; García-Viguera, C.; Oritz, A.; Tomás-Lorente, F. A simple extractive technique for honey flavonoid HPLC analysis. *Apidologie* **1994b**, *25*, 21−30.

Ficarra, P.; Ficarra, R.; Bertucci, C.; Tommasini, S.; Calabrò, M. L.; Costantino, D.; Carulli, M. Direct enantiomeric separation of flavanones by high performance liquid chromatography using various chiral stationary phases. *Plant Med.* **1995**, *61*, 171−176.

Finger, A.; Engelhardt, U. H.; Wray, V. Flavonol glycosides in tea−kaempferol and quercetin rhamnodiglucosides. *J. Sci. Food Agric.* **1991a**, *55*, 313−321.

Finger, A.; Engelhardt, U. H.; Wray, V. Flavonol triglycosides containing galactose in tea. *Phytochemistry* **1991b**, *30*, 2057−2060.

Formica, J. V.; Regelson, W. Review of quercetin and related bioflavonoids. *Food Chem. Toxicol.* **1995**, *33*, 1061−1080.

Franke, A. A.; Custer, L. J.; Cerna, C. M. Narala, K. K. Quantitation of phytoestrogens in legumes by HPLC. *J. Agric. Food Chem.* **1994**, *42*, 1905−1913.

Franke, A. A.; Custer, L. J.; Cerna, C. M.; Narala, K. Rapid HPLC analysis of dietary phytoestrogens from legumes and from human urine. *Proc. Soc. Exp. Biol. Med.* **1995**, *208*, 18−26.

Franke, A. A.; Custer, L. J.; Wang, W.; Shi, C. Y. HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids. *Proc. Soc. Exp. Biol. Med.* **1998**, *217*, 263−273.

Gamache, P.; Ryan, E.; Acworth, I. N. Analysis of phenolic and flavonoid compounds in juice beverages using high-performance liquid chromatography with coulometric array detection. *J. Chromatogr.* **1993**, *635*, 143−150.

Gao, Y.; Cahoon, G. A. High performance liquid analysis of anthocyanins in the red seedless table grape reliance. *Am. J. Enol. Vitic.* **1995**, *46*, 339−345.

Gao, L.; Mazza, G. Rapid method for complete characterization of simple and acylated anthocyanins by high-performance liquid chromatography and capillary gas−liquid chromatography. *J. Agric. Food Chem.* **1994a**, *42*, 118−125.

Gao, L.; Mazza, G. Quantitation and distribution of simple and acylated anthocyanins and other phenolics in blueberries. *J. Food Sci.* **1994b**, *59*, 1057−1059.

Gao, L.; Mazza, G. Characterization of acetylated anthocyanins in lowbush blueberries. *J. Liq. Chromatogr.* **1995a**, *18*, 245−259.

Gao, L.; Mazza, G. Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries. *J. Agric. Food Chem.* **1995b**, *43*, 343−346.

García-Viguera, C.; Zafrilla, P.; Artés, F.; Romero, F.; Abellán, P.; Tomás-Barberán, F. A. Colour and anthocyanin stability of red raspberry jam. *J. Sci. Food Agric.* **1998**, *78*, 565−573.

García-Viguera, C.; Zafrilla, P.; Romero, F.; Abellán, P.; Artés, F.; Tomás-Barberán, F. A. Color stability of strawberry jam as affected by cultivar and storage temperature. *J. Food Sci.* **1999**, *64*, 243−247.

Garrett, S. D.; Lee, H. A.; Friar, P. M. K.; Morgan, M. R. A. Validation of a novel estrogen receptor-based microtitration plate assay for the determination of phytoestrogens in soy-based foods. *J. Agric. Food Chem.* **1999**, *47*, 4106−4111.

Geiger, H. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman & Hall: London, U.K., 1994.

Gil, M. I.; García-Viguera, C.; Artés, F.; Tomás-Barberán, F. A. Changes in pomegranate juice pigmentation during ripening. *J. Sci. Food Agric.* **1995a**, *68*, 77−81.

Gil, M. I.; Cherif, J.; Ayed, N.; Artés, F.; Tomás-Barberán, F. A. Influence of cultivar, maturity stage and geographical location on the juice pigmentation of Tunisian pomegranates. *Z. Lebensm.-Unters.-Forsch.* **1995b**, *201*, 361−364.

Goiffon, J.-P.; Brun, M.; Bourrier, M. J. High-performance liquid chromatography of red fruit anthocyanins. *J. Chromatgr.* **1991**, *537*, 101−121.

Goiffon, J.-P.; Mouly, P. P.; Gaydou, E. M. Anthocyanic pigment determination in red fruit juices, concentrated juices and syrups using liquid chromatography. *Anal. Chem. Acta* **1999**, *382*, 39−50.

Goldberg, D. M.; Tsang, E.; Karumanchiri, A.; Diamandis, E. P.; Soleas, G.; Ng, E. Method to assay the concentrations of phenolic constituents of biological interest in wines. *Anal. Chem.* **1996**, *68*, 1688−1694.

Goldberg, D. M.; Karumanchiri, A.; Tsang, E.; Soleas, G. J. Catechin and epicatechin concentrations of red wines: regional and cultivar-related differences. *Am. J. Enol. Vitic.* **1998**, *49*, 23−34.

Goto, T.; Yoshida, Y. Simultaneous analysis of individual catechins and caffeine in green tea. *Methods Enzymol.* **1999**, *229*, 107−113.

Graham, T. L. A rapid, high-resolution high performance liquid chromatography profiling procedure for plant and microbial aromatic secondary metabolites. *Plant Physiol.* **1991a**, *95*, 584−593.

Graham, T. L. Flavonoid and isoflavonoid distribution in developing soybean seedling tissues and in seed and root exudates. *Plant Physiol.* **1991b**, *95*, 594−603.

Graham, T. L.; Kim, J. E.; Graham, M. Y. Role of constitutive isoflavone conjugates in the accumulation of glyceollin in soybean infected with *Phytophthora megasperma*. *Mol. Plant-Microbe Interact.* **1990**, *3*, 157−166.

Guyot, S.; Marnet, N.; Laraba, D.; Sanoner, P.; Drilleau, J.-F. Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic compounds in different tissue zones of a French cider apple variety (*Malus domestica* var. Kermerrien). *J. Agric. Food Chem.* **1998**, *46*, 1698−1705.

Häkkinen, S. H.; Kärenlampi, S. O.; Marina Heinonen, I.; Mykkänen, H. M.; Riita Törrönen, R. HPLC method for screening of flavonoids and phenolic acids in berries. *J. Sci. Food Agric.* **1998**, *77*, 543−551.

Häkkinen, S.; Heinonen, M.; Kärenlampi, S.; Mykkänen, H.; Ruuskanen, J.; Törrönen, R. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res. Int.* **1999**, *32*, 345−353.

Harborne, J. B.; Grayer, R. J. Flavonoids and insects. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman & Hall: London, U.K., 1994.

Hasegawa, S.; Berhow, M. A.; Fong, C. H. Analysis of bitter principles in *Citrus*. *Modern Methods of Plant Analysis*; Springer: New York: 1996; Vol. 18, pp 59−80.

Hebrero, E.; Santos-Buelga, C.; Rivas-Gonzales, J. C. High performance liquid chromatography-diode array spectroscopy identification of anthocyanins of *Vitis vinifera* variety Tempranillo. *Am. J. Enol. Vitic.* **1988**, *39*, 227−233.

Hebrero, E.; Garcia-Rodriguez, C.; Santos-Buelga, C.; Rivas-Gonzalo, J. C. Analysis of anthocyanins by high performance liquid chromatography-diode array spectroscopy in a hybrid grape variety (*Vitis vinifera* × *Vitis berlandieri* 41B). *Am. J. Enol. Vit.* **1989**, *40*, 283−291.

Heller, W.; Forkmann, G. Biosynthesis of flavonoids. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman & Hall: London, U.K., 1994.

Hempel, J.; Böhm, H. Quality and quantity of prevailing flavonoid glycosides of yellow and green French beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* **1996**, *44*, 2114−2116.

Hendrich, S.; Wang, G.-J.; Lin, H.-K.; Xu, X.; Tew, B.-Y.; Wang, H.-J.; Murphy, P. A. Isoflavone metabolism and bioavailability. In *Antioxidant Status, Diet, Nutrition, and Health*; Papas, A. M., Ed.; CRC Press: Boca Raton, FL, 1999.

Hertog, M. G. L.; Hollman, P. C. H.; Venema, D. P. Optimization of a quantitative HPLC determination of potentially

anticarcinogenic flavonoids in vegetables and fruit. *J. Agric. Food Chem.* **1992**, *40*, 1591−1598.

Hibasami, H.; Achiwa, Y.; Fujikawa, T.; Komiya, T. Induction of programmed cell death (apoptosis) in human lymphoid leukemia cells by catechin compounds. *Anticancer Res.* **1996**, *16*, 1943−1946.

Hilhorst, M. J.; Somsen, G. W.; De Jong, G. J. Potential of capillary electrophoresis for the profiling of propolis. *J. High Resolut. Chromatogr.* **1998**, *21*, 608−612.

Hirota, S.; Shimoda, T.; Takahama, U. Tissue and spacial distribution of flavonol and peroxidase in onion bulbs and stability of flavonol glucosides during boiling of the scales. *J. Agric. Food Chem.* **1998**, *46*, 3497−3502.

Ho, P.; Hogg, T. A.; Silva, M. C. M. Application of a liquid chromatographic method for the determination of phenolic compounds and furans in fortified wines. *Food Chem.* **1999**, *64*, 115−122.

Hong, V.; Wrolstad, R. E. Use of HPLC separation/photodiode array detection for characterization of anthocyanins. *J. Agric. Food Chem.* **1990**, *38*, 708−715.

Hutabarat, L. S.; Mulholland, M.; Greenfield, H. Development and validation of an isocratic high-performance liquid chromatographic method for quantitative determination of phytoestrogens in soya bean. *J. Chromatogr., A* **1998**, *795*, 377−382.

Jones, A. E.; Price, K. R.; Fenwick, G. R. *J. Sci. Food Agric.* **1989**, *46*, 357−364.

Justesen, U.; Knuthsen, P.; Leth, T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photodiode array and mass spectrometric detection. *J. Chromatogr., A* **1998**, *799*, 101−110.

Kader, F.; Rovel, B.; Girardin, M.; Metche, M. Fractionation and identification of the phenolic compounds of Highbush blueberries (*Vaccinium corymbosu*, L.). *Food Chem.* **1996**, *55*, 35−40.

Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M. HL-60 differentiating activity and flavonoid content of the readily extractable fraction prepared from *citrus* juices. *J. Agric. Food Chem.* **1999**, *47*, 128−135.

Khokhar, S.; Venema, D.; Hollman, P. C. H.; Dekker, M.; Jongen, W. A RP-HPLC method for the determination of tea catechins. *Cancer Lett.* **1997**, *114*, 171−172.

Kidøy, L.; Nygård, A. M.; Andersen, Ø.M.; Pedersen, A. T.; Aksnes, D. W.; Kiremire, B. T. Anthocyanins in fruits of *Passiflora edulis* and *P. suberosa*. *J. Food Compos. Anal.* **1997**, *10*, 49−54.

Kingston, D. G. I. Mass spectrometry of organic compounds—VI. Electron-impact spectra of flavonoid compounds. *Tetrahedron* **1971**, *27*, 2691−2700.

Kinoshita, E.; Ozawa, Y.; Aishima, T. Novel tartaric acid isoflavone derivatives that play key roles in differentiating Japanese soy sauces. *J. Agric. Food Chem.* **1997**, *45*, 3753−3759.

Kinoshita, E.; Sugimoto, T.; Ozawa, Y.; Aishima, T. Differentiation of soy sauce produced from whole soybeans and defatted soybeans by pattern recognition analysis of HPLC profiles. *J. Agric. Food Chem.* **1998**, *46*, 877−883.

Kovác, V.; Bourzeix, M.; Heredia, N.; Alonso, E.; Revilla, E. Chromatographic analysis of catechins, procyanidins, and flavonols in white wines made with *Vitis vinifera* × *Vitis amurensis* hybrid cultivars. *Flavors and Off-Flavors*; Proceedings of the 6th International Flavor Conference, Rethymnon, Crete, Greece, July 5−7, 1989; pp 37−52.

Krause, M.; Galensa, R. Improved chiral stationary phase based on cellulose triacetate supported on non-macroporous silica gel diol for the high-performance liquid chromatographic separation of racemic flavanones and diastereomeric flavanone glycosides. *J. Chromatogr.* **1990**, *502*, 287−296.

Krause, M.; Galensa, R. High-performance liquid chromatography of diastereomeric flavanone glycosides in Citrus on a β-cyclodextrin-bonded stationary phase (Cyclobond I). *J. Chromatogr.* **1991**, *588*, 41−45.

Krishnan, H. B. Identification of genistein, an anticarcinogenic compound, in the edible tubers of the American groundnut (*Apios americana* Medikus). *Crop Sci.* **1998**, *38*, 1052−1056.

Kuhr, S.; Engelhardt, U. H. Determination of flavanols, theogallin, gallic acid and caffeine in tea using HPLC. *Z. Lebensm.-Unters.-Forsch.* **1991**, *192*, 526−529.

Kumamoto, M.; Sonda, T. Evaluation of the antioxidative activity of tea by an oxygen electrode method. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 175−177.

Kurzer, M. S. *Contemp. Nutr.* **1992**, *17*, 1−2.

Lamikanra, O. Anthocyanins of *Vitis rotundifolia* hybrid grapes. *Food Chem.* **1989**, *33*, 225−237.

Lamuela-Raventós, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994**, *45*, 1−5.

Larrauri, J. A.; Sánchez-Moreno, C.; Rupérez, P.; Saura-Calixto, F. Free radical scavenging capacity in the aging of selected red Spanish wines. *J. Agric. Food Chem.* **1999**, *47*, 1603−1606.

Lee, H. S.; Wicker, L. Anthocyanin pigments in the skin of lychee fruit. *J. Food Sci.* **1991**, *56*, 466−468.

Lee, H.; Oh, S.-K.; Choi, H.-C.; Kim, S.-U. Identification of anthocyanins from pigmented rice seeds. *Agric. Chem. Biotechnol.* **1998**, *41*, 257−260.

Lewis, C. E.; Walker, J. R. L.; Lancaster, J. E.; Sutton, K. H. Determination of anthocyanins, flavonoids, and phenolic acids in potatoes. I: Coloured cultivars of *Solanum tuberosum* L. *J. Sci. Food Agric.* **1998**, *77*, 45−57.

Liang, Y. R.; Liu, Z. S.; Xu, Y. R.; Hu, Y. L. A study on chemical composition of two special green teas *(Camellia sinensis). J. Sci. Food Agric.* **1990**, *53*, 541−548.

Liggins, J.; Bluck, L. J. C.; Coward, W. A.; Bingham, S. A. Extraction and quantification of daidzein and genistein in food. *Anal. Biochem.* **1998**, *264*, 1−7.

Lin, J.-K.; Lin, C.-L.; Liang, Y.-C.; Lin-Shiau, S.-Y.; Juan, I.-M. Survey of catechins, gallic acid, and methylxanthines in green, oolong, pu-erh, and black teas. *J. Agric. Food Chem.* **1998**, *46*, 3536−3642.

Lin, Y. Y.; Ng, K. J.; Yang, S. Characterization of flavonoids by liquid chromatography—tandem mass spectrometry. *J. Chromatogr.* **1993**, *629*, 389−393.

Lister, C. E.; Lancaster, J. E.; Sutton, K. H. Developmental changes in the concentration and composition of flavonoids in the skin of a red and green apple cultivar. *J. Sci. Food Agric.* **1994**, *64*, 155−161.

López-Serrano, M.; Ros Barceló, A. H_2O_2-mediated pigment decay in strawberry as a model system for studying color alterations in processed plant foods. *J. Agric. Food Chem.* **1999**, *47*, 824−827.

Mabry, T. J.; Markham, K. R.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970.

Marini, D.; Balestrieri, F. Multivariate analysis of flavanone glycosides in citrus juices. *Ital. J. Food Sci.* **1995**, *7*(3), 255−264.

Markham, K. R.; Geiger, H. ^{1}H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuteriodimethyl sulfoxide. In *The Flavonoids*; Harborne, J. B., Ed; Chapman & Hall: London, 1994.

Mazur, W. M.; Duke, J. A.; Wähälä, K.; Rasku, S.; Adlercreutz, H. Isoflavonoids and lignans in legumes: nutritional and health aspects in humans. *Nutr. Biochem.* **1998**, *9*, 193−200.

McDonald, M. S.; Hughes, M.; Burns, J.; Lean, M. E. J.; Matthews, D.; Crozier, A. Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. *J. Agric. Food Chem.* **1998**, *46*, 368−375.

McDowell, I.; Feakes, J.; Gay, D. Phenolic composition of black tea liquors as a means of predicting price and country of origin. *J. Sci. Food Agric.* **1991**, *55*, 627−641.

McDowell, I.; Taylor, S.; Gay, C. The phenolic pigment composition of black tea liquors—Part I: Predicting quality. *J. Sci. Food Agric.* **1995**, *69*, 467−474.

McRae, K. B.; Lidster, A. J.; DeMarco, A. C.; Dick, A. J. Comparison of the polyphenol profiles of apple fruit cultivars by correspondence analysis. *J. Sci. Food Agric.* **1990**, *50*, 329−342.

Messina, M. J. Legumes and soybeans: overview of their nutritional profiles and health effects. *Am. J. Clin. Nutr.* **1999**, *70 (suppl)*, 439S-450S.

Middleton, Jr., E. Biological properties of plant flavonoids: an overview.*Int. J. Pharmacognos* **1996**, *34*, 344−348.

Middleton, Jr., E.; Kandaswami, C. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In *The Flavonoids*; Harborne, J. B., Ed; Chapman & Hall: London, 1994.

Minami, M.; Kitabayashi, H.; Ujihara, A. Quantitative analysis of rutin in buckwheat *(Fagopyrum* sp.) by high-performance liquid chromatography. *J. Fac. Agric. Shinshu University* **1998**, *34*, 91−95.

Mizuno, M.; Tsuchida, H.; Kozukue, N.; Mizuno, S. Rapid quantitative analysis and distribution of free quercetin in vegetables and fruits. *Nippon Shokuhin Kogyo Gakkaishi* **1992**, *39*, 88−92.

Mouly, P.; Gaydou, E. M.; Estienne, J. Column liquid chromatographic determination of flavanone glycosides in *Citrus*. Application to grapefruit and sour orange adulteration. *J. Chromatogr.* **1993**, *634*, 129−134.

Mouly, P. P.; Arzouyan, C. R.; Gaydou, E. M.; Estienne, J. M. Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavanone glycosides. *J. Agric. Food Chem.* **1994**, *42*, 70−79.

Mouly, P.; Gaydou, E. M.; Auffray, A. Simultaneous separation of flavanone glycosides and polymethoxylated flavones in citrus juices using liquid chromatography. *J. Chromatogr., A* **1998**, *800*, 171−179.

Nogata, Y.; Ohta, H.; Yoza, K. I.; Berhow, M.; Hasegawa, S. High-performance liquid chromatographic determination of naturally occurring flavonoids in Citrus with a photodiode-array detector. *J. Chromatogr., A* **1994**, *667*, 59−66.

Ogawa, A.; Arai, H.; Tanizawa, H.; Miyahara, T.; Toto'oka, T. On-line screening method for antioxidants by liquid chromatography with chemiluminescence detection. *Anal. Chim. Acta* **1999**, *383*, 221−230.

Ooghe, W. C.; Ooghe, S. J.; Detavernier, C. M.; Huyghebaert, A. Characterization of orange juice (*Citrus sinensis*) by flavanone glycosides. *J. Agric. Food Chem.* **1994**, *42*, 2183−2190.

Ordaz-Galindo, A.; Wesche-Ebeling, P.; Wrolstad, R. E.; Rodriguez-Saona, L.; Argaiz-Jamet, A. Purification and identification of Capulin (*Prunus serotina* Ehrh) anthocyanins. *Food Chem.* **1999**, *65*, 201−206.

Paganga, G.; Miller, N.; Rice-Evans, C. A. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? *Free Radical Res.* **1999**, *30*, 153−162.

Park, Y.-K.; Lee, C. Y. Identification of isorhamnetin in 4′-glucoside in onions. *J. Agric. Food Chem.* **1996**, *44*, 34−36.

Patil, B. S.; Pike, L. M.; Hamilton, B. K. Changes in quercetin concentration in onion (*Allium cepa* L.) owing to the location, growth stage and soil type. *New Phytol.* **1995a**, *130*, 349−355.

Patil, B. S.; Pike, L. M.; Yoo, K. S. Variation in the quercetin content in different colored onions (*Allium cepa* L.). *J. Am. Soc. Hortic. Sci.* **1995b**, *120*, 909−913.

Pérez-Ilzarbe, J. Hernández, T.; Estrella, I. Phenolic compounds in apples: varietal differences. *Z. Lebensm.-Unters.-Forsch.* **1991**, *192*, 551−554.

Perfetti, G. A.; Frank, Jr., L. J.; Fazio, T.; Page, S. W. Liquid chromatographic methodology for the characterization of orange juice. *J.—Assoc. Off. Anal. Chem.* **1988**, *71*, 469−473.

Pietta, P.; Mauri, P.; Bruno, A.; Rava, A.; Manera, E.; Ceva, P. Identification of flavonoids from *Ginko biloba* L., *Anthemis nobilis* L. and *Equisetum arvense* L. by high-performance liquid chromatography with diode-array UV detection. *J. Chromatogr.* **1991**, *553*, 223−231.

Pietta, P. G.; Mauri, P. L.; Zini, L.; Gardana, C. Optimization of separation selectivity in capillary electrophoresis of flavonoids. *J. Chromatogr., A* **1994**, *680*, 175−179.

Plessi, M.; Bertelli, D.; Rastelli, G.; Albasini, A.; Monzani, A. Fruits of ribes, rubus, vaccinium and prenus genus. Metal contents and genome. *Fresenius' J. Anal. Chem.* **1998**, *361*, 353−354.

Poon, G. K. Analysis of catechins in tea extracts by liquid chromatography—electrospray ionization mass spectrometry. *J. Chromatogr., A* **1998**, *794*, 63−74.

Porter, L. J. Flavans and proanthocyanidins. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman & Hall: London, U.K., 1994.

Powell, C.; Clifford, M. N.; Opie, S. C.; Ford, M. A.; Robertson, A.; Gibson, C. L. Tea cream formation: the contribution of black tea phenolic pigments determined by HPLC. *J. Sci. Food Agric.* **1993**, *63*, 77−86.

Prasongsidh, B. C.; Skurray, G. R. Capillary electrophoresis analysis of *trans*- and *cis*-resveratrol, quercetin, catechin and gallic acid in wine. *Food Chem.* **1998**, *62*, 355−358.

Price, C.; Wrolstad, R. E. Anthocyanin pigments of royal Okanogan huckleberry juice. *J. Food Sci.* **1995**, *60*, 369−374.

Price, K. R.; Casuscelli, F.; Colquhoun, I. J.; Rhodes, M. J. C. Composition and content of flavonol glycosides in broccoli florets (*Brassica olearacea*) and their fate during cooking. *J. Sci. Food Agric.* **1998a**, *77*, 468−472.

Price, K. R.; Rhodes, M. J. C.; Barnes, K. A. Flavonol glycoside content and composition of tea infusions made from commercially available teas and tea products. *J. Agric. Food Chem.* **1998b**, *46*, 2517−2522.

Price, K. R.; Colquhoun, I. J.; Barnes, K. A.; Rhodes, J. C. Composition and content of flavonol glycosides in green beans and their fate during processing. *J. Agric. Food Chem.* **1998c**, *46*, 4898−4903.

Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. Cluster sun exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* **1995**, *46*, 187−194.

Pupin, A. M.; Dennis, M. J.; Toledo, M. C. F. Flavanone glycosides in Brazilian orange juice. *Food Chem.* **1998**, *61*, 275−280.

Raynal, J.; Moutounet, M.; Souquet, J.-M. Intervention of phenolic compounds in plum technology. 1. Changes during drying. *J. Agric. Food Chem.* **1989**, *37*, 1046−1050.

Revilla, E.; Alonso, E.; Bourzeix, M.; Heredia, N. Determination of catechins and procyanidins in red wine. *Flavors and Off-Flavors*; Proceedings of the 6th International Flavor Conference, Rethymnon, Crete, Greece, July 5−7, 1989; pp 53−60.

Ricardo da Silva, J. M.; Rosec, J.-P.; Bourzeix, M.; Heredia, N. Separation of quantitative determination of grape and wine procyanidins by high performance reversed phase liquid chromatography. *J. Sci. Food Agric.* **1990**, *53*, 85−92.

Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

Rivas-Gonzalo, J. C.; Gutierrez, Y.; Hebrero, C.; Santos-Buelga, C. Comparisons of methods for the determination of anthocyanins in red wines. *Am. J. Enol. Vitic.* **1992**, *43*, 210−214.

Robards, K.; Antolovich, M. Analytical chemistry of fruit bioflavonoids. *Analyst* **1997**, *122*, 11R−34R.

Robards, K.; Li, X.; Antolovich, M.; Boyd, S. Characterization of citrus by chromatographic analysis of flavonoids. *J. Sci. Food Agric.* **1997**, *75*, 87−101.

Rodriguez-Saona, L. E.; Mónica Giusti, M.; Wrolstad, R. E. Anthocyanin pigment composition of red-fleshed potatoes. *J. Food Sci.* **1998**, *63*, 458−465.

Rommel, A.; Wrolstad, R. E. Composition of flavonols in red raspberry juice as influenced by cultivar, processing, and environmental factors. *J. Agric. Food Chem.* **1993**, *41*, 1941−1950.

Rommel, A.; Heatherbell, D. A.; Wrolstad, R. E. Red raspberry juice and wine: effect of processing and storage on antho-

cyanin pigment composition, color and appearance. *J. Food Sci.* **1990**, *55*, 1011−1017.
Rommel, A.; Wrolstad, R. E.; Heatherbell, D. A. Blackberry juice and wine: processing and storage effects on anthocyanin composition, color, and appearance. *J. Food Sci.* **1992**, *57*, 385−391.
Rovellini, P.; Cortesi, N.; Fedeli, E. Analysis of flavonoids from *Olea europaea* by HPLC−UV and HPLC-electrospray-MS. *Riv. Ital. Sostanze Grasse* **1997**, *74*, 273−279.
Sabatier, S.; Amiot, M. J.; Tacchini, M.; Aubert, S. Identification of flavonoids in sunflower honey. *J. Food Sci.* **1992**, *57*, 773−774, 777.
Saenger, W. Cyclodextrin-Einschlu−verbindungen in Forschung und Industrie. *Angew. Chem.* **1980**, *92*, 343−361.
Santos, C.; Muñoz, S. S.; Gutiérrez, Y.; Hebrero, E.; Vicente, J. L.; Galindo, P.; Rivas, J. C. Characterization of young red wines by application of HJ biplot analysis to anthocyanin profiles. *J. Agric. Food Chem.* **1991**, *39*, 1086−1090.
Senter, S. D.; Robertson, J. A.; Meredith, F. I. Phenolic compounds of the mesocarp of Cresthaven peaches during storage and ripening. *J. Food Sci.* **1989**, *54*, 1259−1260.
Shao, W.; Powell, C.; Clifford, M. N. The analysis by HPLC of green, black and Pu'er teas produced in Yunnan. *J. Sci. Food Agric.* **1995**, *69*, 535−540.
Siewek, F.; Galensa, R. High-performance chromatographic determination of the degree of glycosidation of flavonols by use of an ultra-violet diode-array detector. *J. Chromatogr.* **1984**, *294*, 385−389.
Soler, C.; Gil, M. I.; García-Viguera, C.; Tomás-Barberán, F. A. Flavonoid patterns of French honeys with different floral origin. *Apidologie* **1995**, *26*, 53−60.
Strack, D.; Wray, V. The anthocyanins. In *The Flavonoids*; Harborne, J. B., Ed.; Chapman & Hall: London, U.K., 1994.
Suárez Vallés, B.; Santamariá Victorero, J.; Mangas Alonso, J. J.; Blanco Gomis, D. High-performance liquid chromatography of the natural phenolic compounds of low molecular weight in apple juice. *J. Agric. Food Chem.* **1994**, *42*, 2732−2736.
Sun, B.; Leandro, C.; Ricardo da Silva, J.; Spranger, I. Separation of grape and wine proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.* **1998**, *46*, 1390−1396.
Takeoka, G. R.; Dao, L. T.; Full, G. H.; Wong, R. Y.; Harden, L. A.; Edwards, R. H.; Berrios, J. de J. Characterization of black bean (*Phaseolus vulgaris* L.) anthocyanins. *J. Argric. Food Chem.* **1997**, *45*, 3395−3400.
Tamura, H.; Hayashi, Y.; Sugisawa, H.; Kondo, T. Structure determination of acylated anthocyanins in Muscat Bailey A grapes by homonuclear Hartmann−Hahn (HOHAHA) spectroscopy and liquid chromatography−mass spectrometry. *Phytochem. Anal.* **1994**, *5*, 190−196.
Tomás-Barberán, F. A.; Ferreres, F.; Blázquez, M. A.; García-Viguera, C.; Tomás-Lorente, F. High-performance liquid chromatography of honey flavonoids. *J. Chromatogr.* **1993**, *634*, 41−46.
Tomás-Lorente, F.; García-Viguera, C.; Ferreres, F.; Tomás-Barberán, F. A. Phenolic compounds analysis in the determination of fruit jam genuineness. *J. Agric. Food Chem.* **1992**, *40*, 1800−1804.
Toyoda, M.; Tanaka, K.; Hoshino, K.; Akiyama, H.; Tanimura, A.; Saito, Y. Profiles of potentially antiallergic flavonoids in 27 kinds of health tea and green tea infusions. *J. Agric. Food Chem.* **1997**, *45*, 2561−2564.
Treutter, D. Separation of naturally occurring mixtures of phenolic compounds from various *Prunus* tissues by reversed-phase high-performance liquid chromatography. *J. Chromatogr.* **1988**, *436*, 490−496.
Treutter, D.; Feucht, W. The pattern of flavan-3-ols in relation to scab resistance of apple cultivars. *J. Hortic. Sci.* **1990**, *65*, 511−517.
Tsuchiya, H. High-performance liquid chromatographic analysis of polyhydroxyflavones using solid-phase borate-complex extraction. *J. Chromatogr., B* **1998**, *720*, 225−230.
Vandercook, C. E.; Tisserat, B. Flavonoid changes in developing lemons grown *in* vivo and *in vitro*. *Phytochemistry* **1989**, *28*, 799−803.
Versari, A.; Barbanti, D.; Biesenbruch, S.; Farnell, P. J. Analysis of anthocyanins in red fruits by use of HPLC/spectral array detection. *Ital. J. Food Sci.* **1997**, *2*, 141−148.
Wang, G.; Kuan, S. S.; Francis, O. J.; Ware, G. M.; Carman, A. S. *J. Agric. Food Chem.* **1990**, *38*, 185−190.
Wang, H.; Murphy, P. A. Isoflavone content in commercial soybean foods. *J. Agric. Food Chem.* **1994a**, *42*, 1666−1673.
Wang, H.; Murphy, P. A. Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. *J. Agric. Food Chem.* **1994b**, *42*, 1674−1677.
Wang, H.; Nair, M. G.; Iezzoni, A. F.; Strasburg, G. M.; Booren, A. M.; Gray, J. I. Quantification and characterization of anthocyanins in Balaton tart cherries. *J. Agric. Food Chem.* **1997**, *45*, 2556−2560.
Watanabe, M. Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. *J. Agric. Food Chem.* **1998**, *46*, 839−845.
Wightman, J. D.; Price, S. F.; Watson, B. T.; Wrolstad, R. E. Some effects of processing enzymes on anthocyanins and phenolics in Pinot noir and Cabernet Savignon Wines. *Am. J. Enol. Vitic.* **1997**, *48*, 39−48.
Xie, B.; Shi, H.; Chien, Q.; Ho, C.-T. Antioxidant properties of fractions and polyphenol constituents from green, oolong, and black teas. *Proc. Natl. Sci. Council, ROC, Part B* **1993**, *17*, 77−84.
Zafrilla, P.; Valero, A.; Garciá-Viguera. Stabilization of strawberry jam colour with natural colorants. *Food Sci. Technol. Int.* **1998**, *4*, 99−105.
Zhu, Q. Y.; Huang, Y.; Tsang, D.; Chen, Z.-Y. Regeneration of α-tocopherol in human low-density lipoprotein by green tea catechin. *J. Agric. Food Chem.* **1999**, *47*, 2020−2025.
Zulu, R. M.; Grayer, R. J.; Ingham, J. L.; Harborne, J. B. Flavonoids from the roots of two *Rhynchosia* species used in the preparation of a Zambian beverage. *J. Sci. Food Agric.* **1994**, *65*, 347−354.

Received for review August 5, 1999. Revised manuscript received December 15, 1999. Accepted December 21, 1999.

JF990872O