High-Performance Liquid Chromatography of the Neutral Phenolic Compounds of Low Molecular Weight in Apple Juice

B. Suárez Vallés,[†] J. Santamaría Victorero,[†] J. J. Mangas Alonso,[†] and D. Blanco Gomis^{*‡}

Instituto de Experimentación y Promoción Agraria, 33300 Villaviciosa, Spain, and Departamento de Química Física y Analítica, Universidad de Oviedo, 33006 Oviedo, Spain

The neutral phenolic compounds, (+)-catechin, (-)-epicatechin, flavan-3-ols polymers (procyanidins), phloridzin, phloretin xyloglucoside, and the flavonol glycosides (isoquercitrin, hyperin, quercitrin, avicularin, and rutin), in apple juice were extracted with ethyl acetate and separated and quantified by reversed-phase HPLC on a C₁₈ column using an acidified water (pH 2.8)-methanol solvent gradient. A diode array detector was used to evaluate the separation and to identify the phenolic compounds. The recovery obtained for the flavanols and some dihydrochalcones ranged between 93% and 106%. On the contrary, the 30% recovery rate obtained for flavonols shows that ethyl acetate liquid-liquid extraction, usually employed in this type of study, is not recommended for the extraction of these last compounds.

Keywords: phenolic compounds; apple juice; HPLC

INTRODUCTION

The study and understanding of the polyphenolic composition and the factors that affect the phenolic compounds are critical in the design of juice products, because they play important role in taste, flavor and color characteristics of fruit juices and alcoholic beverages, and formation of undesirable sediments.

The phenolic constituents of apple juice can be divided into two groups: (a) phenolic acids and their hydroxy-acid esters and derivatives with sugars (Whiting and Coggins, 1975; Van Buren et al., 1976) and (b) flavonoids such as flavan-3-ols and procyanidins, dihydrochalcones, and the glycosides of the flavonols (Schmidt and Neukom, 1969; Lea and Timberlake, 1974, 1978; Duggan, 1969; Dick et al., 1987; Oleszek et al., 1988; Coseteng and Lee, 1987; Burda et al., 1990; Spanos and Wrolstad, 1992).

The methods used for the characterization of phenolic compounds in apple juice, such as paper chromatography, thin layer chromatography (Duggan, 1969), open column chromatography on Sephadex LH-20 and counter-current distribution between ethyl acetate and water (Lea and Timberlake, 1974, 1978) are fairly tedious and not particularly useful for quantification. Gas chromatography is a fast, efficient and accurate technique, but it requires a derivatization step (Keith and Powers, 1966; Moller and Herrmann, 1982). These techniques are now being replaced by high-performance liquid chromatography (HPLC), because it provides high sensitivity with short sample preparation. The phenolic compounds have been separated by normal-phase mode HPLC on silica gel column (Nagels and Parmentier, 1976; Galensa and Hermann, 1980), diol type column (Nagels et al., 1980) and cyano column (Wilson, 1981), but adsorption chromatography led to infinitely longer times (Lea, 1979). Recently, the use of the liquid system allowed the separation of peracetylated flavonoids (Piretti and Doghieri, 1990).

The reversed-phase mode of HPLC (RPLC) supplies a great operational simplicity, high efficiency, column

Table 1. Variety, Geographic Origin, °Brix, pH, and Titratable Acidity (TA) of Laboratory-Prepared Apple Juices

variety	geographic origin	°Brix	pH	TA (% malic)
N ^a Señora	Asturias (Spain)	8.0	3.4	0.55
San Pedro	Asturias (Spain)	10.4	3.4	0.57
San Juan	Asturias (Spain)	8.0	3.5	0.48

stability, and the ability to analyze simultaneously a broad spectrum of both closely-retained and widely-different compounds (Lea, 1979; Piretti and Doghieri, 1990; Wulf and Nagel, 1976). Complete analysis and quantification of phenolic compounds in apple juices by RPLC has not yet been improved, because many phenolics show similar ultraviolet absorption spectra with maxima in a narrow range of 280–360 nm, and, on the other hand, coelution of phenolic acids with the flavonoids and cinnamic esters of interest occurs (Lea, 1980, 1982). Salagoity-Auguste and Bertrand (1984) fractionated the phenolic compounds into neutral and acid groups before injection into the HPLC column.

The objective of the present work was to evaluate a liquid-liquid extraction method, to identify the neutral phenolic compounds present in some Spanish apple varieties, and to quantify them.

EXPERIMENTAL PROCEDURES

Samples. Table 1 lists the geographic origin, variety, and technological characteristics: °Brix, titratable acidity, and pH of the samples. These were obtained in summer 1992 from the Villaviciosa, Spain, Agricultural Experiment Station orchards with the same agroecological conditions. The apples were harvested close to optimal processing maturity, and the juice was produced without previous cold storage to sampling.

Preparation of Apple Juice. Single-variety juices were extracted using three replicates from typical varieties (1 kg of each one) employed for apple juice manufacture (N^a Señora (A), San Pedro (B), San Juan (C)), by means of domestic juicer; a juice yield between 80 ± 5% was obtained. The juice produced was centrifuged at 5000 rpm during 5 min, and no enzyme treatment, filtration, fining and pasteurization was carried out over single-strength juice. The juices were frozen (-20 °C) until their analytical determination.

Standards. The phenolic standards (+)-catechin, (-)-epicatechin, and phloridzin were obtained from Sigma; the

[†] Instituto de Experimentación y Promoción Agraria.

[‡] Universidad de Oviedo.

glycosides of quercetin (isoquercitrin, hyperin, quercitrin, avicularin, and rutin) were provided from Extrasynthèse; procyanidins and phloretin xyloglucoside were kindly provided by Dr. A. Lea (Cadbury Schweppes, Reading, U.K.). Polyphenol standard solutions were prepared in methanol in concentrations of about 14 mM for (-)-epicatechin, 7 mM for (+)-catechin, 5 mM for phloridzin, and 3 mM for rutin, and these were stored at -20 °C.

Preparation of Phenolic Extracts. The juices (50 mL) were adjusted to pH 7.00 with 2 N NaOH and extracted with 50 mL of ethyl acetate at 30 °C by stirring for 5 min, using a casing vessel with a reflux condenser. The solution was then centrifuged at 5000 rpm for 5 min. The organic layer was transferred, and the extraction process was repeated twice with 50 mL of ethyl acetate. The three organic layers were joined, and 125 mL of this solution was evaporated to dryness at 35 °C by means of an helical gas flow (nitrogen) at 1.8 bar by vortexing action (Turbo Vap Evaporator-Zymark). The residue was redissolved in 2 mL of methanol.

HPLC Analysis. The equipment was from Waters and consisted of two pumps 510, universal injector U6K, a photodiode array detector 990 and a Digital 380 data station. The polyphenols were separated by Spherisorb ODS-2 column (250 × 4.6 mm, 3 µm) and Nova-Pak C₁₈ column (300 × 3.9 mm, 4 µm) at 21 ± 0.1 °C; the temperature was kept by means of a temperature control module. The gradient was as follows:

time (min)	flow (mL/min)	B (%)	gradient curve
0	0.6	2	*
50	0.6	42	6
60	0.4	50	6
75	0.4	50	1
77	0.6	2	6

Solvent A was water, adjusted to pH 2.80 with phosphoric acid, and solvent B was methanol. All solvents were of HPLC grade and degassed with helium prior to use.

Identification and Quantification of the Phenolic Compounds. Identification was achieved by comparing the retention times and UV absorbency ratios with those of the standards. The absorbance ratios used for the identification of flavonoids were as follows: 220/275 nm for flavanols and dihydrochalcones, and 260/350 nm for flavonols. UV spectra were also checked by means of a photodiode array detector, to assure peak identity.

Quantitative determinations were carried out by the external standard method. For the compounds lacking of standards, or those which the amount at our disposal was too small, the quantification was achieved from similar compounds. Thus, the procyanidins were quantified as (-)-epicatechin, the phloretin xyloglucoside as phloridzin, and flavonol glycosides as rutin. The calibration curve data were generated by repeated injections of a fixed volume, 2 µL, of standard solutions of polyphenols covering a broad range of concentrations. The resulting peak area data were determined, plotted against concentration, and stored in the data module. Injection volumes of 2 µL were used to analyse the apple juice samples and the amount of each polyphenol was directly obtained from the data module.

RESULTS AND DISCUSSION

We have tested two columns, Spherisorb ODS-2 (250 × 4.6 mm i.d., 3 µm) and Nova-Pak C₁₈ (300 × 3.9 mm i.d., 4 µm), for phenolic separation in apple juice. Chromatographic conditions (pH, ionic strength, percentage of the organic modifier, temperature, and flow rate) were altered in the one-dimensional mode to maximize peak resolution. The different chromatographic parameters studied were in the following ranges: pH 2.25–2.80; ionic strength, 10⁻³–10⁻¹ M; slope of the linear gradient, 0.60–0.80%/min; temperature, 20–40 °C, and flow rate, 0.40–0.60 mL/min. In relation to the pH, the higher value of pH, the better is the resolution between the flavonols and dihydrochal-

Table 2. Retention Time and Resolution of Different Phenolic Standards for the Two Columns Studied

phenolic compd	Spherisorb		Nova-Pak	
	R _t (min)	R _s	R _t (min)	R _s
B1	37.14	<0.80	31.14	>1.25
(+)-catechin	40.74	1.03	35.79	>1.25
B2	44.12	>1.25	38.84	>1.25
C1	47.93	<0.80	43.23	<0.80
tetramer	48.83	<0.80	43.70	<0.80
(-)-epicatechin	49.36	1.01	45.33	1.05
phloretin xyloglucoside	64.58	>1.25	65.61	0.83
phloridzin	67.20	>1.25	70.55	>1.25
rutin	69.42	0.00	66.40	0.83
isoquercitrin	69.42	0.00	67.00	0.00
hyperin	69.42	0.00	67.00	0.00
avicularin			69.80	>2.25
quercitrin	74.19	>1.25	72.64	1.02

cones; at pH 2.70, the phloretin xyloglucoside and rutin partially overlapped, whereas at pH 2.80 an adequate resolution was obtained. On the other hand, an increase of ionic strength brought about longer retention times and worse resolution. Different organic modifiers, namely acetonitrile and tetrahydrofuran (THF), were tested to improve the chromatographic separation of polyphenols, particularly in the last zone of the chromatogram; the use of THF did not contribute to better separation, especially of the procyanidols, and bigger analysis time was noticed. On the other hand, when the strength of the solvent was altered initially (initial percentages of methanol ranging between 2% and 9% were studied) to elute interested compounds early and decrease the analysis time, worse separation of polyphenol compounds was obtained. The use of the analysis time lengthy for an adequate resolution of the neutral phenolic compounds of low molecular weight agrees with the results obtained by Pérez-Illarbe et al. (1991) and Spanos et al. (1990).

Generally, the peak resolution was improved when the work temperature was close to 20 °C and even though the retention times were increased; the best separation was observed at 21 °C. The dihydrochalcones and flavonols were better separated at a lower flow rate; for this reason, the gradient includes a flow rate program starting on 50 min.

As a consequence of these studies and upon the basis of resolution, the operating conditions were fixed as mentioned before.

As can be seen in Figure 1, the Spherisorb ODS-2 column offers an acceptable resolution for flavanols and procyanidins, in the first part of the chromatogram, excluding the procyanidins C1 (trimer) and tetramer because these compounds eluted between unknown cinnamic esters and epicatechin. Nevertheless, in the second part of the chromatogram, where dihydrochalcones and flavonol glycosides were eluted, the resolution is worse. The lower peaks were impure and a lesser sensibility was observed in them compared with the Nova-Pak column, making the characterization of these last eluted compounds (flavonols) difficult. However, the Nova-Pak column provided a good separation of all compounds including the flavonol glycosides (Figure 2). The trimer C1 and tetramer were partially overlapped as can be seen in Table 2, where the retention time and resolution of phenolic standards for the two columns studied are shown.

In any case, with this method it is not possible to separate the quercetin 3-O-galactoside (hyperin) and quercetin 3-O-glucoside (isoquercitrin) (Table 2). In fact, the same problem was reported by other authors (Pérez-Illarbe et al., 1991; Dick, 1986) for this type of

Figure 1. Separation of apple juice phenolics on Spherisorb ODS-2 column (250 × 4.6 mm, 3 μm). Peaks: (1) procyanidin B1; (2) (+)-catechin; (3) cinnamic ester; (4) procyanidin B2; (5) cinnamic ester; (6) trimer C1; (7) cinnamic ester; (8) tetramer; (9) (–)-epicatechin; (10) phloretin xyloglucoside; (11) phloridzin; (12) rutin + isoquercitrin + hyperin; (13) unknown flavonol; (14) quercitrin.

Figure 2. Separation of apple juice phenolics on Nova-Pak C₁₈ column (300 × 3.9 mm, 4 μm). Peaks: (1) procyanidin B1; (2) (+)-catechin; (3) cinnamic ester; (4) procyanidin B2; (5) cinnamic ester; (6) cinnamic ester; (7) trimer C1; (8) tetramer; (9) (–)-epicatechin; (10) phloretin xyloglucoside; (11) rutin; (12) isoquercitrin + hyperin; (13) unknown flavonol; (14) avicularin; (15) phloridzin; (16) unknown flavonol; (17) quercitrin.

column. This chromatographic analysis (Figure 3) shows that, in addition to the compounds identified by comparing the retention times and UV absorbency ratios with those of the standards, four unknown procyanidins or flavan-3-ols (peaks 2, 4, 7, and 12), two cinnamic esters (peaks 5 and 8), and two flavonols (peaks 16 and 19) were present in all the analyzed juices as evidenced by their characteristic UV spectra. In fact, some of these compounds such as procyanidins B3, B4, B5, and other polymers (Pérez-Iñzarbe et al., 1992; Spanos et al., 1990), feruloylglucose and *p*-coumaroylglucose (Pérez-Iñzarbe et al., 1991), and quercetin 3-O-xyloside (Spanos et al., 1990) have been characterized in apple juice recently.

Levels of the quantified compounds in the samples (N^a Señora (A), San Pedro (B), San Juan (C)) are given in Table 3. To determine the accuracy of the method, recovery studies were carried out. Known amounts of each polyphenol were added to a variety of samples and

the resulting spiked samples were subjected to the entire analytical sequence. All analytes were carried out in triplicate at three concentration levels. The average recoveries for flavanols and dihydrochalcones were between 93% and 106% with a CV <5% indicating that the method has a suitable analysis accuracy degree for these type of substances. On the contrary, for most of lipophilic compounds such as flavonols, average recoveries of lower than 30% were experienced. This demonstrated the narrow extraction strength of ethyl acetate, and therefore, it is not appropriate for quantitative studies.

The Spanish varieties tested have fewer polyphenol compounds, namely, (–)-epicatechin, phloridzin, procyanidin B2, and trimer and tetramer procyanidins than English cider apple varieties in agreement with the results reported by Lea (1984) (Table 4). However, the cultivars studied show more concentration of (+)-catechin, (–)-epicatechin, B2, and phloridzin than Granny

Figure 3. HPLC chromatogram of Nª Señora juice. Chromatographic conditions are the same as in Figure 2. Peaks: (1) procyanidin B1; (2) unknown procyanidin; (3) (+)-catechin; (4) unknown procyanidin; (5) cinnamic ester; (6) procyanidin B2; (7) unknown procyanidin; (8) cinnamic ester; (9) trimer C1; (10) tetramer; (11) (-)-epicatechin; (12) unknown procyanidin; (13) phloretin xyloglucoside; (14) rutin; (15) isoquercitrin + hyperin; (16) unknown flavonol; (17) avicularin; (18) phloridzin; (19) unknown flavonol; (20) quercitrin.

Table 3. Flavonoid Contents (Milligrams per Liter) in Apple Juices

phenolic compd	Nª Señora	San Pedro	San Juan
(1) procyanidin B1	23.23 ± 3.08	27.50 ± 2.23	10.80 ± 0.69
(2) unknown procyanidin	5.73 ± 1.48	6.6 ± 1.22	1.70 ± 0.44
(3) catechin	69.54 ± 3.41	46.18 ± 1.60	36.00 ± 0.17
(6) procyanidin B2	138.68 ± 3.88	129.00 ± 1.12	89.96 ± 0.92
(9) + (10) trimer C1 + tetramer	89.67 ± 4.86	119.83 ± 0.69	66.48 ± 0.48
(11) epicatechin	225.61 ± 4.01	193.84 ± 0.69	159.76 ± 0.53
(12) unknown procyanidin	12.83 ± 5.00	15.32 ± 1.73	11.93 ± 3.64
(13) phloretin xyloglucoside	14.19 ± 3.23	37.99 ± 0.84	27.40 ± 1.30
(14) rutin	11.72 ± 3.30	7.02 ± 0.33	12.52 ± 1.80
(15) isoquercitrin + hyperin	8.10 ± 1.55	4.81 ± 0.95	3.42 ± 0.60
(16) unknown flavonol	9.43 ± 0.11	5.21 ± 0.82	6.76 ± 0.19
(17) avicularin	tr ^a	tr	tr
(18) phloridzin	82.79 ± 3.09	93.06 ± 0.63	196.13 ± 0.71
(19) unknown flavonol	27.81 ± 1.07	14.10 ± 1.18	10.57 ± 0.20
(20) quercitrin	16.74 ± 0.88	5.8 ± 0.93	33.64 ± 0.15

^a tr, less than 0.2 mg/L.

Table 4. Concentration of Phenolic Compounds in Different Apple Cultivars

variety ^a	catechin	epicatechin	B2	C1 + tetramer	phloridzin
ECA ^b		380.0	450.0	470.0	190.0
GS ^b	2.1	3.4	0.7	0.8	2.6
GS ^c	11.4	14.3	8.1		3.1
VD ^c	0.2	2.6	<0.1		<0.1
GD ^c	0.3	1.0	<0.1		0.3
GD ^d		40.0	60.0		10.0
GD ^b					3.6
SR ^c	<0.1	<0.1	<0.1		1.4
R ^c	0.1	1.5	<0.1		4.5
E ^d		10.0	40.0		10.0
RIG ^d		140.0	150.0		10.0
RD ^b	4	9.1	1.3	2.7	11.8
Mc ^b	0.2	0.6	0.0	0.0	8.7
S ^b	1.2	1.6	0.0	0.0	5.2

^a ECA, English cider; GS, Granny Smith; VD, Verde Doncella; GD, Golden Delicious; SR, Starking Red; R, Reineta; E, Empire; RIG, Rhode Island Greening; RD, Red Delicious; Mc, McIntosh; S, Spartan. ^b Concentration in µg/mL. ^c Concentration in µg/g. ^d Concentration in µg/g of flesh.

Smith (Spanos et al., 1990; Pérez-Ilzarbe et al., 1991), Verde Doncella (Pérez-Ilzarbe et al., 1991), Golden Delicious (Burda et al., 1990; Pérez-Ilzarbe et al., 1991);

Tomás-Barberán et al., 1993), Starking Red (Pérez-Ilzarbe et al., 1991), Reineta (Pérez-Ilzarbe et al., 1991), Red Delicious (Spanos et al., 1990), McIntosh (Spanos et al., 1990), Spartan (Spanos et al., 1990), and Empire (Burda et al., 1990); on the other hand, the Rhode Island Greening variety showed higher level of B2 procyanidin than the Spanish cultivars controlled (Burda et al., 1990) (Table 4).

In conclusion, experimental results suggested that the liquid-liquid extraction with ethyl acetate of apple juice is a good method to analyze flavan-3-ols and dihydrochalcones. Because the dihydrochalcones are typical compounds of apples, the good accuracy and reproducibility obtained in the analysis show that this method is a suitable indicator of adulteration products. However, this method is not appropriate for the quantification of the glycosides of quercetin. Fractionation of phenolic compounds on Sep-Pak or Sephadex LH-20 represents a good alternative to liquid-liquid extraction. These studies are in progress and they will be the subject of further papers.

ACKNOWLEDGMENT

This work was financially supported by the Comisión Interministerial de Ciencia y Tecnología (Project ALI 92-1027-CO3).

LITERATURE CITED

- Burda, S.; Oleszek, W.; Lee, C. Y. Phenolic compounds and their changes in apples during maturation and cold storage. *J. Agric. Food Chem.* **1990**, *38*, 945-948.
- Coseteng, M. Y.; Lee, C. Y. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. *J. Food Sci.* **1987**, *52*, 985-989.
- Dick, A. J. Apple fruit polyphenols and fruit softening. *Liaison Groupe Polyphenols* **1986**, *13*, 358-364.
- Dick, A. J.; Redden, P. R.; De Macro, A. C.; Lidster, P. D.; Gindley, T. B. Flavonoid glycosides of spartan apple peel. *Food Chem.* **1987**, *35*, 529-531.
- Duggan, M. B. Methods for examination of flavonoids in fruits: Application to flavonol glycosides and aglycones of apples, pears and strawberries. *Anal. Chem.* **1969**, *52*, 1038-1043.
- Galensa, R.; Hermann, K. Analysis of flavonoids by HPLC. *J. Chromatogr.* **1980**, *189*, 217-224.
- Keith, E. S.; Powers, J. J. Gas chromatographic determination of anthocyanins. *J. Food Sci.* **1966**, *31*, 971-979.
- Lea, A. G. H. HPLC of cider procyanidins. *J. Sci. Food Agric.* **1979**, *30*, 833-838.
- Lea, A. G. H. Reversed-phase gradient HPLC of procyanidins and their oxidation products in ciders and wines, optimised by Snyder's procedures. *J. Chromatogr.* **1980**, *194*, 62-68.
- Lea, A. G. H. Reversed-phase HPLC of procyanidins and other phenolics in fresh and oxidising apple juices using a pH shift technique. *J. Chromatogr.* **1982**, *238*, 253-257.
- Lea, A. G. H. Tannin and colour in English cider apples. *Fluess. Obst. Heft.* **1984**, *8*, 1-5.
- Lea, A. G. H.; Timberlake, C. F. The phenolics of cider. 1. Procyanidins. *J. Sci. Food Agric.* **1974**, *25*, 1537-1545.
- Lea, A. G. H.; Timberlake, C. H. The phenolics of ciders: Effect of processing conditions. *J. Sci. Food Agric.* **1978**, *29*, 484-492.
- Moller, B.; Herrmann, K. Analysis of quinic acid esters of hydroxycinnamic acids in plant material by capillary gas chromatography and HPLC. *J. Chromatogr.* **1982**, *241*, 371-379.
- Nagels, L.; Parmentier, F. Kinetic study of possible intermediates in the biosynthesis of chlorogenic acid in *Cestrum Poeppigii*. *Phytochemistry* **1976**, *15*, 703-706.
- Nagels, L.; Van Dongen, W.; De Brucker, J.; De Poster, H. High-performance liquid chromatographic separation of naturally occurring esters of phenolic acids. *J. Chromatogr.* **1980**, *187*, 181-187.
- Oleszek, W.; Lee, C. Y.; Jaworski, A. W.; Price, K. R. Identification of some phenolic components. *J. Agric. Food Chem.* **1988**, *36*, 430-432.
- Pérez-Illarbe, J.; Hernández, T.; Estrella, I. Phenolic compounds in apples: varietal differences. *Z. Lebensm. Unters. Forsch.* **1991**, *192*, 551-554.
- Pérez-Illarbe, J.; Martínez, V.; Hernández, T.; Estrella, I. Liquid chromatographic determination of apple pulp procyanidins. *J. Liq. Chromatogr.* **1992**, *15*, 637-646.
- Piretti, M. V.; Doghieri, P. Separation of peracetylated flavonoid and flavonoid polyphenols by normal-phase HPLC on a cyano-silice column and their determinations. *J. Chromatogr.* **1990**, *514*, 334-342.
- Salagoity-Auguste, M. H.; Bertrand, A. Wine phenolic analysis of low molecular weight components by HPLC. *J. Sci. Food Agric.* **1984**, *35*, 1241-1247.
- Schmidt, H. W. H.; Neukom, H. Identification of the main oligomeric proanthocyanidin occurring in apples. *J. Agric. Food Chem.* **1969**, *17*, 344-346.
- Spanos, G. A.; Wrolstad, R. E. Phenolics of apple, pear, and white grape juices and their changes with processing and storage. *J. Agric. Food Chem.* **1992**, *40*, 1478-1487.
- Spanos, G. A.; Wrolstad, R. E.; Heatherbell, D. A. Influence of processing and storage on the phenolic composition of apple juice. *J. Agric. Food Chem.* **1990**, *38*, 1572-1579.
- Tomás-Barberán, F. A.; García-Viguera, C.; Nieto, J. L.; Ferreres, F.; Tomás-Lorente, F. Dihydrochalcones from apple juices and jams. *Food Chem.* **1993**, *46*, 33-36.
- Van Buren, J.; De Vos, L.; Pilnik, W. Polyphenols in golden delicious apple juice in relation to method of preparation. *J. Agric. Food Chem.* **1976**, *24*, 448-451.
- Whiting, G. C.; Coggins, R. A. Estimation of the monomeric phenolics of ciders. *J. Sci. Food Agric.* **1975**, *26*, 1833-1838.
- Wilson, E. L. High-performance liquid chromatography of apple juice phenolic compounds. *J. Sci. Food Agric.* **1981**, *32*, 257-264.
- Wulf, L. W.; Nagel, C. W. Analysis of phenolic acids and flavonoids by HPLC. *J. Chromatogr.* **1976**, *116*, 271-279.

Received for review March 8, 1994. Revised manuscript received August 4, 1994. Accepted September 13, 1994.*

* Abstract published in *Advance ACS Abstracts*, November 1, 1994.