Importance of Phenolic Compounds for the Characterization of Fruit Juices

Brígida Fernández de Simón,* Javier Pérez-Ilzarbe, Teresa Hernández, Carmen Gómez-Cordovés, and Isabel Estrella

Instituto de Fermentaciones Industriales (CSIC), C/Juan de la Cierva 3, E-28006 Madrid, Spain

Several commercial juices of orange, apple, pineapple, peach, apricot, pear, and grape have been analyzed by HPLC and TLC to establish their phenolic composition. Benzoic acids and aldehydes, 3-flavanols, flavonols, chalcones, cinnamic acids, and their derivatives in the form of esters and glycosides have been studied. Hydroxycinnamic acid esters with tartaric acid are typical of grape, phloridzin is typical of apple, and isorhamnetin glycosides are typical of pear. Myricetin is only found in peach, and luteolin and apigenin glucosides are found only in orange. Apricot could be detected by the presence of two coumarins and pineapple by the presence of sinapic acid and the absence of the flavonoids studied in this work.

INTRODUCTION

The study of the polyphenolic composition of fruits is of great interest owing to the qualitative and quantitative differences appearing as a function of the species, cultivar, degree of ripening, and environmental conditions of growing, ripening, and storage (Spanos and Wrolstad, 1990a; Fernández de Simón et al., 1992). Phenolics are also important because of their contribution to the sensory quality of fruits (color, astringency, bitterness, and flavor) (Herrmann, 1990), which may be affected during the technological processes used for obtaining the juices and other transformation products (Raynal et al., 1986; Spanos and Wrolstad, 1990b; Spanos et al., 1990). Some of these compounds also have important pharmacological properties (Cody et al., 1988; Herrmann, 1990) and are used for therapeutic purposes. All of these aspects justify the increasing interest in fruit phenolics which has been manifested in the past few years (Macheix et al., 1990).

As a result of recent advances in chromatographic techniques, especially HPLC, phenolics have been individually studied, mainly those of the different flavonoid families (Harborne et al., 1975; Fleuriet and Macheix, 1976; Markham, 1982; Cheynier and Rigaud, 1986; Hrazdina, 1986; Tomás-Lorente et al., 1988; González-SanJosé et al., 1990). Some of them have been used for detecting adulterations, such as phloretin and isorhamnetin derivatives in distinguishing apple and pear juices (Wald and Galensa, 1989) or relationships between naringin and neohesperidin in mixtures of orange and grapefruit (Rousseff, 1988). A quercetin triglycoside is a suitable indicator of adulteration of black currant products with red currants (Siewek et al., 1984).

In contrast, low molecular weight phenolics have been scarcely studied, except the cinnamic acids and their esters with quinic and tartaric acids and some of their glycosides (Ribéreau-Gayon, 1963; Wardale, 1973; Mosel and Herrmann, 1974a,b; Whiting and Coggins, 1975; Macheix et al., 1977; Melin et al., 1979; Möller and Herrmann, 1982; Billot, 1983; Macheix and Fleuriet, 1986; Risch and Herrmann, 1988a,b; Peleg et al., 1991). It is thought that the free acids are formed by partial degradation of the combined forms during extraction or processing of fruits (Ramírez-Martínez and Luh, 1973; Fleuriet and Macheix, 1976). Acids different from cinnamic ones have been identified in the following fruits: grape, gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, and syringic (Fernández de Simón et al., 1992); black currant, salicylic, vanillic, 2,5-dihydroxybenzoic, and shikimic (Tanchev et al., 1986); apple, protocatechuic and *p*-hydroxybenzoic (Bilyk et al., 1988); and bilberry, *p*-hydroxybenzoic, *m*-hydroxybenzoic, gallic, protocatechuic, vanillic, and syringic (Azar et al., 1987).

In the present work, 3-flavanols, flavonols, chalcones, benzoic acids and aldehydes, cinnamic acids, and their derivatives in the form of esters and glycosides have been analyzed in several commercial juices and "nectars" to investigate the presence of marker compounds that could be useful in their characterization and differentiation.

MATERIALS AND METHODS

The Spanish Technical-Sanitary Regulations (*Código Alimentario*, 1985) distinguish between juices and nectars. The former are the liquids obtained from fruit by industrial processes, without dilution or fermentation. The nectars are obtained from juices after the addition of a syrup of the same Brix degree of that of the original juice in a proportion between 40 and 60%. Addition of concentrated grape juice is also permitted if it does not exceed the quantity of treated juice.

In this work, we have analyzed juices of commercial origin and from different packers: four apple juices, three peach nectars, three apricot nectars, three pear nectars, three grape juices, two orange nectars and four orange juices, two pineapple nectars and two pineapple juices, one peach plus apple juice, two peach plus grape juices, and one pear plus grape juice.

1. Extraction. All samples were processed in the same way: each juice (100 mL) was concentrated to 25 mL with a rotatory evaporator, always keeping the bath temperature under 35 °C. The process should not exceed 40 min. The concentrated juices were extracted two times with 15 mL of diethyl ether and then two times with 15 mL of ethyl acetate; fractions were pooled and evaporated to dryness, and the residue was redissolved in 2 mL of methanol/water (1:1 v/v).

2. Two-Dimensional TLC Analysis. *Analysis of Low Molecular Weight Phenolics and 3-Flavanols (Gómez-Cordovés et al., 1978; Díez de Bethencourt et al., 1980).* The adsorbent was cellulose MN300. Method 1 solvents were 2% formic acid and isopropyl alcohol/ammonium hydroxide/water (8:1:1 v/v/v). Method 2 solvents were 2% acetic acid and 20% potassium chloride. Detection of the spots was by UV light at 254 and 360 nm, before and after saturation with ammonia vapor, and spraying with 25% lead(II) acetate. Other spraying reagents were diazotized *p*-nitroaniline [mixture in (2:8 v/v) proportions of 0.5% *p*-nitroaniline in 2 N HCl and 20% sodium acetate, plus drops of 5% sodium nitrite] and then sodium carbonate 15%, chlor-

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Table I. Ranges of Concentration (in Milligrams per Liter) of Certain Nonflavonoid Phenolic Compounds in Commercial Juices and Nectars[a]

	peach	peach + grape	apricot	apple	apple + peach	pear	pear + grape	grape	orange	pineapple
1. *p*-hydroxybenzoic ac	0.01–0.16	0.98–3.42	0.06–0.39	t	0.09		t	0.81–2.60		0.17–0.72
2. protocatechuic ac		0.32–0.69								
3. gallic ac		0.38–0.84					0.01	0.86–2.01		
4. *p*-hydroxybenzoic ald	0.02–0.06			0.02–0.12					t	0.02–0.11
5. vanillic ald	0.01–0.08	0.01–0.04	0.01–0.16	t	0.01	0.02–0.12	0.03		t	t
6. syringic ald	0.01–0.04	t	0.01–0.07	t		0.03–0.11	0.03		0.10–0.25	0.10–0.38
7. 3,4-dihydroxybenzoic ald	0.03–0.18	0.10–0.20	0.04–0.08	0.05–0.26	0.10	0.02–0.06	0.04	0.03–0.18		t
8. *p*-coumaric ac	0.06–0.15	0.11–0.22	0.07–0.11	0.56–0.77	0.06	0.08–0.12	0.39	0.26–1.91	0.10–0.30	0.11–0.44
9. ferulic ac	nd–0.05	0.02–0.07	0.05–0.18	t	0.19	t	t	0.10–0.12	0.23–0.54	0.11–1.77
10. caffeic ac	0.02–0.31	0.20–0.37	0.09–0.27	0.86–1.05	0.18	0.01–0.07	0.04	0.38–0.81		0.08–0.16
11. sinapic ac									0.36–1.11	0.22–0.64
12. scopoletin			0.03–0.07						t	
13. aesculetin	t		0.02–0.05	0.20–0.45						
14. chlorogenic ac	0.22–7.12	1.18–1.48	0.65–3.09	2.49–9.14	3.52	1.14–7.88	11.56		0.13–2.04	
15. *p*-coumaroylquinic ac		0.27–0.48[b]		0.81–2.58	0.52	0.17–0.43	0.46			0.25–0.39
16. feruloylglucose				0.02–0.14		nd–0.30	0.03		0.08–0.19	0.09–0.14
17. caffeoyl ester	0.11–1.68[b]	0.14–0.37[c]	0.36–1.06[b]		0.99[b]			0.10–1.78[c]		
18. feruloyl ester		0.07–0.16[c]						0.74–1.01[c]		
19. *p*-coumaroyl ester		0.05–0.07[c]						0.78–1.61[c]		

[a] ac, acid; ald, aldehyde; nd, not detected; t, traces (only detected by TLC). [b] Possible 3′-caffeoylquinic acid. [c] Tartaric ester.

hydric vanillin (1% vanillin in 70% HCl), and sulfuric catechin [0.4% catechin in acetone/water/sulfuric acid (50:37.5:12.5 v/v/v)].

Analysis of Flavonols (Markham, 1982). The adsorbent was cellulose MN300. Method 1 solvents were BAW [upper phase of *n*-butyl alcohol/acetic acid/water (4:1:5 v/v/v)] and acetic acid 15%. Method 2 (monodimensional) solvent was Forestal [acetic acid/water/hydrochloric acid (30:10:3 v/v/v)] in plates previously washed with 15% acetic acid. Detection of the spots was by UV light, before and after saturation with ammonia vapor, and spraying with Neu's reagent (Neu, 1957) and UV light.

3. HPLC Analysis. The equipment was from Waters Associates and consisted of two M-6000A pumps, one 720 system controller, one U6K universal injector, and an absorbance detector Model M-440. A 600E pump system controller and a 991 photodiode array detector were also used for the analysis of certain low molecular weight phenolic compounds.

Analysis of Low Molecular Weight Phenolics and 3-Flavanols. A stainless steel C_{18} Nova-Pak column (300 × 3.9 mm) was used. Solvent A was 2% acetic acid and solvent B methanol/acetic acid/water (30:2:68 v/v/v). Detection was performed simultaneously at 280 and 340 nm. The gradient was as follows:

time, min	flow, mL/min	% A	% B	gradient curve
0	0.6	100	0	
2	0.6	100	0	5
10	0.6	60	40	5
15	0.6	50	50	5
20	0.5	40	60	5
30	0.4	30	70	5
40	0.4	25	75	5
45	0.3	15	85	5
50	0.7	15	85	5
60	0.8	15	85	5
65	0.6	100	0	5

Analysis of Flavonol Aglycons. A stainless steel C_{18} Nova-Pak column (150 × 3.9 mm) was used. The solvent used was water/methanol/acetic acid (57.5:37.5:5 v/v/v), and the flow rate was 0.7 mL/min. Detection was at 365 and 254 nm.

Analysis of Flavonol Glycosides. A stainless steel C_{18} Nova-Pak column (150 × 3.9 mm) was used. The solvent was a mixture, in 65:35 (v/v) proportions, of 2.5% acetic acid and tetrahydrofuran/water/acetic acid (50:47.5:2.5 v/v/v). The flow rate was 0.7 mL/min, and detection was at 365 and 254 nm.

4. Identification of the Compounds. Identification in HPLC analysis was achieved by comparing the retention times and UV absorbance ratios with those of the standards (from Fluka, Aldrich, and Sarsynthèse). In TLC analysis, identification was carried out by comparing R_f values and colors given with the spraying reagents or under UV light with those of the standards.

For the identification of the hydroxycinnamic acid derivatives, whose standards were not available, the pure compounds were collected from the chromatograph outlet. They were then subjected to enzymatic hydrolysis with pectolytic enzyme (Rapidase CX), and the hydrolysate was analyzed by HPLC and GLC. The phenolic moiety was identified by HPLC in the same conditions described above, and the nonphenolic moiety was analyzed by GLC of the trimethylsilyl derivatives (Villarroya, 1992). Likewise, the aglycon of some flavonol glycosides has been identified after acid hydrolysis with 5 N HCl (reflux for 1 h at 100 °C).

5. Quantitation of the Compounds. Quantitative determinations were carried out by the external standard method, the calibration curves having been described elsewhere (Fernández de Simón et al., 1990).

Hydroxycinnamic acid esters were quantitated with the calibration lines of the free acids, since their UV responses were expected to be very similar (Möller and Herrmann, 1982). This was confirmed by their similar UV spectra, obtained by diode array detection. In the same way, isorhamnetin glycosides and a kaempferol glycoside found in apricot were quantitated as rutin.

RESULTS AND DISCUSSION

Nonflavonoid Phenolics. Concentration intervals of the quantified nonflavonoid phenolic compounds in the juices and nectars are given in Table I.

Three benzoic acids (*p*-hydroxybenzoic, protocatechuic, and gallic) were quantitated, although they were present in small concentrations. Only the former was widespread among fruits. Gallic acid was only found in grape juices or in juices mixed with grape, so it could be considered as typical of grape. Also, small quantities of four benzoic aldehydes (namely *p*-hydroxybenzoic, vanillic, syringic, and 3,4-dihydroxybenzoic) were found.

Small quantities of two coumarins were quantitated by HPLC: scopoletin and aesculetin in apricot and aesculetin in apple juices. However, those compounds are better detected by TLC due to their intense fluorescence under UV light, and only aesculetin in peach and scopoletin in orange juices were found. So, we could differentiate apricot juices as the only ones containing both scopoletin and aesculetin. By means of TLC qualitative analysis, umbelliferone was found only in orange juices.

The cinnamic acids, caffeic, *p*-coumaric, ferulic, and sinapic acids, have been identified in their free forms, as

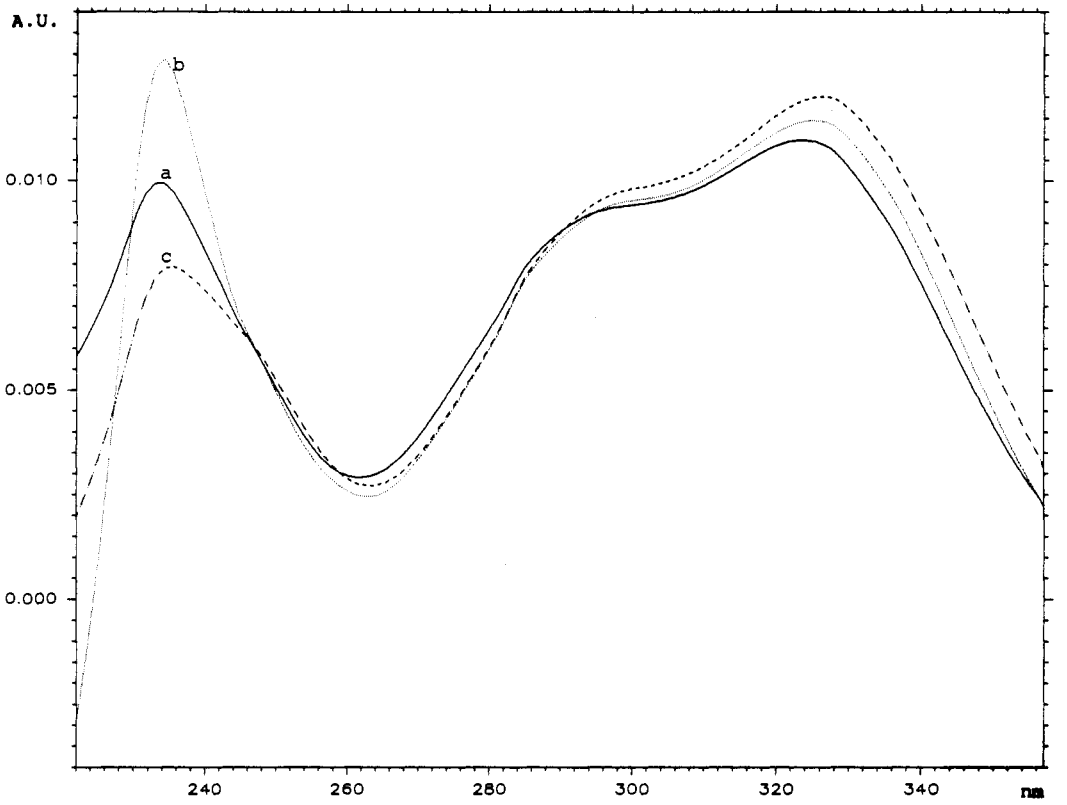

Figure 1. UV spectra of (a) caffeic acid, (b) possible 3′-caffeoylquinic acid, and (c) 5′-caffeoylquinic acid.

Table II. Ranges of Concentration (in Milligrams per Liter) of Certain Flavonoid Phenolic Compounds in Commercial Juices and Nectars[a]

	peach	peach + grape	apricot	apple	apple + peach	pear	pear + grape	grape	orange	pineapple
1. (+)-catechin	0.12–4.64	0.28–2.56		0.02–0.83	3.44			0.22–2.03		
2. (−)-epicatechin	0.41–3.60	1.09–1.46		0.02–0.74	4.30	nd–0.39		0.06–1.40		
3. myricetin	0.92–0.99	2.05–4.14								
4. quercetin	0.03–0.11	0.07–0.13	0.04–0.16	0.08–0.43	0.14	0.03–0.14	0.05	0.07–0.50		
5. kaempferol	0.03–0.04	0.05–0.08		0.03–0.06				0.06–0.27		
6. Q 3-*O*-rutinoside	0.03–0.11	0.17–0.22	0.37–1.52	0.06–0.10	0.23	0.01–0.14	0.07	0.05–0.16	0.30–1.22	
7. Q 3-*O*-galactoside	0.01–0.04	0.12–0.18		0.75–3.78	0.36	0.15–2.46	0.04	0.71–2.44		
8. Q 3-*O*-glucoside	0.04–0.16	0.25–1.37	0.02–1.28	0.01–0.08	0.11	nd–0.16	0.04	0.06–0.23		
9. Q 3-*O*-arabinoside	0.01–0.12	0.04–0.22		0.24–0.70	0.07	0.27[b]				
10. Q 3-*O*-rhamoside				1.33–2.68	0.36	0.35[b]		0.03–0.20		
11. Ph 2′-*O*-glucoside				13.81–26.9	3.79	2.54[b]				
12. K 3-*O*-rutinoside								0.01–2.72		
13. kaempferol glycoside[c]			0.08–0.75							
14. M 3-*O*-rhamoside								0.01–0.07		
15. luteolin 7-*O*-glucoside									0.09–0.97	
16. apigenin 7-*O*-glucoside									2.40–7.82	
17. isorhamnetin glycoside[c]						0.07–4.00	0.47			
18. isorhamnetin glycoside[c]						0.30–0.32	0.90			
19. isorhamnetin glycoside[c]						0.03–1.29	0.05			

[a] Q, quercetin; Ph, phloretin; K, kaempferol; M, myricetin; nd, not detected. [b] Supposed mixture with apple. [c] Expressed as rutin.

well as caffeoyl, *p*-coumaroyl, and feruloyl derivatives. Sinapic acid only occurred in orange and pineapple juices, while the rest of the free acids were found almost in every fruit juice.

In respect to hydroxycinnamic acid derivatives, the most widely distributed was chlorogenic acid (5′-caffeoylquinic acid). It was the one that reached highest concentrations, being the major compound in the fruits in which it was present, except in orange. No chlorogenic acid has been found in pineapple or in grape. Grape constitutes a particular case, since the hydroxy acid that esterifies the cinnamic acids is not quinic but tartaric, a fairly rare acid in fruits. Grape juice can be detected by the presence of caffeoyltartaric, *p*-coumaroyltartaric, and feruloyltartaric acids, while the presence of hydroxycinnamic acid esters with quinic acid would imply adulteration with other fruits.

Another caffeic acid ester has been found in peach and apricot, which was tentatively identified as 3′-caffeoylquinic acid, on the basis of its chromatographic and spectral features. This compound is reported to elute earlier than chlorogenic acid in reversed-phase HPLC columns (Court, 1977; Möller and Herrmann, 1982), and it shows a UV spectrum (obtained with the diode array detector) very similar to those of caffeic and chlorogenic acids (Figure 1). Several works have already stated the usefulness of diode array detection for the identification of phenolic acid esters (Winter and Herrmann, 1986; Pérez-Ilzarbe et al., 1991). In the same way, in both orange and pineapple we found several peaks, with UV spectra very similar to those of the free cinnamic acids, which could be other derivatives with sugars or hydroxy acids and whose identification is to be completed in future work.

The mixed juices of peach and grape contained tartaric esters of cinnamic acids coming from the grape and chlorogenic acid and possibly 3′-caffeoylquinic acid coming from the peach. The mixed juice of pear and grape contained high levels of chlorogenic acid but no tartaric acid esters. On the other hand, it contained a small quantity of gallic acid. That is way we think the proportions of grape juice in the mixture was very small, since the concentrations of all of the compounds were very low.

Flavonoids. The identified compounds are listed in Table II, with the concentration intervals found in the different juices and nectars. None of them was found in pineapple juices.

The 3-flavanol monomers (+)-catechin and (−)-epicatechin were found in peach, apple, and grape and in mixtures of peach with grape and peach with apple. No flavanols were found in the mixtures of grape and pear.

The great variation in the contents of flavanols is noteworthy. This was probably due not only to known cultivar differences (Risch and Herrmann, 1988a; McRae et al., 1990) but also to the different degrees of pressing, for these compounds are mainly located in the skin and seeds (Billot, 1983; Pérez-Ilzarbe et al., 1991; Fernández de Simón et al., 1992). Besides, flavanols are natural substrates for polyphenol oxidase and are involved in browning, polymerization, and haze formation phenomena leading to losses during storage or technological processing of fruits (Spanos and Wrolstad, 1990a,b; Spanos et al., 1990).

Those are the reasons not to find (+)-catechin nor (−)-epicatechin in pear and apricot juices, although those fruits have been reported to contain important quantities of flavanols (Billot, 1983; Risch and Herrmann, 1988a). No catechins were found in pineapple and orange juices.

Three flavonol aglycons have been found: myricetin, quercetin, and kaempferol. Myricetin only occurred (and in high levels) in juices containing peach, while quercetin occurred in all of them except those of orange and pineapple. In respect to flavonol glycosides, and like the aglycons, the most common were those of quercetin. Again, pineapple juices contained no quercetin glycosides.

A non-quercetin glycoside is phloridzin (phloretin 2′-*O*-glucoside), which attained high concentrations in juices containing apple, thus allowing detection of apple. One of the samples of pear juice contained phloridzin as the main flavonoid, together with quercetin 3-*O*-rhamnoside and quercetin 3-*O*-arabinoside, which are typical of apple and not of pear. We should assume that this nectar was a mixture with apple juice.

Kaempferol glycosides were only detected in the pure juices of grape and apricot, and myricetin 3-*O*-rhamnoside was only present in pure grape juices. There were three isorhamnetin glycosides which only occurred in juices containing pear, permitting detection of the presence of this juice in the mixtures.

With respect to orange, it could be differentiated as the only studied fruit containing luteolin and apigenin glucosides. However, flavanones seem to be the most representative phenolics in this fruit (Rousseff et al., 1987; Rousseff, 1988) but were not analyzed in this work.

The mixtures with grape could not be detected by their flavonoid glycosidic patterns, for the mixed juices contained the same compounds as the pure juices of pear and peach (pear being detected by the isorhamnetin glycosides and peach by myricetin). Nevertheless, the presence of grape juice could be detected by the identification of gallic acid or hydroxycinnamic esters with tartaric acid. The mixture of peach plus apple could be proved by high concentrations of phloridzin like in the apple and by the presence of myricetin like in the peach. There was also the occurrence of quercetin 3-*O*-rhamnoside, typical of apple but not of peach.

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LITERATURE CITED

Azar, M.; Vérette, E.; Brun, S. Identification of some phenolic compounds in bilberry juice. (*Vaccinium myrtillus*). *J. Food Sci.* **1987**, *52*, 1255–1257.

Billot, J. Evolution of phenolic compounds during the ripening of Passe Crassane pear. *Physiol. Veg.* **1983**, *21*, 527–535.

Bilyk, A.; Hicks, K. B.; Bills, D. D.; Sapers, G. M. Application of HPLC and dual wavelength detection to the analysis of phenolic compounds in apples. *J. Liq. Chromatogr.* **1988**, *11*, 2829–2841.

Cheynier, V.; Rigaud, J. HPLC separation and characterization of flavonols in the skins of *Vitis vinifera* var. Cinsault. *Am. J. Enol. Vitic.* **1986**, *37*, 248–252.

Código Alimentario. Boletín Oficial del Estado: Madrid, 1985; pp 220–230.

Cody, V.; Middleton, E., Jr.; Harborne, J. B.; Beretz, A. *Plant Flavonoids in Biology and Medicine II. Biochemical, cellular and mechanical properties*; Liss: New York, 1988.

Court, W. A. High-performance reversed-phase liquid chromatography of naturally occurring phenolic compounds. *J. Chromatogr.* **1977**, *130*, 287–291.

Díez de Bethencourt, C.; Gómez-Cordovés, C.; Blanco, M.; de la Calera, J. G. Low molecular weight phenolic compounds. Their influence on the quality of vinegars. *Rev. Agroquim. Tecnol. Aliment.* **1980**, *20*, 247–256.

Fernández de Simón, B.; Pérez-Ilzarbe, J.; Hernández, T.; Gómez-Cordovés, C.; Estrella, I. HPLC study of the efficiency of extraction of phenolic compounds. *Chromatographia* **1990**, *30*, 35–37.

Fernández de Simón, B.; Hernández, T.; Estrella, I.; Gómez-Cordovés, C. Variation in phenol content in grapes during ripening: low-molecular-weight phenols. *Z. Lebensm. Unters. Forsch.* **1992**, *194*, 351–354.

Fleuriet, A.; Macheix, J. J. Effect of anaerobic conditions on the phenolic compounds of "cherry" tomatoes (*Lycopersicum esculentum* var. Cerasiforme). *Physiol. Veg.* **1976**, *14*, 407–414.

Gómez-Cordovés, C.; Garrido, D.; Díez, C. Study of the phenolic compounds of oak-aged spirits. *Bull. Liaison—Groupe Polyphenols* **1978**, *8*, 369–372.

González-SanJosé, M. L.; Barrón, L. J. R.; Díez, C. Evolution of anthocyanins during maturation of Tempranillo grape variety (*Vitis vinifera*) using polynomial regression models. *J. Sci. Food Agric.* **1990**, *51*, 337–343.

Harborne, J. B.; Mabry, T. J.; Mabry, H. *The flavonoids*; Academic Press: New York, 1975.

Herrmann, K. Significance of hydroxycinnamic acid compounds in food. II. Effects on the activity of food enzymes, alleged antithiamine effect, bitter taste, pharmacological effectiveness and therapeutic uses, and significance as antimutagens and anticarcinogens. *Chem. Mikrobiol. Technol. Lebensm.* **1990**, *12*, 161–167.

Hrazdina, G. Biosynthesis and accumulation of phenolics in grapes. *Bull. Liaison—Groupe Polyphenols* **1986**, *13*, 462–465.

Macheix, J. J.; Fleuriet, A. Hydroxycinnamic derivatives in fruits. *Bull. Liaison—Groupe Polyphenols* **1986**, *13*, 337–351.

Macheix, J. J.; Rateau, J.; Fleuriet, A.; Bureau, D. Hydroxycinnamic esters in fruits. *Fruits* **1977**, *32*, 397–405.

Macheix, J. J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL 1990.

Markham, K. R. *Techniques of flavonoid identification*; Academic Press: London, 1982; pp 16–32.

McRae, K. B.; Lidster, P. D.; 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.

Melin, C.; Billot, J.; Dupin, J. F. Phenolic compounds in Bigarrean Napoleon cherries. I. Hydroxycinnamic derivatives. *Physiol. Veg.* **1979**, *17*, 557–572.

Möller, D.; Herrmann, K. Analysis of quinic acid esters of hydroxycinnamic acids in plant material by capillary gas chromatography and high-performance liquid chromatography. *J. Chromatogr.* **1982**, *241*, 371–379.

Mosel, H. D.; Herrmann, K. The phenolics of fruits. IV. The phenolics of blackberries and raspberries and their changes during development and ripeness of the fruits. *Z. Lebensm. Unters. Forsch.* **1974a**, *154*, 324–327.

Mosel, H. D.; Herrmann, K. Changes in catechins and hydroxycinnamic acid derivatives during development of apples and pears. *J. Sci. Food Agric.* **1974b**, *25*, 251–256.

Neu, R. A new reagent for detection and identification of flavones in paper chromatograms. *Naturwissenschaften* **1957**, *43*, 82–84.

Peleg, H.; Naim, M.; Rousseff, R. L.; Zehavi, U. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*) *J. Sci. Food Agric.* **1991**, *57*, 417–426.

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

Ramírez-Martínez, J. R.; Luh, B. S. Phenolic compounds in frozen avocados. *J. Sci. Food Agric.* **1973**, *24*, 219–225.

Raynal, J.; Ginestet, C.; Souquet, J. M.; Moutounet, M. Evolution of phenolic constituents in the epicarp of Ente's prune during drying. *Bull. Liaison—Groupe Polyphenols* **1986**, *13*, 482–487.

Ribéreau-Gayon, P. Phenolic acids in *Vitis vinifera*. *C. R. Acad. Sci. Paris* **1963**, *256*, 4108–4111.

Risch, B.; Herrmann, K. Contents of hydroxycinnamic acid derivatives and catechins in pome and stone fruit. *Z. Lebensm. Unters. Forsch.* **1988a**, *186*, 225–230.

Risch, B.; Herrmann, K. Hydroxycinnamic acid derivatives in Citrus fruit. *Z. Lebensm. Unters. Forsch.* **1988b**, *187*, 530–534.

Rousseff, R. L. Liquid chromatographic determination of naringin and neohesperidin as a detector of grapefruit juice in orange juice. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 798–802.

Rousseff, R. L.; Martin, S. F.; Youtsey, C. O. Quantitative survey of narirutin, naringin, hesperidin, and neohesperidin in citrus. *J. Agric. Food Chem.* **1987**, *35*, 1027–1030.

Siewek, F.; Galensa, R.; Herrmann, K. Detection of adulteration of black currant products by products from red currants by means of HPLC of flavonol glycosides. *Z. Lebensm. Unters. Forsch.* **1984**, *179*, 315–321.

Spanos, G. A.; Wrolstad, R. E. Influence of variety, maturity, processing and storage on the phenolic composition of pear juice. *J. Agric. Food Chem.* **1990a**, *38*, 817–824.

Spanos, G. A.; Wrolstad, R. E. Influence of processing and storage on the phenolic composition of Thompson Seedless grape juice. *J. Agric. Food Chem.* **1990b**, *38*, 1565–1571.

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.

Tanchev, S.; Ioncheva, N.; Genov, N.; Malchev, E. Identification of phenolic acids in black currants juice (*Ribes nigrum*) and their transformations during their thermal treatment. *Bull. Liaison—Groupe Polyphenols* **1986**, *13*, 370–373.

Tomás-Lorente, F.; Ferreres, F.; Tomás-Barberán, F. A.; Albaladejo, J.; Guzmán, G. HPLC analysis for the characterization of jams and juices. *Aliment., Equipos Tecnol.* **1988**, *7*, 167–169.

Villarroya, B. Influence of the climate on the main components of *Vitis vinifera* grape during its maturation. Ph.D. Thesis, Universidad Autónoma de Madrid, Facultad de Ciencias, 1992; pp 84–86.

Wald, B.; Galensa, R. Detection of adulteration of apple juice and pear juice. *Z. Lebensm. Unters. Forsch.* **1989**, *188*, 107–114.

Wardale, D. A. Effect of phenolic compounds in *Lycopersicum esculentum* on the synthesis of ethylen. *Phytochemistry* **1973**, *12*, 1523–1530.

Whiting, G. C.; Coggins, R. A. Estimation on the monomeric phenolics of ciders. *J. Sci. Food Agric.* **1975**, *26*, 1833–1838.

Winter, M.; Herrmann, K. Esters and glucosides of hydroxycinnamic acids in vegetables. *J. Agric. Food Chem.* **1986**, *34*, 616–620.

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