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Varietal Differences among the Polyphenol Profiles of Seven Table Grape Cultivars Studied by LC−DAD−MS−MS

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Polyphenols present in red table grape varieties Red Globe, Flame Seedless, Crimson Seedless, and Napoleon, and the white varieties Superior Seedless, Dominga, and Moscatel Italica were analyzed by HPLC−DAD−MS. The anthocyanins peonidin 3-glucoside, cyanidin 3-glucoside (and their corresponding *p*-coumaroyl derivatives), malvidin 3-glucoside, petunidin 3-glucoside, and delphinidin 3-glucoside were found. In addition, caffeoyltartaric acid, *p*-coumaroyltartaric acid, and the flavonols quercetin 3-glucuronide, quercetin 3-rutinoside, quercetin 3-glucoside, kaempferol 3-galactoside, kaempferol 3-glucoside, and isorhamnetin 3-glucoside were detected. Flavan-3-ols were also detected, and were identified as gallocatechin, procyanidin B1, procyanidin B2, procyanidin B4, procyanidin C1, catechin, and epigallocatechin. These phenolics were present only in the skin, as the flesh of these grape cultivars was almost devoid of these compounds. Anthocyanins were the main phenolics in red grapes ranging from 69 (Crimson Seedless) to 151 (Flame Seedless) mg/kg fresh weight of grapes, whereas flavan-3-ols were the most abundant phenolics in the white varieties ranging from 52 (Dominga) to 81 (Moscatel Italica) mg/kg fresh weight of grapes. Total phenolics ranged from 115 (Dominga) to 361 (Flame Seedless) mg/kg fresh weight of grapes. This means that a serving of unpeeled table grapes (200 g) could provide up to 72 mg of total phenolics (Flame Seedless). These results indicate that the intake of unpeeled table grapes should be recommended in dietary habits as a potential source of antioxidant and anticarcinogenic phenolic compounds.

KEYWORDS: Anthocyanins; hydroxycinnamic acid derivatives; flavan-3-ols; flavonols; HPLC−DAD−MS; stilbenoids; table grapes; total phenolics; *Vitis vinifera*

INTRODUCTION

In the past few years, epidemiological, clinical, and in vitro studies have shown the role of polyphenolic compounds (present in fruits, vegetables, and wine) in preventing cardiovascular disease mortality (*1*−*5*). Flavonoids and other phenolic compounds have been reported to have multiple biological effects such as antioxidant (*6*, *7*), antiinflammatory (*8*, *9*), inhibition of platelet aggregation (*10*), antimicrobial (*11*), and even "anti-aging" (*5*) activities. Other in vivo studies showed that antioxidant flavonoids were inversely associated with mortality from heart disease (*12*). Furthermore, delayed tumor onset was observed in transgenic mice upon feeding with red wine phenolics (*13*).

Grapes constitute one of the major sources of phenolic compounds among different fruits (*14*). In this context, the antioxidant activity of grapes has been positively correlated with their phenolic composition: anthocyanins, flavonols, flavan-3-ols, hydroxybenzoates, and, in general, phenolic compounds (*15*) (**Figure 1**). In addition, these compounds have proved to inhibit in vitro the oxidation of human low-density lipoproteins (*16*), an important step in atherogenic plaque formation which is determinant for development of cardiovascular and coronary disease (*17*). Also established is the effectiveness of phenolic compounds from grape extracts to inhibit hydroperoxide formation as well as to inhibit both lipid and protein oxidation (*18*).

Some studies have previously reported the phenolic composition of wine grape varieties (*19*−*21*). Usually, these studies have been carried out in order to correlate phenolic compounds concentration with antioxidant activity. However, information concerning the identification and quantification of each phenolic compound of table grape varieties is rather scarce.

The objective of the present study is to identify by HPLC−DAD−MS−MS the composition of potential health-promoting phenolic compounds from seven table grape varieties. Four red (Red Globe, Flame Seedless, Crimson Seedless, and Napoleon) and three white (Superior Seedless, Dominga, and Moscatel Italica) table grape varieties were analyzed.

MATERIALS AND METHODS

Reagents. Cyanidin 3-rutinoside was purchased from Polyphenols A. S. (Sandnes, Norway), quercetin 3-rutinoside was purchased from Merck (Darmstadt, Germany), and resveratrol, catechin, and chlorogenic acid were purchased from Sigma (Madrid, Spain). Formic acid and

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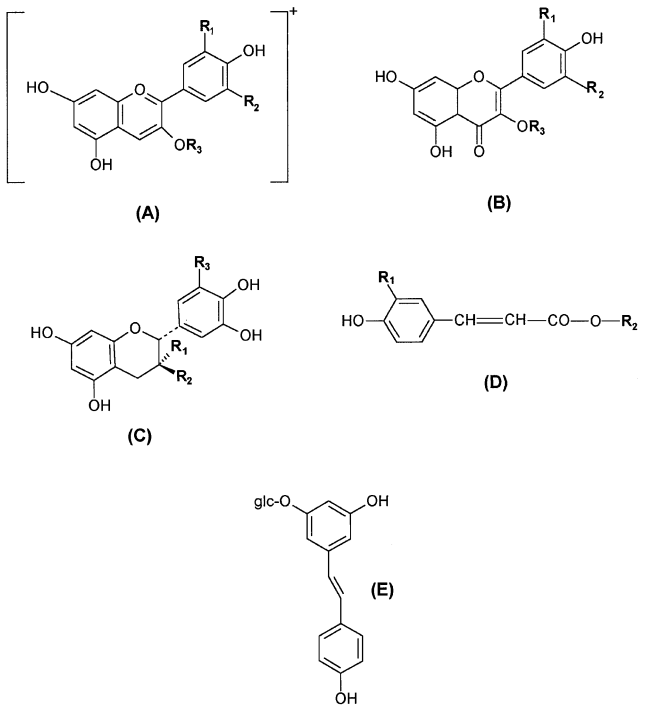

Figure 1. Representative structures of the phenolic compounds described in the present study. (A) Anthocyanins: $R_1 = R_2 = -OH$, R_3 = glucose, delphinidin 3-glucoside; $R_1 = -OH$, $R_2 = H$, R_3 = glucose, cyanidin 3-glucoside; $R_1 = -OCH_3$, $R_2 = -OH$, R_3 = glucose, petunidine 3-glucoside; $R_1 = -OCH_3$, $R_2 = H$, R_3 = glucose, peonidin 3-glucoside; $R_1 = R_2 = -OCH_3$, R_3 = glucose, malvidin 3-glucoside; $R_1 = -OH$, $R_2 = H$, R_3 = *p*-coumaroylglucose, cyanidin 3-*p*-coumaroylglucoside; $R_1 = -OCH_3$, $R_2 = H$, R_3 = *p*-coumaroylglucose, peonidin 3-*p*-coumaroylglucoside. (B) Flavonols: $R_1 = -OH$, $R_2 = H$, R_3 = glucose, quercetin 3-glucoside; $R_1 = -OH$, $R_2 = H$, R_3 = glucose-rhamnose, quercetin 3-rutinoside; $R_1 = R_2 = H$, R_3 = galactose, kaempferol 3-galactoside; $R_1 = R_2 = H$, R_3 = glucose; kaempferol 3-glucoside; $R_1 = -OCH_3$, $R_2 = H$, R_3 = glucose, isorhamnetin 3-glucoside. (C) Flavan-3-ols: $R_1 = H$, $R_2 = -OH$, $R_3 = H$, (+)-catechin; $R_1 = H$, $R_2 = -OH$, $R_3 = -OH$, gallocatechin; $R_1 = -OH$, $R_2 = H$, $R_3 = -OH$, epigallocatechin; epicatechin–catechin, procyanidin B_1; epicatechin–epicatechin, procyanidin B_2; catechin–epicatechin, procyanidin B_4; epicatechin–epicatechin–epicatechin, procyanidin C_1. (D) Hydroxycinnamic acid derivatives: $R_1 = -OH$, R_2 = tartaric acid, caffeoyltartaric acid; $R_1 = H$, R_2 = tartaric acid, *p*-coumaroyltartaric acid. (E) *trans*-Piceid.

methanol (MeOH) were of analytical grade and also supplied by Merck. Ultrapure water from a Milli-Q system (Millipore Corp., Bedford, MA) was used throughout this research.

Grapes. The table grapes were harvested in summer (July−October) 2001 in different locations from Murcia (Spain): Superior Seedless (Cieza, July 1), Flame Seedless (Molina de Segura, July 16), Red Globe and Moscatel Italica (Aledo, July 31), Crimson Seedless (Blanca, September 9), Dominga (Totana, November 7), and Napoleon (Alhama, November 14). All grape berries were transported to the laboratory and processed the same day.

Extraction of Phenolic Compounds. Grapes were peeled with a sharp knife, and the skins were stored at −20 °C until analyzed. Skin represented approximately 10% of the total fresh weight of grape berry, so data were divided by 10 to express the phenolic content in mg/kg fresh weight. Samples were homogenized in an Ultraturrax T-25 equipment (Janke and Kunkel, Ika-Labortechnick) at 24 000 rpm for 1 min after addition of 4 mL of a solution of MeOH/formic acid (97:3) per g of grape skin. The extracts were centrifuged at 5000*g* for 5 min in a Centromix centrifuge (Selecta, Barcelona), filtered through a 0.45-μm membrane filter Millex-HV_{13} (Millipore), and analyzed by HPLC.

HPLC Analysis of Phenolics. A sample of 20 μL of the supernatant above-obtained was analyzed using a Merck-Hitachi HPLC system with a pump model L-7100 and a diode array detector Merck-Hitachi 7455. Separations were achieved on a Licrochart column (Merck) (RP-18, 12 × 0.4 cm; 5 μm particle size). The mobile phase consisted of water with 5% formic acid (solvent A) and HPLC grade methanol (solvent B) at a flow rate of 1 mL/min. Elution was performed as previously described by Cantos et al. (*22*). HPLC experiments were repeated three times. Phenolic content was expressed as mg/kg of fresh weight taking into account that skin represented about 10% of the total berry weight.

Phenolic Identification and Quantification. Chromatograms were recorded at 510, 360, 320, and 280 nm. Phenolic compounds were identified by their UV spectra recorded with a diode array detector and by LC−MS. Some of these phenolics have been previously identified with authentic markers (*22*), and other were identified by their MS spectra and their corresponding daughter MS/MS fragments.

Anthocyanins were quantified at 510 nm as cyanidin 3-rutinoside, flavonols at 360 nm as quercetin 3-rutinoside, stilbenoids at 320 nm as resveratrol, hydroxycinnamic acid derivatives at 320 nm as chlorogenic, acid and flavan-3-ols at 280 nm as catechin.

HPLC−MS−MS. Chromatographic separation was carried out on an RP C_{18} LiChroCART column (25 × 0.4 cm, particle size 5 μm, Merck, Darmstadt, Germany) using water/formic acid (95:5, v/v) (A) and methanol (B) as the mobile phases. Elution was performed as HPLC analysis conditions above detailed. The HPLC system equipped with a DAD detector and mass detector in series consisted of a HPLC binary pump (G1312A), an autosampler (G1313A), a degasser (G1322A), and a photodiode array detector (G1315B) controlled by software (v. A08.03) from Agilent Technologies (Waldbronn, Germany). The mass detector was an ion-trap mass spectrometer (G2445A, Agilent Technologies, Waldbronn, Germany) equipped with an electrospray ionization (ESI) system and controlled by software (v. 4.0.25). The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. Mass scan (MS) and daughter (MS−MS) spectra were measured from *m*/*z* 100 up to *m*/*z* 1500. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas, and the collision energy was set at 50%. Mass spectrometry data were acquired in the negative ionization mode for anthocyanins, flavonols, hydroxycinnamic acids, and flavan-3-ols. UV chromatograms were recorded at 510, 360, 320, and 280 nm.

RESULTS AND DISCUSSION

Anthocyanins. Few qualitative differences in anthocyanins from red table grape varieties were found. Seven anthocyanin-glucosides (two of them acylated) were identified in grape skins: delphinidin 3-glucoside, cyanidin 3-glucoside, cyanidin 3-*p*-coumaroylglucoside, petunidin 3-glucoside, peonidin 3-*p*-coumaroylglucoside, peonidin 3-glucoside, and malvidin 3-glucoside (**Table 1**; **Figure 2A**). The only qualitative difference was the absence of both acylated derivatives in Flame Seedless as well as the absence of the cyanidin 3-*p*-coumaroylglucoside in Napoleon (**Table 2**). However, significant quantitative differences in the anthocyanin profile were found. The main anthocyanin in all the varieties was peonidin 3-glucoside, in contrast to wine grapes in which the main anthocyanin has been reported to be malvidin 3-glucoside (*23*). The other most abundant anthocyanins were cyanidin 3-glucoside and malvidin 3-glucoside. The latter two occurred in similar amounts except in Red Globe in which cyanidin 3-glucoside was three times more abundant than malvidin 3-glucoside (**Table 2**). Petunidin 3-glucoside and delphinidin 3-glucoside were minor glucosides with a mean contribution of about 3% and 2% of the total anthocyanins, respectively (**Table 2**). The above quantitative profile of anthocyanins was not accomplished by Flame Seedless which contained the highest amount in anthocyanins distributed in equal proportion, including peonidin 3-glucoside and delphinidin 3-glucoside which represented around 22% of total anthocyanins in this variety (**Table 2**). Total anthocyanins

Table 1. HPLC–DAD–MS Analysis of Grape Skin Phenolics

structure[a]	molecular ion m/z^- (MS)	fragment m/z^- (MS–MS)	HPLC Rt (min)
dp 3-glc	463	300	23.5
cy-3-glc	447	284	26.3
pt-3-glc	477	314	28.7
pn-3-glc	461	299	31.1
mv-3-glc	491	329	32.8
cy-*p*-coum	593	284	46.0
pn-*p*-coum	607	453, 299	46.9
q-3-gluc	477	301	33.5
q-3-glc	463	300	34.1
q-3-rut	609	463, 300	34.4
k-3-gal	447	284	37.3
k-3-glc	447	284	39.2
i-3-glc	477	314	40.5
cafta	311	179	10.3
couta	295	163	13.3
piceid	389	227	26.8
(epi)catechin	289	289	11.9
procyanidin B1	577	289	10.3

[a] Abbreviations: dp-3-glc, delphinidin 3-glucoside; cy-3-glc, cyanidin 3-glucoside; pt-3-glc, petunidin 3-glucoside; pn-3-glc, peonidin 3-glucoside; mv-3-glc, malvidin 3-glucoside; cy-*p*-coum, cyanidin 3-*p*-coumaroylglucoside; pn-*p*-coum, peonidin 3-*p*-coumaroylglucoside; q-3-gluc, quercetin 3-glucuronide; q-3-glc, quercetin 3-glucoside; q-3-rut, quercetin 3-rutinoside; k-3-gal, kaempferol galactoside; k-3-glc, kaempferol-3-glucoside; i-3-glc, isorhamnetin 3-glucoside; cafta, caffeoyltartaric acid; couta, *p*-coumaroyltartaric acid.

ranged from 151 mg/kg of fresh weight of grapes (fw) for Flame Seedless (the richest source) to 68 mg/kg of fw for Crimson Seedless (the poorest variety together with Napoleon). Total anthocyanins, as well as the different proportion of every anthocyanin in the total anthocyanin profile, has been previously described in 32 red table grape varieties including both Red Globe and Flame Seedless (*24*). This previous study reported total anthocyanin values similar to those found in the present study (**Table 2**), even though the proportion of each anthocyanin regarding the total anthocyanin profile was different from that reported here (**Table 2**). In addition, in this previous study, not only coumaroyl−anthocyanin esters but also both caffeoyl− and acetyl−anthocyanin esters were found, although in small amount. The lack of these compounds as well as the other differences in the seven table grapes assayed here could be related to agronomic factors such as soil composition, irrigation, light intensity, etc., as agronomic factors have been proposed as important determinants of the phenolic composition of fruits and vegetables, including the possible variation of the phenolic profile of grape varieties (*25*).

Flavonols. The main flavonols found in all the table grape varieties were quercetin 3-glucuronide, quercetin 3-glucoside, and quercetin 3-rutinoside (the two latter coeluted in a single peak under these chromatographic conditions and thus they were quantified as a single peak; **Figure 1B**, peak 11). A common pattern in the quantitative profile of the main flavonols was not found. Quercetin 3-glucuronide was the dominant flavonol in Flame Seedless and Napoleon varieties, whereas it was found in the same concentration as the other two quercetin derivatives in Crimson Seedless and Moscatel Italica varieties. Red Globe, Superior Seedless, and Dominga grapes contained quercetin 3-glucuronide in smaller amounts than quercetin 3-glucoside plus quercetin 3-rutinoside (**Table 3**).

Three other minor compounds were identified as two kaempferol−hexosides and isorhamnetin 3-glucoside. Both kaempferol derivatives shared the same mass and UV-spectra, although their retention times were different. The UV spectrum

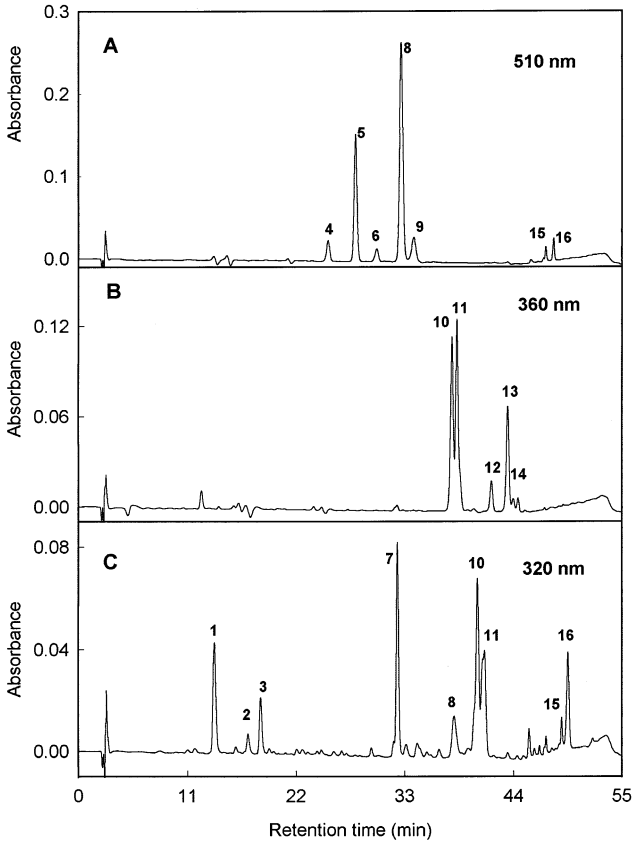

Figure 2. HPLC chromatograms of skin extracts of mature table grapes. (A) Flame variety, 510 nm; (B) Superior variety, 360 nm; (C) Napoleon variety, 320 nm. (**1**) caffeoyltartaric acid; (**2**) hydroxycinnamic acid derivative-*a*; (**3**) *p*-coumaroyltartaric acid; (**4**) delphinidin 3-glucoside; (**5**) cyanidin 3-glucoside; (**6**) petunidine 3-glucoside; (**7**) *trans*-piceid; (**8**) peonidin 3-glucoside; (**9**) malvidin 3-glucoside; (**10**) quercetin 3-glucuronide; (**11**) quercetin 3-glucoside + quercetin 3-rutinoside; (**12**) kaempferol 3-galactoside; (**13**) kaempferol 3-glucoside; (**14**) isorhamnetin 3-glucoside; (**15**) cyanidin 3-*p*-coumaroylglucoside; (**16**) peonidin 3-*p*-coumaroylglucoside.

of the first eluted compound (peak 12; **Figure 2B**) revealed that the hexoside substitution was at 3 position, because substitution at 7 position provokes a characteristic shift in the maximum of UV spectrum which was not observed. Peak 13 (**Figure 2B**) was coincident with the retention time, UV spectrum, and m/z^- of an authentic standard of kaempferol-3-glucoside. As the compound corresponding to peak 12 eluted earlier than peak 13, this difference was due to the hexoside residue. It can be assumed that peak 12 is kaempferol 3-galactoside (**Figure 2B**; **Table 1**). The above minor flavonols were detected in trace amounts in most of the varieties. However, they were quantified in the white varieties Superior Seedless and Moscatel Italica. In both cases kaempferol 3-glucoside was the most abundant (**Table 3**). Previous reports described the presence of myricetin derivatives in both wine (*26*) and table (*21*) grapes. However, the flavonol myricetin was not found in any of the seven table grape varieties studied here. This discrepancy could be justified by the use of different extraction protocols, as organic media extract higher amounts of both flavan-3-ols and flavonols, whereas water extracts higher amounts of anthocyanins and procyanidins (*21*).

Total flavonol content ranged from 64 mg/kg of fw for Superior Seedless to 13 mg/kg of fw for Crimson Seedless. In general, white grape varieties showed a higher flavonol contribution to total phenols than red grape varieties. This contribu-

Table 2. Anthocyanins Content of Table Grape Varieties[a]

anthocyanin[b]	Red Globe	Flame	Crimson	Napoleon
dp-3-glc	4.7 (1.0)	34.3 (2.7)	1.1 (0.1)	1.9 (0.3)
cy-3-glc	28.9 (4.2)	32.7 (7.1)	6.6 (2.0)	11.1 (3.2)
pt-3-glc	2.71 (0.9)	17.9 (0.8)	0.9 (0.1)	1.4 (0.4)
pn-3-glc	65.4 (0.2)	32.4 (5.1)	45.2 (5.2)	40.6 (0.2)
mv-3-glc	9.25 (0.2)	33.4 (7.3)	8.8 (1.4)	17.8 (4.2)
cy-*p*-coum	1.4 (0.4)	0.0 (0.0)	1.2 (0.3)	0.0 (0.0)
pn-*p*-coum	2.9 (0.1)	0.0 (0.0)	4.7 (0.7)	5.9 (1.5)
ta	115.3 (1.1)	150.7 (2.2)	68.5 (6.9)	75.7 (0.4)

[a] Values are expressed as mg/kg of fresh weight of grape berry (skin + flesh). Standard deviation is reported in parentheses ($n = 3$). [b] Abbreviations used: dp-3-glc, delphinidin 3-glucoside; cy-3-glc, cyanidin 3-glucoside; pt-3-glc, petunidin 3-glucoside; pn-3-glc, peonidin 3-glucoside; mv-3-glc, malvidin 3-glucoside; cy-*p*-coum, cyanidin 3-*p*-coumaroylglucoside; pn-*p*-coum, peonidin 3-*p*-coumaroylglucoside; ta, total anthocyanins.

Table 3. Flavonols Content of Table Grape Varieties[a]

flavonol[b]	Red Globe	Flame	Crimson	Napoleon	Superior	Dominga	Moscatel Italica
q-3-gluc	24.0 (2.1)	34.2 (2.6)	5.0 (1.1)	22.7 (1.7)	20.9 (1.7)	5.66 (0.6)	18.4 (3.5)
q-3-glc/rut	37.3 (7.5)	19.6 (3.5)	7.8 (2.8)	9.7 (4.0)	26.0 (1.2)	27.0 (2.0)	20.4 (1.7)
k-3-gal	tr[c]	tr	tr	tr	3.4 (0.4)	tr	0.5 (0.1)
k-3-glc	tr	tr	tr	tr	12.3 (1.4)	tr	4.7 (1.2)
i-3-glc	tr	tr	tr	tr	1.4 (0.1)	tr	3.7 (0.5)
tf	61.3 (9.6)	53.8 (6.0)	12.8 (3.5)	32.4 (5.5)	64.0 (4.6)	32.7 (2.7)	47.7 (6.1)

[a] Values are expressed as mg/kg of fresh weight of grape berry (skin + flesh). Standard deviation is reported in parentheses ($n = 3$). [b] Abbreviations: q-gluc, quercetin 3-glucuronide; q-3-glc/rut, quercetin 3-glucoside + quercetin 3-rutinoside; k-3-gal, kaempferol 3-galactoside; k-3-glc, kaempferol 3-glucoside; i-3-glc, isorhamnetin 3-glucoside; tf, total flavonols. [c] Traces.

Table 4. Phenolics Distribution Regarding Total Phenolic Compounds in Table Grape Varieties

	Red Globe	Flame	Crimson	Napoleon	Superior	Dominga	Moscatel Italica
% anthocyanins	51	42	52	56	0	0	0
% flavonols	27	15	10	24	47	28	33
% OH–cinnamates[a]	4	13	7	7	7	22	11
% flavan-3-ols	18	30	31	13	46	50	56

[a] (OH–Cinnamates), hydroxycinnamic acid derivatives.

tion ranged from 47% for Superior Seedless to 10% for Crimson Seedless (**Table 4**). Previous studies on phenolic content in table and wine grape varieties have been carried out but followed a different approach (*27*, *28*). These studies did not quantify individual phenolics, but quantified phenolic groups, i.e., anthocyanins, flavonols, hydroxycinnamic acid derivatives, and flavan-3-ols (**Figure 1**). According to previous studies the same flavonol contribution to total phenolics was found in Flame Seedless (15%) in agreement with our results. However the flavonol contribution previously reported in Red Globe was 14% (*27*), in contrast with the contribution described in the present study (27%) (**Table 4**). Again, and as a tentative hypothesis to justify the above discrepancy, agronomic factors and extraction protocols could be involved (*21*, *25*).

It should be stressed that the content of flavonols is important because of their antioxidant activity. In fact, quercetin is one of the most effective antioxidant flavonoids (*20*, *29*) with anticancer activity (*30*, *31*).

Hydroxycinnamic Acid Derivatives. Caffeoyltartaric and *p*-coumaroyltartaric acid were found in all the grape varieties (**Figure 2C**). The identification of the third hydroxycinnamic acid derivative was not possible because of its poor ionization in our LC−MS assay conditions. Caffeoyltartaric acid represented about 60% of total hydroxycinnamic acid derivatives in all the varieties except in Superior Seedless in which the contribution was about 40%.

The total amount of hydroxycinnamic acid derivatives ranged from 48 mg/kg of fw for Flame Seedless to 8.4 mg/kg of fw for Red Globe (**Table 5**). Previous data concerning the content of hydroxycinnamic acid derivatives have been reported for Red Globe and Flame Seedless varieties (*27*, *28*). However, total hydroxycinnamic acid derivatives represented about 5% and 12% of total phenols in Red Globe and Flame, respectively, in contrast to our results (13% for Red Globe and 4% for Flame Seedless). No significant differences were found between mg/kg fw in white and red table grapes, and their contribution to total phenols.

Flavan-3-ols. Catechin, gallocatechin, epigallocatechin, procyanidin B1, procyanidin B2, procyanidin B4, and procyanidin C1 were identified by LC−MS (**Table 1**). However, HPLC conditions were not optimal to quantitatively analyze flavan-3-ols because separation of catechin and its corresponding polymers requires specific HPLC conditions (*32*, *33*). Therefore, catechin and procyanidins were quantified as a single flavan-3-ol compound (**Table 5**).

The total amount of flavan-3-ols ranged from 109 mg/kg of fw for Flame Seedless to 18 mg/kg of fw for Napoleon (**Table 5**) which was not in agreement with either Meyer et al. (*27*), who did not found flavan-3-ols in most of the varieties studied, or with Yi et al. (*28*), who reported the lack of flavan-3-ols in Red Globe variety as well as a content of 7-fold less amount of flavan-3-ols in Flame Seedless variety. We have previously emphasized that this variability could be tentatively justified by extraction protocols. These authors used water to redissolve the already extracted compounds. Flavan-3-ols, except catechin and epicatechin, show low solubility in aqueous media. Therefore, this could explain the absence of procyanidins and flavan-3-ols in these previous studies (*27*, *28*).

The contribution of flavan-3-ols to total phenolics was higher in white grapes than that in red ones (**Table 4**). In addition, the high variability in flavan-3-ols content could be important, as these phenolics have been described as the most important antioxidants in grapes (*34*, *35*) together with their possible role

Table 5. Hydroxycinnamic Acid Derivatives, Flavan-3-ols, and Total Phenolics Content of Table Grape Varieties[a]

	Red Globe	Flame	Crimson	Napoleon	Superior	Dominga	Moscatel Italica
cafta	5.2 (0.7)	28.6 (3.4)	5.7 (0.8)	5.7 (0.2)	3.5 (0.5)	15.9 (2.2)	10.4 (1.7)
derv-*a*	1.1 (0.2)	4.4 (0.2)	1.1 (0.2)	1.0 (0.2)	1.8 (0.4)	2.5 (0.3)	2.1 (0.4)
couta	2.1 (0.3)	14.6 (1.3)	2.7 (0.2)	2.8 (0.2)	3.7 (0.5)	6.6 (0.3)	3.8 (0.4)
TH	8.4 (0.2)	47.6 (4.8)	9.5 (0.5)	9.5 (0.5)	9.0 (0.9)	25.0 (0.9)	16.3 (2.0)
Tfla	40.4 (4.1)	109.1 (0.4)	41.1 (10.8)	18.3 (3.0)	62.7 (3.5)	57.2 (6.0)	81.1 (10.7)
Tphen	225.4 (9.4)	361.2 (8.0)	131.9 (6.3)	135.9 (6.3)	135.7 (5.8)	114.9 (6.6)	145.1 (9.5)

[a] Values are expressed as mg/kg of fresh weight of grape berry (skin + flesh). Standard deviation is reported in parentheses ($n = 3$). Abbreviations: Cafta, caffeoyltartaric acid; derv-*a*, hydroxycinnamic acid derivative; couta, *p*-coumaroyltartaric acid; TH, total hydroxycinnamic acid derivates; Tfla, total flavan-3-ols; Tphen, total phenolics.

in the reduction of mortality from heart disease (*36*). Recent studies have proved that a supplement of catechin (7.5 mg in daily catechin intake) was associated with a tendency for a 20% reduction in ischaemic heart disease mortality risk (*37*).

Grape seeds are very rich in flavan-3-ols (*38*). However, current dietary habits do not involve the ingestion of seeds, so that the possible health-promoting role of grape seeds should remain under discussion. In this context, seedless and seed-containing grapes could be equally healthy. However, seed-containing grapes could be useful to make juice because during the crushing the juice is enriched with flavan-3-ols coming from the seeds (*20*, *27*). Another interesting approach for using seed extracts is the development of functional foods and nutraceuticals (*34*).

Stilbenoids. *trans*-Piceid (*trans*-resveratrol 3-*O*-β-glucoside) was detected in Superior Seedless (7.3 mg/kg of fw) and Napoleon (1.3 mg/kg of fw) varieties. No more stilbenoids were detected. It should be noted that stilbenoids are phytoalexins which are induced under stress conditions (pathogen attack, UV−C light, etc.). Moreover, stilbenoid induction requires elapse of a certain amount of time to be detected in a significant amount as previously reported (*39*). Therefore, it is logical to find the lack of stilbenoids in grapes recently harvested and immediately analyzed.

Total Phenolics. Total phenolics content was determined as the sum of anthocyanins, flavonols, hydroxycinnamic acid derivatives, and flavan-3-ols (**Table 5**). The red varieties Flame Seedless and Red Globe presented the highest total phenolic contents (361 and 225 mg/kg fw, respectively) due to their high anthocyanin content (151 and 115 mg/kg fw, respectively) which is in agreement with previous studies (*21*, *28*, *40*). However, Crimson Seedless and Napoleon, which contained less anthocyanins (70 and 76 mg/kg fw, respectively), presented approximately the same amount of total phenolic compounds as white varieties (~130 mg/kg of fw). Therefore, white varieties which are richer in flavan-3-ols than red varieties, compensate for the lack of anthocyanins as supported by other authors (*27*). In fact, the correlation between antioxidant activity and anthocyanin content in red grape juice has been reported, whereas antioxidant activity was related to flavan-3-ols content in white grape juice (*41*).

Previous studies reported much higher total phenolics values than those found in the present study (*27*, *28*, *40*). However, these studies used the Folin−Ciocalteu method to quantify total phenolics. This method is quiet unspecific because compounds such as sugars, ascorbic acid, aromatic amines, etc. could interfere in the measurement, leading to an overestimation of total phenolic content (*42*).

It should be also noted that other nonflavonoid phenolics have been described in grapes, such as hydroxybenzoic and gallic acid derivatives (*21*), which were not detected in the present study.

From the nutritional point of view, a serving (200 g) of (unpeeled) table grape could supply an amount of phenolic compounds which could range from 72 mg (Flame variety) to 23 mg (Dominga variety) of total phenolic compounds. For instance, the red variety Flame Seedless (the richest variety in total phenolics) provides 30 mg of anthocyanins, 11 mg of flavonols, 9.5 mg of hydroxycinnamic acid derivatives, and 22 mg of flavan-3-ols per serving of grapes.

Bearing all this in mind, a serving of table grape (200 g) could provide enough phenolics content to potentially promote health benefits according to in vivo studies which show the inverse relation between catechin and quercetin rich diets and the risk of ischaemic heart disease and the regulation of cancer promoter genes, respectively (*37*, *43*).

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