JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Phenolic Composition of the Brazilian Seedless Table Grape Varieties BRS Clara and BRS Morena

Ellen Silva Lago-Vanzela,[†] Roberto Da-Silva,[†] Eleni Gomes,[†] Esteban García-Romero,[‡] and Isidro Hermosín-Gutiérrez[*,§]

[†]Departamento de Química e Ciências Ambientais, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, Rua Cristovão Colombo 2265, Jardim Nazareth, CEP 15054-000, São José do Rio Preto, SP, Brazil

[‡]Instituto de la Vid y el Vino de Castilla-La Mancha, Carretera de Albacete s/n, 13700 Tomelloso, Spain

[§]Instituto Regional de Investigación Científica Aplicada, Escuela Universitaria de Ingeniería Técnica Agrícola, Universidad de Castilla-La Mancha, Ronda de Calatrava 7, 13071 Ciudad Real, Spain

ABSTRACT: The detailed phenolic composition (anthocyanins, flavonols, hydroxycinnamic acid derivatives, stilbenes, and flavan-3-ols) in the skin and flesh of the new BRS Clara and BRS Morena seedless table grapes has been studied using HPLC-DAD-ESI-MS/MS. The two grapes, especially BRS Morena, contained high amounts of phenolic compounds, mainly located in their skins and qualitatively not different from those found in *Vitis vinifera* grapes. In addition, BRS Morena (a teinturier variety) showed qualitatively different phenolic compositions in its skin and flesh, mainly affecting the anthocyanin and flavonol profiles. Consistent with high phenolic contents, high antioxidant capacity values were registered for both grape varieties, especially for BRS Morena. Proanthocyanidins and hydroxycinnamoyl-tartaric acids were the major phenolic compounds found in BRS Clara and were also important in BRS Morena, although anthocyanins were the main phenolic compounds in the latter case. These results suggest that the entire grapes, including the skin, may potentially possess properties that are beneficial to human health. In this context, the BRS Morena grape can be considered as a high resveratrol producer.

KEYWORDS: anthocyanins, flavonols, proanthocyanidins, hydroxycinnamic acid, stilbenes, seedless table grapes, antioxidant capacity, phenolic compounds

INTRODUCTION

The inclusion of table grapes and derived products in the diet of Western countries has increased significantly in recent decades,[1] largely due to the mounting evidence that the phenolic compounds of these grapes present functional characteristics and properties that are beneficial to human health.[2−5] In this niche market, seedless grapes have gained importance because, in addition to nutritional value, they are easy to consume.

Advancements in genetic knowledge, along with an increase in market demand for new quality products, have contributed to the introduction of several seedless grapes into worldwide markets, such as Superior Seedless or Festival, Crimson Seedless, Thompson Seedless, Catalunha, Vênus, Marroo Seedless, and Perlette. The phenolic composition of certain grapes has been studied, and results have shown that they are important sources of phenolic compounds.[1,6−8]

Due to the growth in the demand for seedless grapes, the Brazilian Agricultural Research Corporation (EMBRAPA) developed two new varieties of seedless grapes that are adapted to the tropical regions found in the country. BRS Clara is a complex hybrid variety (76.32% *Vitis vinifera*, 14.04% *Vitis rupestris*, 3.95% *Vitis aestivalis*, 3.51% *Vitis berlandieri*, 1.75% *Vitis labrusca*, and 0.43% *Vitis cinerea*) that originated from a crossing between CNPUV 154-147 and Centennial Seedless (pedigree: Seibel 6468 × Seibel 6905 = Seyve Villard 12327; Moscatel Rosado × Beauty Seedless = CG 87746; Seyve Villard 12327 × CG 87746 = CNPUV 154-147; Gold × Q.25-6 (F2 Emperor × Pirovano 75) = Centennial Seedless; CNPUV 154-147 × Centennial Seedless = BRS Clara), which is known for its soft and pleasant taste, its yellow-green color, the crunchy texture of its pulp, and its high glucometric potential.[9] In contrast, BRS Morena comes from the crossing of two *V. vinifera* varieties (Marroo Seedless × Centennial Seedless) and is a grape with a red-colored flesh, so it is also known as a teinturier variety, a type of variety that has a high fertility, moderate vigor, and a preferred taste (it was praised by consumers during the validation tests). It also has a pulp with a firm and crunchy texture.[10]

As far as we know, no further studies have been developed to identify phytochemical constituents of these seedless grapes, even though these constituents contribute to the aforementioned biological activities. Therefore, the goal of this study was to thoroughly examine the phenolic composition of the edible parts (flesh and skin) of BRS Clara and BRS Morena grapes using HPLC-DAD-ESI-MS/MS. The study comprises the phenolic classes of anthocyanins, flavonols, hydroxycinnamic acid derivatives, stilbenes, and flavan-3-ols (monomers, dimers, and the polymeric proanthocyanidins also called tannins). This study also evaluates total phenolic content and antioxidant activity in the fruit.

Received: May 4, 2011
Revised: June 27, 2011
Accepted: June 30, 2011
Published: June 30, 2011

dx.doi.org/10.1021/jf201753k | *J. Agric. Food Chem.* 2011, 59, 8314–8323

MATERIALS AND METHODS

Chemicals. All solvents were of HPLC quality, and all chemicals were of analytical grade (>99%). The water used was of Milli-Q quality. The following commercial standards from Phytolab (Vestenbergsgreuth, Germany) were used: malvidin 3-glucoside, caffeic and *p*-coumaric acids, *trans*-caftaric acid, *trans*-piceid, (−)-epigallocatechin, and (−)-gallocatechin. The following commercial standards from Extrasynthese (Genay, France) were used: cyanidin 3-glucoside, procyanidins B1 and B2, kaempferol, quercetin, isorhamnetin, myricetin, syringetin, and the 3-glucosides of kaempferol, quercetin, isorhamnetin, and syringetin. The following commercial standards from Sigma (Tres Cantos, Madrid, Spain) were used: gallic acid, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, (−)-epicatechin 3-gallate, and (−)-gallocatechin 3-gallate. Other noncommercial flavonol standards (myricetin 3-glucoside, quercetin 3-glucuronide) were either kindly supplied by Dr. Ulrich Engelhardt (Institute of Food Chemistry, Technical University of Braunschweig, Germany) or isolated from Petit Verdot grape skins (laricitrin 3-glucoside) used in a previous study.[11] The *trans* isomers of resveratrol and piceid (resveratrol 3-glucoside) were transformed into their respective *cis* isomers using UV irradiation (366 nm light for 5 min in quartz vials) with 25% MeOH solutions of the *trans* isomers.

All of these standards were used for identification. Anthocyanins were quantified as equivalents of malvidin 3-glucoside (mg/kg of fresh grape weight). Flavonols (as μmol/kg fresh grape weight) were quantified using the calibration curve of each standard when available; in other cases, the closest flavonol was used for quantification, making molecular mass correction (3-glucosides for the respective 3-galactosides or 3-glucuronides). Hydroxycinnamic acid derivatives were quantified using their respective common hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acids) as standards and making molecular mass correction.

Grapes. During the 2010 harvest season, healthy BRS Clara and BRS Morena grapes (at optimum ripeness for harvesting) were donated by the Experimental Station of Tropical Viticulture of EMBRAPA (Brazilian Agricultural Research Corporation), in the city of Jales (northwest of the city of São Paulo, Brazil), which lies at 20° 15′ 08″ S and 50° 33′ 29″ W, 500 m above sea level (referred to datum WGS84, World Geodetic System 1984). Once in the laboratory, 5 kg from each grape batch was separated for analysis. The BRS Clara grapes showed the following characteristics (average values for triplicates): sugar content, 18.9 °Brix; total acidity, 5.1 g as tartaric acid/kg of fresh grape weight; pH, 3.85; berry size, 15 × 20 mm. The average characteristics for BRS Morena grapes were sugar content, 16.3 °Brix; total acidity, 6.1 g as tartaric acid/kg of fresh grape weight; pH, 3.69; and berry size, 16 × 20 mm.

Sample Preparation. Two hundred grams of each grape, BRS Morena and BRS Clara, was carefully peeled by hand, and the resulting skins (yield, 25.06 and 24.05% of fresh fruit weight, respectively) were immediately frozen at −80 °C for 12 h and then freeze-dried for 24 h and weighed (results were 8.94 and 10.77 g, respectively). The dried skins were homogenized in a porcelain mortar with the aid of a pestle and further divided into four subsamples, three of which were used for chemical analysis. The subsamples (approximately 2 g per subsample) were immersed in 50 mL of a solvent mixture of methanol, water, and formic acid (50:48.5:1.5 v/v) and then maintained under an ultrasonic bar for 10 min. Samples were then centrifuged at 2500*g* and 5 °C for 10 min. A second extraction of the resulting pellets was completed using the same volume of the solvent mixture (50 mL), and the combined supernatants for each sample were maintained at −18 °C until the beginning of the analysis. Aliquots of skin extracts were diluted with 0.1 N HCl (1:10, v/v), filtered (0.20 μm, polyester membrane, Chromafil PET 20/25, Macherey-Nagel, Düren, Germany), and directly injected onto the HPLC to determine the anthocyanins.

Anthocyanins usually cause great interference in the HPLC-DAD analysis of grape flavonols. The use of ECX SPE cartridges (40 μm, 500 mg, 6 mL; Scharlab, Sentmenat, Barcelona, Spain), which combine a mixture of reverse-phase adsorbent and cationic-exchanger material, allowed the isolation of grape flavonols.[12] To carry out this step, 3 mL of BRS Morena skin extracts and 3 mL of BRS Morena flesh extracts were concentrated in a rotary evaporator (37 °C) to eliminate any excess methanol. These extracts were then diluted with 3 mL of 0.1 N HCl, and the prepared samples were passed through the SPE cartridges, which had been previously conditioned with 5 mL of methanol and 5 mL of water. After the cartridges had been washed (5 mL of 0.1 N HCl acid and 5 mL of water), the anthocyanin-free flavonol fractions were eluted with 3 × 5 mL of methanol. The flavonol extracts were dried in a rotary evaporator (37 °C) and redissolved in 3 mL of water with 20% methanol and directly injected into the HPLC equipment. Because BRS Clara is a white grape, this last step was not necessary, and its skin extracts were directly injected for HPLC analysis of nonanthocyanin phenolics.

The separated flesh of both fruits (BRS Morena yield, 74.94% of fresh fruit weight; and BRS Clara yield, 75.95% of fresh fruit weight) was immediately homogenized with 100 mL of a solvent mixture of methanol, water, and formic acid (50:48.5:1.5 v/v) to avoid oxidation. This homogenization was followed by 30 min of agitation in the absence of light at room temperature. The flesh extract was then centrifuged at 10000*g* and 5 °C for 20 min. The supernatants were dried separately in a rotary evaporator (37 °C) to eliminate excess methanol, and the volume was brought up to 100 mL with water.

To remove the sugars and other polar, nonphenolic compounds present in the flesh extracts, 3 mL of extract was diluted with 3 mL of 0.1 N HCl, and the prepared sample was then passed through C18 SPE cartridges (Sep-Pak Vac, 3 cm^3/500 mg, 55−105 μm; Waters Corp., Milford, MA) that had been previously conditioned with 5 mL of methanol and 5 mL of water. After the cartridges had been washed with 5 mL of 0.1 N HCl and 5 mL of water, the sample was eluted with 3 × 5 mL of methanol. The eluate was dried in a rotary evaporator (37 °C), redissolved in 3 mL of 20% methanol in water, and directly injected into the HPLC equipment.

Flavan-3-ols were isolated from grape skin extracts by SPE on C18 cartridges (Sep-pak Plus C18, Waters Corp.; cartridges filed with 820 mg of adsorbent): a mixture of 2 mL of grape skin extract with 0.5 mL of a solution of 20 mg/L of (−)-gallocatechin 3-gallate (internal standard) and 6 mL of water was passed through the C18 cartridge previously conditioned with methanol (10 mL) and water (10 mL); after drying of the cartridge under reduced pressure, methanol (15 mL) and ethyl acetate (5 mL) were sequentially added for recovery of adsorbed phenolics; after solvent evaporation in a rotary evaporator (40 °C), the residue was dissolved in methanol (4 mL) and stored at −18 °C until used.

Total Phenolic Content and Antioxidant Capacity. Skin and flesh extracts were used to determine total phenolic content and antioxidant capacity. Total phenolic content was measured as milligrams of gallic acid equivalents following the Folin−Ciocalteu method.[13] Antioxidant capacity was determined as millimoles of Trolox equivalents according to the DPPH method.[14] The results were initially expressed in kilograms of fresh grape weight, although other values were also calculated in the case of antioxidant capacity (μmol/g of fresh weight skin; μmol or μM/g dry weight skin) to better compare the data to those of the literature.

HPLC-DAD-ESI-MS/MS Identification of Grape Phenolic Compounds. HPLC identification of grape skin and flesh phenolic compounds was performed using an Agilent 1200 series system equipped with DAD (Agilent, Germany) and coupled to an AB Sciex 3200 Q TRAP (Applied Biosystems) electrospray ionization mass spectrometry system (ESI-MS/MS). The chromatographic system was managed by an Agilent Chem Station (version B.01.03) data-processing station. The mass spectral data were processed with Analyst MDS software (Applied Biosystems, version 1.5). The samples were injected into a Zorbax Eclipse XDB-C18 reversed-phase column (4.6 × 250 mm; 5 μm particle; Agilent)

after their filtration (0.20 μm, polyester membrane, Chromafil PET 20/25, Machery-Nagel, Düren, Germany).

In the case of anthocyanins, the aforementioned prepared grape skin and flesh extracts were injected (50 μL) into the chromatographic column thermostated at 40 °C. The chromatographic conditions were adapted from the OIV method for the analysis of anthocyanins in red wines.[12] The solvents were water/acetonitrile/formic acid (87:3:10, v/v/v, solvent A; 40:50:10, v/v/v, solvent B), and the flow rate was 0.63 mL/min. The linear gradient for solvent B was as follows: 0 min, 6%; 15 min, 30%; 30 min, 50%; 35 min, 60%; 38 min, 60%; 46 min, 6%. For identification, the ESI-MS/MS in positive ionization mode was operated using a combination of +EMS (enhanced mass spectrum; MS conditions) and +EPI (enhanced product ion; MS/MS conditions) experiments, setting the following parameters: scan, 100−1500 Da (250 Da/s); declustering potential, 65 V; entrance potential, 10 V; collision energy, 10 (arbitrary units); curtain gas, 15 psi; collision gas, medium; ion spray voltage, 4000 V; temperature, 450 °C; ion source gas 1, 70 (arbitrary units); ion source gas 2, 50 (arbitrary units); and Q3 barrier, 12 V.

HPLC identification of grape skin and flesh flavonols, hydroxycinnamic acid derivatives, and stilbenes was performed on the same chromatographic system as described for anthocyanins. However, the chromatographic conditions used were conditions that had been previously reported.[11] The solvents were solvent A, acetonitrile/water/formic acid, 3:88.5:8.5, v/v/v; solvent B, acetonitrile/water/formic acid, 50:41.5:8.5, v/v/v; and solvent C, methanol/water/formic acid, 90:1.5:8.5, v/v/v. The flow rate was 0.63 mL/min, the column was thermostated at 40 °C, and the injection volume was 50 μL. The linear solvent gradient was as follows: 0 min, 96% A and 4% B; 7 min, 96% A and 4% B; 38 min, 70% A, 17% B, and 13% C; 52 min, 50% A, 30% B, and 20% C; 52.5 min, 30% A, 40% B, and 30% C; 57 min, 50% B and 50% C; 58 min, 50% B and 50% C; 65 min, 96% A and 4% B. For identification, the ESI-MS/MS was used in negative ionization mode using a combination of −EMS (enhanced mass spectrum; MS conditions) and −EPI (enhanced product ion; MS/MS conditions) experiments, with the following parameters: scan, 100−650 Da (1000 Da/s); declustering potential, −45 V; entrance potential, −12 V; collision energy, −20 (arbitrary units); curtain gas, 15 psi; collision gas, high; ion spray voltage, −4000 V; temperature, 425 °C; ion source gas 1, 70 (arbitrary units); ion source gas 2, 50 (arbitrary units); and Q3 barrier, 12 V.

HPLC-DAD Quantification of Grape Phenolic Compounds. Anthocyanins and flavonols were quantified using an Agilent 1100 series system, equipped with DAD (G1315B) and coupled to an Agilent ChemStation (version B.01.03) data-processing station. The same column and chromatographic parameters (column model and temperature, solvents and gradient, injection volume) used for their identification were applied. For quantification, DAD chromatograms were extracted at 520 nm (anthocyanins), 360 nm (flavonols), and 320 nm (hydroxycinnamic acid derivatives and stilbenes).

Identification and Quantification of Grape Skin Flavan-3-ols Using Multiple Reaction Monitoring HPLC-ESI-MS/MS. The same chromatographic system employed for the identification of the other phenolic compounds was also used for both the identification and quantification of flavan-3-ols. The samples were injected (100 μL) into a Zorbax Eclipse XDB-C18 reversed-phase column (4.6 × 250 mm; 5 μm particle; Agilent) after filtration (0.20 μm, polyester membrane, Chromafil PET 20/25, Machery-Nagel), thermostated at 16 °C. The solvents used were water/methanol/formic acid (89:10:1, v/v/v, solvent A) and methanol (solvent B), and the flow rate was 0.5 mL/min. The linear gradient for solvent B was as follows: 0 min, 1%; 2 min, 1%; 60 min, 23%; 75 min, 70%; 80 min, 95%; 90 min, 95%; 95 min, 1%; 100, 1%. Two MS scan types were used: enhanced MS (EMS) for compound identification; and multiple reaction monitoring (MRM) for quantification. MS conditions for both scan types were as follows: ion spray voltage, −4000 V; ion source temperature, 450 °C; collision gas, high; curtain gas, 15 psi; ion source gas 1, 70 (arbitrary units); ion source gas 2, 50 (arbitrary units); declustering potential, −35 V; entrance potential, −10 V; collision energy, −30 (arbitrary units); collision cell exit potential, −3 (arbitrary units).

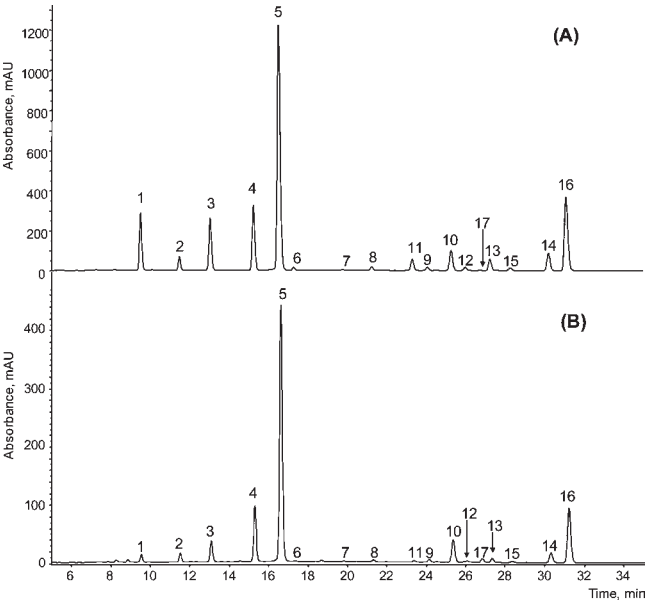

Figure 1. Anthocyanin chromatographic profile (DAD at 520 nm) of BRS Morena grape skin (A) and flesh (B). Peak numbering is as in Table 1.

In the case of the analysis of flavan-3-ol monomers and dimer procyanidins B1 and B2, 0.50 mL of the SPE-C18 grape skin extract was diluted with 2.5 mL of water in a chromatographic vial that was sealed, and the extract was then injected. The selected mass transitions (m/z pairs) for MRM scan and quantification (in some cases two most intense transitions were available, thus gaining in sensitivity) were as follows: (+)-catechin and (−)-epicatechin (289−245); procyanidins B1 and B2 (577−425 and 577−407); (−)-epigallocatechin and (−)-gallocatechin (305−221 and 305−219); (−)-epicatechin 3-gallate (441−289); and (−)-gallocatechin 3-gallate (457−331 and 457−305). Calibration curves for each flavan-3-ol were obtained to calculate the respective molar response factors against (−)-gallocatechin 3-gallate used as internal standard. Analyses were performed in duplicate.

The structural information of proanthocyanidins was obtained following the method of acid-catalyzed depolymerization induced by pyrogallol, a recently proposed alternative nucleophile trapping agent that offers similar results when compared to the classic phloroglucinol method, but which also functions under milder experimental conditions.[15] In this study, 0.25 mL of pyrogallol reagent solution (100 g/L of pyrogallol and 20 g/L ascorbic acid in methanolic 0.4 N HCl) was added to 0.25 mL of SPE-C18 grape skin extract, and the mixture was then maintained at 35 °C for 20 min. After the reaction was interrupted with the addition of 2 mL of 40 mM sodium acetate, the reaction mixture was analyzed as described above for monomeric and dimer flavan-3-ols. In addition to the MRM transitions for monomeric flavan-3-ols (terminal units of proanthocyanidins), the following m/z pairs (in some cases the two most intense transitions were available) were also selected, corresponding to the pyrogallol adducts of the extension units of proanthocyanidins: (+)-catechin and (−)-epicatechin adducts (413−287); (−)-epigallocatechin adduct (429−303 and 429−261); and (−)-epicatechin 3-gallate adduct (565−413). The calibration curve of the adduct formed between pyrogallol and (−)-epicatechin was obtained through depolymerization experiments of both procyanidins (B1 and

Table 1. Data for the Identification of the Anthocyanins in the BRS Morena Grape Using HPLC-DAD-ESI-MS/MS (Positive Ionization Mode) and Molar Proportions (Mean ± Standard Deviation, $n = 3$) of Each Anthocyanin in the Two Parts of the Grape[a]

				molar %[b]	
peak	anthocyanin[c]	UV−vis (nm)	molecular and product ions (m/z)	skin, $n = 3$	flesh, $n = 3$
1	dp-3-glc	277, 298 (sh), 346, 440 (sh), 524	465; 303	5.79 ± 0.23 a	1.37 ± 0.01 b
2	cy-3-glc	280, 292 (sh), 325 (sh), 380 (sh), 440 (sh), 517	449; 287	0.66 ± 0.01 a	1.49 ± 0.05 b
3	pt-3-glc	276, 298 (sh), 348, 440 (sh), 527	479; 317	6.68 ± 0.04 a	4.10 ± 0.03 b
4	pn-3-glc	280, 292 (sh), 325 (sh), 380 (sh), 440 (sh), 518	463; 301	4.66 ± 0.00 a	10.75 ± 0.03 b
5	mv-3-glc	276, 298 (sh), 348, 440 (sh), 528	493; 331	43.84 ± 0.29 a	54.38 ± 0.14 b
6	dp-3-acglc	277, 298 (sh), 349, 440 (sh), 526	507; 303	0.51 ± 0.01 a	0.19 ± 0.02 b
7	cy-3-acglc	440 (sh), 519[d]	491; 287	0.10 ± 0.02	0.11 ± 0.00
8	pt-3-acglc	276, 298 (sh), 349, 440 (sh), 529	521; 317	0.79 ± 0.01 a	0.46 ± 0.01 b
9	pn-3-acglc	280, 330, 380 (sh), 440 (sh), 519	505; 301	0.56 ± 0.03 a	0.71 ± 0.01 b
10	mv-3-acglc	278, 298 (sh), 349, 440 (sh), 529	535; 331	7.10 ± 0.23	6.55 ± 0.01
11	dp-3-cmglc	282, 298 (sh), 316 (sh), 440 (sh), 530	611; 303	1.87 ± 0.03 a	0.35 ± 0.00 b
12	cy-3-cmglc	283, 313, 440 (sh), 522	595; 287	0.50 ± 0.06 a	0.60 ± 0.04 b
13	pt-3-cmglc	282, 298 (sh), 316 (sh), 440 (sh), 531	625; 317	2.47 ± 0.03 a	0.95 ± 0.02 b
14	mv-3-*cis*-cmglc	280, 296 (sh), 306 (sh), 440 (sh), 535	639; 331	0.59 ± 0.01 a	0.36 ± 0.03 b
15	pn-3-cmglc	283, 313, 440 (sh), 521	609; 301	2.72 ± 0.06 a	2.56 ± 0.00 b
16	mv-3-*trans*-cmglc	284, 298 (sh), 316 (sh), 440 (sh), 532	639; 331	20.99 ± 0.17 a	14.34 ± 0.06 b
17	mv-3-cfglc	281, 298 (sh), 333, 440 (sh), 529	655; 331	0.17 ± 0.03 a	0.96 ± 0.05 b
total concentration (mg/kg)[e]				526.36 ± 67.14 a	16.18 ± 4.29 b

[a] Peak numbers are as in Figure 1. [b] "a" and "b" indicate significant differences (Student t test; $\alpha = 0.05$) between skin and flesh composition. [c] dp, delphinidin; cy, cyanidin; pt, petunidin; pn, peonidin; mv, malvidin; glc, glucoside; acglc, 6″-(acetyl)glucoside; cfglc, 6″-(caffeoyl)glucoside; cmglc, 6″-(*p*-coumaroyl)glucoside (*trans* configuration if not indicated). [d] Only visible maxima were able to be measured. [e] As malvidin 3-glucoside equivalents (mv-3-glc).

B2), which allowed the calculation of their molar response factor against (−)-gallocatechin 3-gallate, which was used as internal standard. The factor response of the other adducts was assumed to be proportional to those obtained by their respective monomer precursors. Analyses were performed in duplicate.

RESULTS AND DISCUSSION

Anthocyanins. Anthocyanins occurred only in the BRS Morena grapes; however, because it is a teinturier variety, they were present in both the skin and flesh. The typical chromatographic profiles of the anthocyanins of BRS Morena grape skin and flesh are shown in Figure 1, panels A and B, respectively. Their spectral characteristics (molecular and product ions under ESI-MS/MS, online DAD UV−vis λ_{max} values) are presented in Table 1. With the help of extracted ion chromatograms (EIC) at the m/z ratios corresponding to the different anthocyanidins (aglycones), we were able to detect a total of 17 anthocyanins in both edible parts (flesh and skin) of the grape. The identification of the anthocyanins was completed on the basis of coincident spectral data, with authentic standards and with data reported previously in the literature.[16]

In summary, only 3-glucosides of the same five anthocyanidins usually found in *V. vinifera* grape varieties (delphinidin, cyanidin, petunidin, peonidin, and malvidin) were detected in the BRS Morena grape. The completed series of nonacylated anthocyanins (peaks 1−5), acetylated anthocyanins (peaks 6−10), and *p*-coumaroylated anthocyanins (peaks 11−15, corresponding to the major *trans* isomers; and also the *cis* isomer of the malvidin derivative, peak 16), along with the caffeoylated derivative of malvidin (peak 17), were identified. These results show that the anthocyanin profile of this grape resembles the anthocyanin profiles shown by the most widespread *V. vinifera* red grape varieties.[17−21] These results are in agreement with those found for the genealogy of BRS Morena,[10,22] which is from a cross between *V. vinifera* grape varieties (Marroo Seedless × Centennial Seedless). It is commonly accepted that the anthocyanin profile of a given variety is closely linked to its genetic inheritance, although environmental factors may have some influence on this profile.[20,23] The anthocyanin profile (molar percentage in which each anthocyanin appears; Table 1) was also dominated by malvidin-type derivates in the case of both the skin and flesh of the BRS Morena grape (63 and 77%, respectively), but it was also found in the form of nonacylated derivates (61 and 72%, respectively), *p*-coumaroyl derivatives (29 and 19%, respectively), and acetyl derivatives (9 and 8%, respectively); caffeoyl derivatives were minor anthocyanins (<1%).

This type of anthocyanin profile, especially that found in the skin of the BRS Morena grape, was also similar to the profiles of anthocyanins shown by Marroo Seedless and Black Seedless table grapes, which, in other studies, were found to be approximately 61 and 49% of the malvidin-type anthocyanins, respectively, and higher proportions of *p*-coumaroyl anthocyanins (around 14% in both cases) within the acylated anthocyanins.[6] The similarity to the Marroo Seedless grape profile was expected, considering that this grape was used in the hybridization process during the production of the BRS Morena grape. However, the profile that was found differs from that which has been reported for other table grapes, such as Red Globe, Crimson Seedless, and Napoleon, which all presented peonidin 3-glucoside as the

Table 2. Data for the Identification of the Flavonols in the BRS Morena and BRS Clara Grapes Using HPLC-ESI-MS/MS (Negative Ionization Mode) and Molar Proportions (Mean ± Standard Deviation) of Each Flavonol in Both Parts of the Grape (Skin and Flesh) [a]

			molar %[b]			
			Morena		Clara	
peak	flavonol[c]	pseudomolecular and product ions (m/z)	skin, $n = 3$	flesh, $n = 3$	skin, $n = 3$	flesh, $n = 3$
18	M-3-glcU	493; 317	3.29 ± 0.09	ND	ND	ND
19	M-3-gal	479; 317	1.05 ± 0.03	NQ	ND	ND
20	M-3-glc	479; 317	43.19 ± 0.70 b	8.41 ± 0.61 a	ND	ND
21	Q-3-gal	463; 301	1.27 ± 0.26	ND	6.32 ± 0.97	4.90 ± 0.35
22	Q-3-glcU	477; 301	13.95 ± 0.47 b	10.51 ± 0.62 a	47.59 ± 0.96 B	45.00 ± 0.10 A
23	Q-3-glc	609; 301	12.35 ± 1.21 a	34.58 ± 1.03 b	35.64 ± 0.41 A	40.23 ± 0.30 B
A	Q-3-rut	463; 301	0.25 ± 0.02	0.24 ± 0.01	4.14 ± 0.05 B	3.07 ± 0.02 A
24	L-3-glc	493; 331	11.42 ± 0.30 a	16.55 ± 0.16 b	ND	ND
25	K-3-gal	447; 285	0.29 ± 0.05	ND	1.03 ± 0.03	NQ
26	K-3-glcU	461; 285	ND	ND	0.76 ± 0.02	NQ
27	K-3-glc	447; 285	1.21 ± 0.10 a	1.86 ± 0.00 b	3.97 ± 0.08	4.36 ± 0.39
28	I-3-gal	477; 315	0.61 ± 0.26	ND	ND	ND
29	I-3-glc	477; 315	3.69 ± 0.27 a	7.01 ± 0.31 b	0.55 ± 0.03 A	2.44 ± 0.18 B
30	S-3-glc	507; 345	7.43 ± 0.32 a	20.84 ± 0.11 b	ND	ND
total concentration (μmol/kg)			114.02 ± 6.30 b	2.16 ± 0.11 a	139.73 ± 3.68 B	3.20 ± 0.05 A

[a] Peak numbers are as in Figure 2. [b] ND, nondetectable. NQ, nonquantifiable. "a" and "b" and "A" and "B" indicate significant differences (Student t test; $\alpha = 0.05$) between skin and flesh composition of Morena and Clara varieties, respectively. [c] M, myricetin; Q, quercetin; L, laricitrin; K, kaempferol; I, isorhamnetin; S, syringetin; glcU, glucuronide; gal, galactoside; glc, glucoside; rut, rutinoside (6″-rhamnosylglucoside).

predominant anthocyanin.[1] It also differs from the profiles of the Flame Seedless[1] and Ruby Seedless[6] varieties, which presented more balanced proportions of acylated anthocyanidin 3-glucosides, particularly in the case of peonidin- and cyanidin-type 3-glucosides.

When BRS Morena was compared to more widespread *V. vinifera* grape varieties used in the production of red wine, its anthocyanin profile resembled those of grape varieties of Spanish origin, such as Tempranillo, Garnacha, and Bobal.[17] In contrast, the typical French varieties (Cabernet Souvignon and Merlot) are characterized by the presence of a higher proportion of acetylated anthocyanins than *p*-coumaroylated anthocyanins. Other French varieties (Syrah or Petit Verdot) have similar and relatively high proportions in the case of both types of derivatives (acetylated derivatives and *p*-coumaroylated derivatives).

Significant differences among the anthocyanin profiles of the skin and flesh of the BRS Morena grape were observed and largely involved higher proportions of nonacylated anthocyanins (except for delphinidin- and petunidin-type 3-glucosides) in the flesh, as well as the acylated derivates malvidin 3-(6″-caffeoyl)-glucoside and peonidin 3-(6″-acetyl)glucoside. Anthocyanins have also been found to be unevenly distributed within the skin and flesh of other varieties of teinturier grapes. When compared to its own skin, the flesh of the Yan 73 grape hybrid variety (Muscat Hamburg × Alicante Bouschet) showed a much lower ratio of 3′,5′-substituted to 3′-substituted anthocyanins and a much higher ratio of methoxylation of the anthocyanin B-ring to nonmethoxylation. The values of acylated anthocyanin content in the skin were also higher than those found in the flesh.[24] The anthocyanin composition of Garnacha Tintorera (*V. vinifera*) grape showed a slight predominance of malvidin-type anthocyanins in the skin, followed by equally high proportions of peonidin-type derivates, whereas peonidin 3-glucoside was the major anthocyanin in the flesh.[16]

The anthocyanin content in grapes depends on the variety, and its maturity stage, as well as on seasonal conditions, production area, and cultural practices.[25] The anthocyanin content of the BRS Morena grape (approximately 542 mg/kg as malvidin 3-glucoside) was found in amounts that were similar to those found in other varieties that are commonly used for making red wines. For instance, the reported total anthocyanin content for Cencibel (Tempranillo) grape was 690 ± 30 mg/kg, but with values ranging from 210 to 1500 mg/kg.[26] In addition, the total anthocyanin content of red grapes also depends on the analyzed variety:[27] Cabernet Sauvignon (range, 686−883 mg/kg; average, 784 ± 58 mg/kg); Merlot (range, 843−1296 mg/kg; average, 1021 ± 139 mg/kg); Syrah (range, 826−1316 mg/kg; average, 1024 ± 142 mg/kg); Tempranillo (range, 651−1002 mg/kg; average, 782 ± 103 mg/kg); Garnacha (range, 348−482 mg/kg; average, 411 ± 41 mg/kg); and Cariñena (range of 485−759 mg/kg; average value of 639 ± 70 mg/kg). The anthocyanin content of BRS Morena was also within the range for certain table grapes (between 509 and 669 mg/kg), such as Michele Palieri, Monuka, Moscatel Hamburgo, Ribol, Pella, Bel Air, Black Seedless, Marroo Seedless, and Pasiga.[6] Although both edible parts of the BRS Morena grape (skin and flesh) were pigmented, the amount of anthocyanins in the flesh was remarkably lower (16.18 ± 4.29 mg/kg, as malvidin 3-glucoside) than the amount in the skin (526.36 ± 67.14 mg/kg).

Flavonols. Only flavonol 3-glycosides were found in BRS Morena and BRS Clara grapes. The identification of the occurring flavonols was largely completed using their MS/MS data

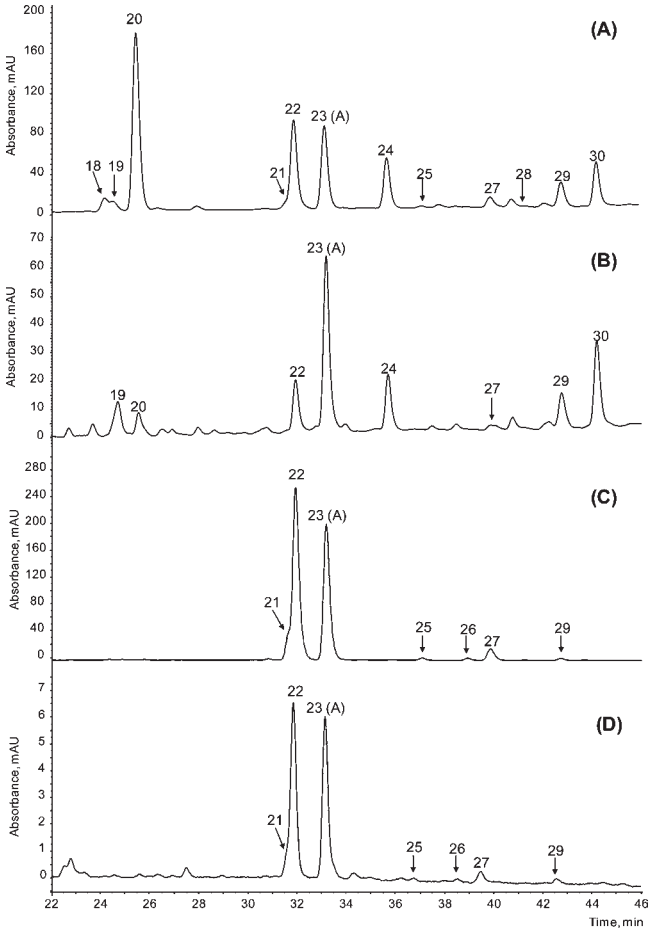

Figure 2. Flavonol chromatographic profile (DAD at 360 nm) of BRS Morena grape skin (A) and flesh (B) and BRS Clara grape skin (C) and flesh (D). Peak numbering is as in Table 2.

(Table 2), which were in agreement with those provided by the literature.[11,12,28] Moreover, the online DAD UV−vis spectra (data not shown) helped to confirm the type of flavonoid structure, and the visible maxima appeared at the expected wavelength values for flavonol 3-glycosides:[11] 348−349 nm for the kaempferol-type; 353−354 nm for quercetin- and isorhamnetin-types; and 355−359 nm for myricetin-, laricitrin- and syringetin-types. BRS Morena grape flavonols consisted of the six flavonoid aglycones that have been previously found in red grape varieties,[11,12] but, in our study, only the 3-glucoside series was completely identified, and they were the only kind of 3-glycosides found for the laricitrin and syringetin aglycones; isorhamnetin was also found as 3-galactoside. The 3-glucuronides occurred for only the nonmethoxylated aglycones (kaempferol, quercetin, and myricetin) and, finally, rutin (quercetin 3-rutinoside) was found partially coeluting with quercetin 3-glucoside as a minor flavonol.

Because BRS Morena is a teinturier grape variety, it was expected that flavonols would be found in its flesh, as has been reported for the *V. vinifera* Garnacha Tintorera teinturier variety.[16] As in the latter case, the flavonol profiles shown by the skin and flesh of BRS Morena were different (Figure 2A,B). However, nonteinturier grape varieties, such as French Merlot, also seem to contain flavonols in their flesh.[29] Whereas myricetin-type flavonols (mainly the 3-glucoside derivative) predominate in BRS Morena skin (around 47.5%), only myricetin 3-glucoside was present in the flesh in quantifiable amounts and in much lower proportions (only 8.4%). Quercetin-type flavonols were the second most common flavonols found in BRS Morena skin (27.8%) with similarly high proportions of the 3-glucuronide and 3-glucoside derivatives (12−14% each). Their amounts increased in the flesh (45.3%), particularly that of quercetin 3-glucoside, which accounted for the highest individual proportion of all the flavonols (34.6%). The large decrease in the proportions of myricetin-type flavonols in the flesh was accompanied by the increase of the other types of flavonols and, more notably, for their methoxylated analogues (syringetin-type, from 7.4% in skin to 20.8% in flesh; laricitrin-type, from 11.4 to 16.6%) but also for isorhamnetin-type flavonols (from 4.3 to 7.1%).

Despite the aforementioned differences between skin and flesh flavonol profiles, the most notable difference was the very low amount in which flavonols were found in the flesh of the BRS Morena grape when compared to its skin (2.2 vs 114.0 μmol/kg in the skin, or a ratio of approximately 1:50), results that have been similarly reported for the Garnacha Tintorera grape variety.[16] The level of flavonols found in the BRS Morena grape (around 116 μmol/kg) was within the range described for other red table grapes (68.5−150.7 μmol/kg fresh fruit)[1] and was slightly lower than those found for several varieties of *V. vinifera* red wine grapes (129−346 μmol/kg).[12]

The BRS Clara grape contained flavonols not only in the skin, as expected, but also in the flesh, with a ratio flesh to skin of approximately 1:40. The flavonol profile of the BRS Clara grape was characterized by only 3-glycoside derivatives and a lack of B-ring trisubstituted flavonoid structures (myricetin, laricitrin, and syringetin). These results are in agreement with those that have been previously reported for *V. vinifera* white wine grape varieties.[28] The following flavonol 3-glycosides were identified through the use of ESI-MS/MS data (Table 2), as well as through the confirmation of online DAD UV−vis spectra (data not shown): the complete expected series of kaempferol-type derivatives (3-glucoside, 3-galactoside, and 3-glucuronide) and quercetin-type derivatives (3-glucoside, 3-galactoside, 3-glucuronide, and 3-rutinoside) and only the isorhamnetin 3-glucoside derivative (3-galactoside and, less frequently, 3-glucuronide derivatives have been found in other white wine grapes).[28] In contrast to the flavonol profiles found for the BRS Morena grape, the flavonol profiles found in the skin and flesh of the BRS Clara grape were very similar (Figure 2C,D), with few but significant differences that mainly involved increases in the proportions of the 3-glucosides of quercetin and its methoxylated analogue, isorhamnetin (Table 2). As mentioned previously, flavonols concentrated in the skin of the BRS Clara grape and their contents can be considered important (approximately 140 μmol/kg), because the content found in 22 varieties of white grapes varied between 8 and 160 μmol/kg, and only 4 varieties (Jaén, Malvar, Moscatel grano menudo, and Viognier) had values that exceeded the values obtained for BRS Clara.[28] Flavonols are excellent natural antioxidants. Because there is a high content of flavonols in the BRS Clara grape, we suggest that it be consumed as a table grape with the skin.

Hydroxycinnamic Acid Derivatives (HCAD) and Stilbenes. The expected hydroxycinnamoyl-tartaric acids were the only HCAD found in both BRS Morena and BRS Clara grapes. The identification of caftaric acid (only the *trans* isomer), coutaric acids (*trans* and *cis* isomers), and fertaric acid (only the *trans* isomer) was largely completed using their ESI-MS/MS data

Table 3. Data for the Identification of the Hydroxycinnamic Acid Derivatives (HCAD) and Stilbenes (Resveratrol and Its 3-Glucoside, Piceid) in the BRS Morena and BRS Clara Grapes Using HPLC-ESI-MS/MS (Negative Ionization Mode) and Molar Proportions of Each HCAD, Total Content of HCAD, and Stilbene Concentrations (Milligrams per Kilogram) in Both Parts of the Grapes (Skin and Flesh; Mean ± Standard Deviation)

		molar %[a]			
		Morena		Clara	
compound[b]	psedomolecular and product ions (m/z)	skin, $n = 3$	flesh, $n = 3$	skin, $n = 3$	flesh, $n = 3$
trans-CAFT	311; 179, 149, 135	61.91 ± 1.46 b	53.02 ± 1.17 a	82.29 ± 0.66 A	89.40 ± 0.22 B
trans-COUT	295; 163, 149, 119	23.14 ± 0.49 b	6.35 ± 1.14 a	9.12 ± 0.52 B	4.29 ± 0.07 A
cis-COUT	295; 163, 149, 119	12.41 ± 0.85 a	20.98 ± 2.22 b	7.79 ± 0.27 B	2.65 ± 0.24 A
trans-FERT	325; 193, 149	2.55 ± 0.12 a	19.65 ± 2.26 b	0.81 ± 0.11 A	3.67 ± 0.39 B
total HCAD (μmol/kg)		101.1 ± 12.5 b	8.0 ± 1.0 a	85.1 ± 8.8 B	34.5 ± 2.7 A
trans-piceid[c]	389; 227	2.56 ± 0.64	ND	0.23 ± 0.01	ND
trans-resveratrol[c]	227	3.91 ± 1.00	ND	ND	ND

[a] "a" and "b" and "A" and "B" indicate significant differences (Student t test; $\alpha = 0.05$) between skin and flesh composition. [b] CAFT, caftaric acid; COUT, coutaric acid; FERT, fertaric acid.ND, nondetectable. [c] Concentrations in mg/kg.

(Table 3) and was confirmed by their online DAD UV−vis spectra (data not shown), which were in agreement with the data from the literature.[30] Additional confirmation was obtained by the injection of a standard of *trans*-caftaric acid. HCAD were present in both grape parts and were more commonly located in the skins (ratio of flesh to skin of 1:12 for BRS Morena and 1:2.5 for BRS Clara). The BRS Morena grape contained slightly higher amounts of HCAD than the BRS Clara grape did (101 and 85 μmol/kg, respectively). These results are in agreement with the literature, reporting 2−100 times more HCAD content in the skin of *V. vinifera* grape than in the flesh that corresponds to calculated grape skin HCAD contents in the range of 19−278 μmol/kg,[31] although some grape varieties were reported as reaching values as high as 800 μmol/kg.[16] The HCAD molar profile also differed according to grape variety and the grape part being considered. The predominant HCAD was always *trans*-caftaric acid (especially in the case of BRS Clara) (Table 3), followed by coutaric acid (*trans* and *cis* isomers) and *trans*-fertaric acid. These data are consistent with the data reported for *V. vinifera*.[31] The proportion of *trans*-caftaric acid was significantly lower in the flesh of the BRS Morena grape. This low proportion, along with a significant decrease in the proportion of *trans*-coutaric acid, resulted in significant increases in the proportion of *cis*-coutaric and more pronounced increases in the proportions of *trans*-fertaric acids in the flesh. These changes occurred when these acids were minor compounds in the skins. However, the total sum of coutaric acid decreased in the flesh HCAD profile of the BRS Morena grape (from 35.55% in the skin to 27.33% in the flesh). In the case of the BRS Clara grape, the flesh contained significantly higher proportions of *trans*-caftaric and *trans*-fertaric acids than the skin. The flesh also presented significantly lower proportions of both isomers of coutaric acid.

Finally, resveratrol and its 3-glucoside (piceid) were found in the skin of the BRS Morena grape and solely in the form of *trans* isomers, whereas BRS Clara skin contained only small amounts of *trans*-piceid (Table 3). Other known grape stilbenes (e.g., pterostilbene, piceatannol, viniferins, pallidol, parthenocissin, and hopeaphenol) were investigated by MS/MS, but no evidence of their occurrence was found. There is a lack of data regarding the content of resveratrol and its isomers in nonvinifera grapes. However, according to the classification proposed for *V. vinifera* grape varieties,[32] the amounts of stilbenes found in the BRS Morena grape suggested that it may be considered a high resveratrol producer. This class of grape variety is characterized by the following mean value contents of resveratrol-type derivatives (mg/kg of grape): *trans*-resveratrol, 2.37 (3.91 in the BRS Morena grape); *cis*-piceid, 4.19 (not detected in the BRS Morena grape); *trans*-piceid, 4.18 (2.56 in the BRS Morena grape); total resveratrols, 10.74 (6.47 in the BRS Morena grape). In contrast, the BRS Clara grape seems to belong to the pool of low resveratrol producers. This class of grape variety is characterized by the following mean values (mg/kg of grape): *trans*-resveratrol, 0.53 (not detected in the BRS Clara grape); *cis*-piceid, 0.29 (not detected in the BRS Clara grape); *trans*-piceid, 0.48 (0.23 in the BRS Clara grape); and total resveratrols, 1.30 (0.23 in the BRS Clara grape). However, it is necessary to bear in mind that stilbene grape concentration is affected not only by genotype but also by environmental and cultural factors,[33] and more data are needed to confirm the suggested classification of BRS Clara and Morena grapes with regard to their potential of resveratrol production.

Flavan-3-ols. Five flavan-3-ol monomers were found in the skins of BRS Morena and BRS Clara grapes (Table 4), the most common of which was (+)-catechin (C), followed by lower amounts of (−)-gallocatechin (GC), (−)-epicatechin (EC), (−)-epigallocatechin (EGC), and (−)-epicatechin 3-gallate (ECG). The dimer procyanidin B1 (PB1) was also found in amounts comparable to those of (+)-catechin, along with lower amounts of procyanidin B2 (PB2). Significant differences between the two grape varieties were found in terms of the content of (+)-catechin, which was higher in the BRS Clara grape; the BRS Clara grape also presented lower contents of the minor monomers (GC, EC, and EGC) and the other dimer (PB2). The total sum of flavan-3-ol monomers and dimers was low, although it was higher in the case of the BRS Clara grape (18.08 vs 14.98 for BRS Morena, as mg/kg of (+)-catechin equiv). The main fraction of flavan-3-ols was present in both grape varieties as oligomers and polymers, jointly referred to as grape proanthocyanidins (PA). They accounted for 391 and 264 mg/kg (as (+)-catechin equiv; Table 5) for BRS Morena and BRS Clara, respectively. These values were lower than the usual values reported for the total PA content in skins of *V. vinifera* grape varieties, although some samples of Spanish Syrah grape skins

Table 4. Monomeric Flavan-3-ol and Dimer B-Type Procyanidin Contents (Mean ± Standard Deviation, mg/kg Grape) in the Skin of BRS Morena and BRS Clara Grapes

flavan-3-ol	BRS Morena,[a] $n = 3$	BRS Clara,[a] $n = 3$
(−)-epicatechin	1.08 ± 0.20 b	0.50 ± 0.10 a
(+)-catechin	6.25 ± 0.52 a	10.62 ± 0.27 b
(−)-epigallocatechin	0.36 ± 0.06 b	0.08 ± 0.02 a
(−)-gallocatechin	1.47 ± 0.11 b	0.68 ± 0.03 a
(−)-epicatechin 3-gallate	0.07 ± 0.01	0.08 ± 0.02
procyanidin B1	10.23 ± 0.84	11.78 ± 1.48
procyanidin B2	1.28 ± 0.14 b	0.47 ± 0.05 a
total (mg/kg)[b]	14.98 ± 1.38 a	18.08 ± 1.20 b

[a] "a" and "b" indicate significant differences (Student t test; $\alpha = 0.05$) between grape varieties. [b] As (+)-catechin equivalents.

were reported as having only 282 ± 40 mg/kg, as (+)-catechin equiv.[34]

The structural analysis of the proanthocyanidins of BRS Morena and BRS Clara grapes produced results (Table 5) that, in general, were consistent with previously reported data for *V. vinifera* grape varieties.[35−37] The mean degree of polymerization (mDP) was higher in the BRS Morena grape (9.90 vs 7.03 in BRS Clara). The BRS Morena grape also had a higher proportion of prodelphinidin units (26.34 vs 12.91% in BRS Clara) and a lower degree of galloylation or 3-gallate ester units (2.50 vs 3.17% in BRS Clara). The extension units that formed the skin proanthocyanidins of the two BRS grape varieties (Table 5) were the same as the four found in *V. vinifera* grape skins:[35] In our study, (−)-epicatechin was the main extension unit, with a higher proportion in BRS Clara (64.72 vs 57.67% in BRS Morena). Our analysis also revealed (−)-epigallocatechin (12.83% in BRS Clara and 26.02% in BRS Morena), as well as (+)-catechin and (−)-epicatechin 3-gallate, which were minor extension units found in the skins of both grapes (each contributed to no more than approximately 5%). With regard to the terminal units, it is common to find studies that report on only (+)-catechin as the main terminal unit (which was significantly higher in BRS Clara), whereas (−)-epicatechin has been reported in lower values,[34] which was consistent with our findings (Table 5). However, we were able to quantify very low percentages (<0.4%) of terminal units corresponding to the other flavan-3-ol monomers ((−)-epigallocatechin and (−)-epicatechin 3-gallate), which also contributed as extension units. The latter finding might be very likely linked to the higher sensitivity of the MRM technique used for the detection and quantification of flavan-3-ols.

Total Phenolic Content and Antioxidant Capacity. The BRS Morena and BRS Clara grape varieties showed total phenolic contents of 1008 and 577 mg (as gallic acid equivalents)/kg of fresh fruit, respectively. This content was distributed between 86.2% in the skin and 13.8% in the flesh for the BRS Morena grape and between 76.5% in the skin and 23.5% in the flesh for the BRS Clara grape (Table 6). The contents of the phenolic compounds found were significantly higher than those reported for other table grapes (70−361 mg as gallic acid/kg of fresh fruit).[1] The value found in BRS Morena was within the range described for *V. vinifera* red grapes (731−3486 mg as gallic acid/kg of fresh fruit),[37] results that reinforced their genetic origins. The aforementioned results confirm once again that the edible parts of these grapes, especially the skin, constitute a rich source of phenolic compounds.

Table 5. Structural Characterization of the Skin Proanthocyanidins (Mean ± Standard Deviation) of BRS Morena and BRS Clara Grapes

proanthocyanidin[a]	BRS Morena,[b] $n = 3$	BRS Clara,[b] $n = 3$
total PA (mg/kg)[c]	391.4 ± 29.4 b	264.0 ± 9.8 a
mDP	9.90 ± 0.56 b	7.03 ± 0.07 a
% galloylation	2.50 ± 0.09 a	3.17 ± 0.05 b
% prodelphinidin	26.34 ± 0.12 b	12.91 ± 0.71 a
% extension-EC	57.67 ± 0.25 a	64.72 ± 0.52 b
% extension-C	3.80 ± 0.09 a	5.21 ± 0.15 b
% extension-EGC	26.02 ± 0.14 b	12.83 ± 0.69 a
% extension-ECG	2.39 ± 0.10 a	3.02 ± 0.06 b
% terminal-EC	0.82 ± 0.11 b	0.47 ± 0.09 a
% terminal-C	8.87 ± 0.46 a	13.52 ± 0.22 b
% terminal-EGC	0.32 ± 0.04 b	0.08 ± 0.02 a
% terminal-ECG	0.11 ± 0.01 a	0.14 ± 0.01 b

[a] mDP, mean degree of polymerization; % galloylation, % of 3-gallate units; % prodelphinidin, % of epigallocatechin units; and % of each of the flavan-3-ol monomers as extension and terminal units; EC, (−)-epicatechin; C, (+)-catechin; EGC, (−)-epigallocatechin; ECG, (−)-epicatechin 3-gallate. [b] "a" and "b" indicate significant differences (Student "t" test; $\alpha = 0.05$) between grape varieties. [c] As (+)-catechin equivalents, calculated by total sum of the concentrations of extension and terminal units.

With regard to total antioxidant capacity (Table 6), BRS Morena and BRS Clara grapes exhibited high values (39.62 ± 1.11 and 15.93 ± 0.24 mmol/kg as Trolox equiv, respectively) that were mainly located at the skins (92.0% in BRS Morena and 86.8% in BRS Clara). It is difficult to find similar data for other grapes and even more difficult to compare them because of the differences in the assay methods used (DPPH vs ABTS, for example) or because of the differences in the grape material of reference (fresh weight grape, fresh weight skins, or dry weight skins). For this reason, we calculated the values for antioxidant capacity of the skins of BRS Morena grapes using other units (as Trolox equiv): 146 μmol/g fresh weight skin; 680 μmol/g dry weight skin; and 150 μM/g dry weight skin. In the case of the BRS Clara grape, the values in other units were as follows: 58 μmol/g fresh weight skin; 312 μmol/g dry weight skin; and 63 μM/g dry weight skin. In this context, and considering only data regarding DPPH values, the antioxidant capacity found in the BRS Morena grape skin was approximately 6 times higher than that of skins of *Vitis rotundifolia* grapes (20.5−26.6 μmol/g fresh weight skin).[38] The value found in BRS Clara was approximately 2 times higher. The values found in BRS Morena and BRS Clara were approximately 15 and 6 times higher, respectively, than those found in some *Vitis aestivalis* and *V. vinifera* grapes (8−9 μmol/g fresh weight of a mixture of skin and flesh).[39] In a recent study, DPPH antioxidant capacity values in the range of 94−276 μM Trolox equiv/g of dry weight skin were reported for several European (*V. vinifera*), American (*V. rotundifolia*), and Euro-American and Euro-Asian hybrids, as well as for Asian *Vitis* species.[40] BRS Clara antioxidant capacity values were higher and closer to the upper limit of the aforementioned range, but BRS Morena values were 2−3 times higher. On the basis of these results, it can be suggested that the skin of new grape varieties developed by EMBRAPA, especially BRS Morena, have a high potential antioxidant capacity that is linked to its equally high content of phenolic compounds. However, this high potential of

Table 6. Total Phenolic Content and Antioxidant Capacity of BRS Morena and BRS Clara Grape Skin and Flesh

	BRS Morena[a]		BRS Clara[a]	
	skin, $n = 3$	flesh, $n = 3$	skin, $n = 3$	flesh, $n = 3$
total phenolic content (mg/kg, as gallic acid equiv)	869.2 ± 16.4 b	138.9 ± 0.2 a	441.2 ± 15.3 B	135.5 ± 0.1 A
antioxidant capacity (mmol/kg, as Trolox equiv)	36.46 ± 0.92 b	3.16 ± 0.19 a	13.83 ± 0.19 B	2.10 ± 0.05 A

[a] "a" and "b" and "A" and "B" indicate significant differences (Student t test; $\alpha = 0.05$) between skin and flesh composition.

antioxidant capacity needs to be evaluated using different vintages and under different cultivation conditions, because the phenolic content of grapes is strongly affected by both genotype and environmental factors.

AUTHOR INFORMATION

Corresponding Author

*Phone:++34-926-295253. Fax: ++34-926-295351. E-mail: isidro.hermosin@uclm.es.

Funding Sources

This work has been partially financed by funding from the IVICAM (Project PREG-10-002). We thank the following Brazilian agencies: FAPESP, for financial assistance; CNPq, for a productivity scholarship (R.D.-S.); CAPES, for a scholarship; and the PDEE program (E.S.L.-V.).

ACKNOWLEDGMENT

The authors are grateful to Dra. Rosimeire de Lellis Naves and Dr. Umberto Almeida Camargo (EMBRAPA) for supplying the grape samples.

REFERENCES

(1) Cantos, E.; Espín, J. C.; Tomás-Barberán, F. A. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. *J. Agric. Food Chem.* **2002**, *50*, 5691–5696.

(2) Yadav, M. Biological and medicinal properties of grapes and their bioactive constituents: an update. *J. Med. Food* **2009**, *12*, 473–483.

(3) Yang, J.; Martinson, T. E.; Liu, R. H. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chem.* **2009**, *116*, 332–339.

(4) Anastasiadi, M.; Pratsinis, H.; Kletsas, D.; Skaltsounis, A.; Haroutounian, S. A. Bioactive non-coloured polyphenols content of grapes, wines and vinification byproducts: evaluation of the antioxidant activities of their extracts. *Food Res. Int.* **2010**, *43*, 805–813.

(5) Xia, E; Deng, G.; Guo, Y; Li, H. Biological activities of polyphenols from grapes. *Int. J. Mol. Sci.* **2010**, *11*, 622–646.

(6) Carreño, J.; Almela, L.; Martínez, A.; Fernández-López, J. A. Chemotaxonomical classification of red table grapes based on anthocyanin profile and external colour. *Lebensm. Wiss. Technol.* **1997**, *30*, 259–265.

(7) Molina-Quijada, D. M. A.; Medina-Juárez, L. A.; González-Aguilar, G. A.; Robles-Sánchez, R. M.; Gámez-Meza, N. Phenolic compounds and antioxidant activity of table grape (*Vitis vinifera* L.) skin from northwest Mexico. *CYTA — J. Food* **2010**, *8*, 57–63.

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

(9) Camargo, U. A.; Nachtigal, J. C.; Maia, J. D. G.; Oliveira, P. R. D.; Protas, J. F. S. BRS Clara: nova cultivar de uva branca de mesa sem semente; Technical Communication 46, 2003; http://www.cnpuv.embrapa.br/publica/comunicado/cot046.pdf (accessed July 12, 2010).

(10) Camargo, U. A.; Nachtigal, J. C.; Maia, J. D. G.; Oliveira, P. R. D.; Protas, J. F. S. BRS Morena: nova cultivar de uva preta de mesa sem semente; Technical Communication 47, 2003; http://www.cnpuv.embrapa.br/publica/comunicado/cot047.pdf (accessed July 12, 2010).

(11) Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Gómez, M. V.; Velders, A. H.; Hermosín-Gutiérrez, I. Flavonol 3-*O*-glycosides series of *Vitis vinifera* cv. Petit verdot red wine grapes. *J. Agric. Food Chem.* **2009**, *57*, 209–219.

(12) Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I. Flavonol profiles of *Vitis vinifera* red grapes and their single-cultivar wines. *J. Agric. Food Chem.* **2007**, *55*, 992–1002.

(13) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin−Ciocalteu reagent. In *Methods in Enzymology: Polyphenols and Flavonoids*; Sies et al. , Eds.; Academic Press: San Diego, CA, 1999; pp 152−178.

(14) Brand-Williams, W.; Cuvelier, M. E.; Berst, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm. Wiss. Technol.* **1995**, *28*, 25–30.

(15) Bordiga, M.; Travaglia, F.; Coïsson, J. D.; Locatelli, M.; Arlorio, M.; Martelli, A. Pyrogallol: a new trapping nucleophile in proanthocyanidins analysis. *In Final Papers of 32nd World Congress of Vine and Wine;* Kubanovic, V., Ed.; Ministry of Agricultural, Fisheries and Rural Development: Zagreb, Croatia, 2009.

(16) Castillo-Muñoz, N.; Fernández-González, M.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I. Red-color related phenolic composition of Garnacha Tintorera (*Vitis vinifera* L.) grapes and red wines. *J. Agric. Food Chem.* **2009**, *57*, 7883–7891.

(17) Hermosín-Gutiérrez, I.; García-Romero, E. Antocianos de variedades tintas cultivadas en La Mancha: perfiles varietales característicos de la uva y de los vinos monovarietales, y evolución durante la maduración de la baya. *Alimentaria* **2004**, *41*, 127–139.

(18) Mazzuca, P.; Ferranti, P.; Picariello, G.; Chianese, L.; Addeo, F. Mass spectrometry in the study of anthocyanins and their derivatives: differentiation of *Vitis vinifera* and hybrid grapes by liquid chromatography/electrospray ionization mass spectrometry and tandem mass spectrometry. *J. Mass Spectrom.* **2005**, *40*, 83–90.

(19) Liang, Z.; Wu, B.; Fan, P.; Yang, C.; Duan, W.; Zheng, X.; Liu, C.; Li, S. Anthocyanin composition and content in grape berry skin in *Vitis* germplasm. *Food Chem.* **2008**, *111*, 837–844.

(20) Gómez-Plaza, E.; Gil-Muñoz, R.; Hernández-Jiménez, A.; López-Roca, J. M.; Ortega-Regules, A.; Cutillas, A. M. Studies on the anthocyanin profile of *Vitis vinifera* intraspecific hybrids (Monastrell × Cabernet Sauvignon). *Eur. Food Res. Technol.* **2008**, *227*, 479–484.

(21) Papini, P. C.; Mazza, G.; Gatti, M.; Bavaresco, L. Anthocyanin and aroma profiling of the 'Albarossa' grapevine crossbreed (*Vitis vinifera* L.) and its parent varieties 'Barbera' and 'Nebbiolo di Dronero'. *Vitis* **2010**, *49*, 121–127.

(22) Leão, P. C. S. Recursos genéticos de videira (*Vitis* ssp.): análise da diversidade e caracterização da coleção de germoplasma da Embrapa Semi-Árido; Tese (Doutor em Genética e Melhoramento), Minas Gerais - Universidade Federal de Viçosa, 2008; 126 pp.

(23) Poudel, P. R.; Mochioka, R.; Beppu, K.; Kataoka, I. Influence of temperature on berry composition of interspecific hybrid wine grape 'Kadainou R-1' (*Vitis ficifolia* var. ganebu × *V. vinifera* 'Muscat of Alexandria'). *J. Jpn. Soc. Hortic. Sci.* **2009**, *78*, 169–174.

(24) He, J.; Liu, Y.; Pan, Q.; Cui, X.; Duan, C. Different anthocyanin profiles of the skin and the pulp of Yan73 (Muscat Hamburg × Alicante Bouschet) grape berries. *Molecules* **2010**, *15*, 1141–1153.

(25) Mazza, G.; Miniati, E. *Anthocyanins in Fruits, Vegetables, And Grains*; CRC Press: Boca Raton, FL, 1993.

(26) Verdejo Fernández, C.; Alcaide Quintana, J.; Alía Robledo, J. M.; Montero Riquelme, F.; Brasa Ramos, A.; Hermosín Gutiérrez, I. Contribución al estudio para la zonificación de la denominación de origen Valdepeñas: análisis de la uva de la vendimia 2004. *Enologos* **2007**, *45*, 58–66.

(27) Fragoso, S.; Aceña, L.; Guasch, J.; Busto, O.; Mestres, M. Application of FT-MIR spectroscopy for fast control of red grape phenolic ripening. *J. Agric. Food Chem.* **2011**, *59*, 2175–2183.

(28) Castillo-Muñoz, N.; Gómez-Alonso, S.; García-Romero, E.; Hermosín-Gutiérrez, I. Flavonol profiles of *Vitis vinifera* white grape cultivars. *J. Food Compos. Anal.* **2010**, *23*, 699–705.

(29) Pereira, G. E.; Gaudillere, J. P.; Pieri, P.; Hilbert, G.; Maucourt, M.; Deborde, C.; Moing, A.; Rolin, D. Microclimate influence on mineral and metabolic profiles of grape berries. *J. Agric. Food Chem.* **2006**, *54*, 6765–6775.

(30) Buiarelli, F.; Coccioli, F.; Merolle, M.; Jasionowska, R.; Terracciano, A. Identification of hydroxycinnamic acid−tartaric acid esters in wine by HPLC−tandem mass spectrometry. *Food Chem.* **2010**, *123*, 827–833.

(31) Cheynier, V.; Moutounet, M.; Sarni-Manchado, P. Los compuestos fenólicos. In *Enología. Fundamentos Científicos y Tecnológicos*, 2nd ed.; Flanzy, C., Ed.; AMV Ediciones and Ediciones Mundi-Prensa: Madrid, Spain, 2003; pp 114−136.

(32) Gatto, P.; Vrhovsek, U.; Muth, J.; Segala, C.; Romualdi, C.; Fontana, P.; Pruefer, D.; Stefanini, M.; Moser, C.; Mattivi, F.; Velasco, R. Ripening and genotype control stilbene accumulation in healthy grapes. *J. Agric. Food Chem.* **2008**, *56*, 11773–11785.

(33) Bavaresco, L.; Pezzutto, S.; Gatti, M.; Mattivi, F. Role of the variety and some environmental factors on grape stilbenes. *Vitis* **2007**, *46*, 57–61.

(34) Busse-Valverde, N.; Gómez-Plaza, E.; López-Roca, J. M.; Gil-Muñoz, R.; Fernández-Fernández, J. I.; Bautista-Ortín, A. B. Effect of different enological practices on skin and seed proanthocyanidins in three varietal wines. *J. Agric. Food Chem.* **2010**, *58*, 11333–11339.

(35) Souquet, J.; Cheynier, V.; Broussaud, F.; Moutounet, M. Polimeric proanthocyanidins from grape skins. *Phytochemistry* **1996**, *43*, 509–512.

(36) Labarbe, B.; Cheynier, V.; Brossand, F.; Souquet, J.; Moutounet, M. Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.* **1999**, *47*, 2719–2723.

(37) Katalinic, V.; Mozina, S. S.; Skroza, D.; Generalic, I.; Abramovic, H.; Milos, M.; Ljubenkov, I.; Piskernik, S.; Pezo, I.; Terpinc, P.; Boban, M. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 *Vitis vinifera* varieties grown in Dalmatia (Croatia). *Food Chem.* **2010**, *119*, 715–723.

(38) Sandhu, A. K.; Gray, D. J.; Lu, J.; Gu, L. Effects of exogenous abscisic acid on antioxidant capacities, anthocyanins, and flavonol contents of muscadine grape (*Vitis rotundifolia*) skins. *Food Chem.* **2011**, *126*, 982–988.

(39) Hogan, S.; Zhang, L.; Li, J.; Zoecklein, B.; Zhou, K. Antioxidant properties and bioactive components of Norton (*Vitis aestivalis*) and Cabernet Franc (*Vitis vinifera*) wine grapes. *LWT − Food Sci. Technol.* **2009**, *421*, 269–1274.

(40) Xu, C.; Zhang, Y.; Cao, L.; Lu, J. Phenolic compounds and antioxidant properties of different grape cultivars grown in China. *Food Chem.* **2010**, *119*, 1557–1565.