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Phenolic Composition of Malbec Grape Skins and Seeds from Valle de Uco (Mendoza, Argentina) during Ripening. Effect of Cluster Thinning

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ABSTRACT: The phenolic composition of Malbec (*Vitis vinifera* L.) grape skins and seeds during ripening and the effect of cluster thinning (CT) in two consecutive seasons (2008−2009) were evaluated by high-performance liquid chromatography−diode array detection/electrospray ionization−mass spectrometry (HPLC-DAD/ESI-MS). Removal of 50% of clusters was performed at 40 days (T1), 80 days (T2), and 100 days after flowering (T3) in a vineyard located in southern Mendoza (Argentina). Yield components, with the exception of cluster weight, were significantly affected by CT in both seasons, but no statistically significant differences were found among treatments. Cluster thinning and its timing had little or no influence on physical parameters and fruit chemical composition, and the differences with respect to the control were mainly due to the season. At harvest in 2008, T1 encouraged the biosynthesis of individual anthocyanins in skins, generating 44.0, 39.6, and 41.2% more glucosylated, acetylated, and total anthocyanins, respectively, as compared to the control, whereas in seeds, T1 and T2 mainly changed the concentrations of (+)-catechin, epicatechin-3-gallate, procyanidin B4, dimer gallate 1, trimer gallate 2, and tetramer. Conversely in 2009, T1 significantly affected the content of flavanols and flavonols in skins, whereas in seeds, T1 and T2 modified the level of (+)-catechin, procyanidins B4 and B6, and trimer gallate 2. Moreover, in 2008 the grapes had a higher concentration of most phenolic compounds, indicating a greater potential for more complex wines. Finally, dihydroquercetin-3-glucoside was the major compound among all nonanthocyanin phenolics detected in Malbec skins and represented 25.7% (2008) and 39.9% (2009) of the total content of those compounds at harvest. This finding could represent a distinctive feature of this grape variety.

KEYWORDS: phenolic compound, Malbec, skin, seed, ripening, anthocyanins, flavanols, flavonols, dihydroflavonols

INTRODUCTION

Phenolic compounds constitute one of the most important quality parameters of grapes and wines, because they contribute to organoleptic characteristics such as color, astringency, and bitterness. These compounds are also active in biochemical processes and have nutraceutical effects on human health, including antimicrobial, anticarcinogenic, and antioxidant properties.[1,2] In grape berries, phenolic compounds are present mainly in skins and seeds. The concentration and presence of these compounds in grapes depend mainly on genetic, soil, climate, and viticulture factors, among others.[3,4] Several authors have suggested that the concentration of some secondary metabolites, such as anthocyanins, flavanols, and flavonols, is dependent, to some extent, on the plant yield and the leaf area/berry ratio.[4,5]

Some agronomical practices such as summer pruning and cluster thinning (fruit removal) have been proposed to improve berry grape quality, by means of modifying some attributes of the berries such as sugar content, pH, total acidity, flavors, and color during ripening.[6] Cluster thinning is a practice applied to regulate the yield levels and to help ripen the crop under poor climatic conditions or excessive crop demand.[7] Nevertheless, the literature reports contrasting results with cluster thinning leading to better fruit quality is some cases,[7,8] but with no clear effect in others.[6,9,10] Moreover, it is potentially an expensive process in terms of labor and lost yield. In addition, the amount of fruit removed and the timing of the operation may be important. Removing crop early in the season (at bloom or soon thereafter) may not lead to the desired result because the reduced sink size might in turn lead to lower leaf photosynthesis rates, so that the remaining berries may not have extra sugar available for import. If, however, photosynthesis remains unchanged, surplus photoassimilates could also be used to fuel more shoot (and root) growth. This growth would counteract the benefits of lower crop load because of its negative effect on vigor and canopy microclimate. Therefore, it might be beneficial to delay thinning until shoot growth has slowed and assimilates may be diverted to the fruit.[10]

Malbec (*Vitis vinifera* L.) is a middle-maturing grape variety of French origin that is now mainly produced in Mendoza. This cultivar is well adapted to the different local ecosystems and nowadays is considered to be the emblematic cultivar for wine production in Argentina.[11] In Mendoza's Malbec vineyards, cluster thinning is commonly applied to modify fruit growth and

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composition. However, to our knowledge, to date there is no published information about the influence of this viticultural practice on the individualized phenolic composition of Malbec grapes and the evolution of these compounds during ripening. Considering this, the aim of the present work was to study the phenolic composition of Malbec grape skins and seeds and to evaluate the effect of cluster thinning timing in two consecutive seasons.

MATERIALS AND METHODS

Standards and Reagents. Standards of gallic acid [149-91-7], syringic acid [530-57-4], caffeic acid [331-39-5], ethyl gallate [831-61-8], (+)-catechin [7295-85-4], (−)-epicatechin [490-46-0], resveratrol [501-36-0], myricetin [529-44-2], quercetin-3-glucoside [21637-25-2], and *p*-dimethylaminocinnamaldehyde [6203-18-5] were purchased from Sigma-Aldrich (St. Louis, MO), whereas protocatechuic acid [99-50-3], quercetin [117-39-5], kaempferol-3-glucoside [480-10-4], and malvidin-3-glucoside chloride [7228-78-6] were supplied by Extrasynthese (Lyon, France). Sodium chloride, sodium metabisulfite, sodium hydroxide, and tartaric acid were purchased from Anedra (Buenos Aires, Argentina). Ammonium iron(II) sulfate and butanol were obtained from Dalton (Mendoza, Argentina). Ethyl ether and ethyl acetate were acquired from Sintorgan (Buenos Aires, Argentina). Sodium sulfate anhydrous, hydrochloric acid, acetic acid, formic acid, ethanol, chromatography grade methanol, and acetonitrile were purchased from Merck (Darmstadt, Germany). All reactives were of analytical grade or superior. Ultrapure water was obtained from an RiO/Elix3-Sinergy185 purification system (Millipore, Sao Pablo, Brazil). Cellulose filter (3 μm pore size) and 0.45 μm pore size nylon membrane were supplied by Microclar (Buenos Aires, Argentina).

Instrumentation. The pH was measured in a TPX-1 equipment (Altronix, Buenos Aires, Argentina), and soluble solids (°Brix) were measured with a refractometer, model ATC-1 (Atago, Tokyo, Japan). Skin grinding was performed using a mixer homogenizer (Omni International, Germany). Seeds milling was carried out through an ultracentrifugal mill model ZM 200 (Retsch, Newtown, PA). The extract maceration was made in an orbital shaker (Decalab, Buenos Aires, Argentina) and the centrifugation with CM4080 equipment (Rolco, Buenos Aires, Argentina). Absorbance measurements were made with a Perkin-Elmer UV−vis spectrophotometer model Lambda 25 (PerkinElmer, Hartford, CT). The chromatographic system consisted of a Perkin-Elmer series 200 high-performance liquid chromatograph equipped with a photodiode array detector, a quaternary pump, and an autosampler (HPLC-DAD; PerkinElmer, Shelton, CT). A reversed phase Chromolith Performance C_{18} column (100 mm × 4.6 mm i.d., 2 μm; Merck) was used for individual anthocyanin analysis. A reversed phase Nova-Pak C_{18} column (300 mm × 3.9 mm i.d., 4 μm; Waters Corp., Milford, MA) was applied in low molecular weight phenolic compound analysis.

Plant Material and Experimental Conditions. The experiments were performed in 2008 and 2009 seasons, in a commercial vineyard located at an altitude of 1100 m at Altamira (69° 07′ W and 33° 43′ S), San Carlos, Mendoza, Argentina. The grapevines of *V. vinifera* L. cv. Malbec were planted in 2000, own-rooted, trained on a vertical trellis system, pruned as Guyot, and arranged in north−south oriented rows spaced 2 m apart, with 1.2 m between plants on the row. The vineyard was managed according to standard viticultural practices for the cultivar and region. Winter pruning was carried out leaving 12 buds per vine. Canopy management practices, all manually performed, included trunk deshooting and removal of double shoots. Shoots were not trimmed, but positioned twice between the wires, and no leaf removal was conducted. The plants were maintained with no soil−water restriction during the whole experiment by a drip irrigation system. Average seasonal (September−March) water received per vine has been estimated at about 400 mm. Drip irrigation was applied with pressure-compensated emitters (2 L/h) located in a single row 0.75 m apart. Irrigation started before budbreak and finished about a week before harvest. Four cluster thinning (CT) treatments were imposed as a completely randomized design with three replicates. The experimental unit consisted of 30 plants, which were selected on the basis of their homogeneity in the row. Treatments were early thinning [T1, at pea size, approximately 40 days after flowering (DAF)], veraison thinning (T2, at veraison, 80 DAF), late thinning (T3, 100 DAF), and no thinned control (C). For CT treatments, 50% of the clusters of each plant were removed at 40 (T1), 82 (T2), and 103 (T3) DAF in 2008 and at 39 (T1), 78 (T2), and 101 (T3) DAF in 2009. Flowering was on November 13, 2008, and November 17, 2009, according to the stage 23 described by Coombe.[12] The distal cluster was removed, leaving only one bunch per shoot at most, as were clusters of weak shoots. Yield components were assessed at harvest. The number of clusters, total vine yield per vine, and canopy surface area/yield ratio were determined in 10 vines per treatment. Cluster weight was calculated in 10 clusters per treatment. Crop yield prediction per hectare was based on past weight of clusters recorded by the vineyard and confirmed at harvest. Climatic conditions of the two seasons 2008 and 2009 were very different, particularly in the rainfall amounts and temperatures (Table 1). These meteorological conditions significantly affected the grape ripening period. The 2008 season had higher rainfall records and lower temperatures than the 2009 season, so the ripening was slower and the time of harvest about 30 days later. Due to the foregoing, the berry sampling dates in both seasons were different.

Berry Sampling and General Analytical Parameters. Three hundred berries per experimental unit were randomly collected, from different positions within clusters and plants, in nylon bags. Sampling corresponded to 85, 113, and 154 DAF in 2008 and to 67, 98, and 121 DAF in 2009. The last sampling in both seasons is considered as the harvest time (about 25 °Brix). The samples were kept in dry ice to prevent dehydration and transported to the laboratory, where they were weighed, frozen, and conserved at −80 °C. One hundred berries per experimental unit were defrosted at room temperature, and skins were separated from pulp and seeds by hand. The pulps were collected in nylon bags and crushed by finger pressing to obtain the juice, and later this juice was used to determine soluble solids (°Brix), pH, and titratable acidity (g/L of tartaric acid) as described by Zoecklein et al.[13]

Extract Preparation of Skins and Seeds. Berry phenolics were extracted as described in previous papers.[14−16] Briefly, skins and seeds were separated by hand from 100 berries, weighed, and ground with 30 mL of ultrapure water. Forty milliliters of hydroalcoholic solution (ethanol/water, 12:88, v/v) containing 5 g/L of tartaric acid was added to the ground material (skins or seeds), and the weight of the resulting suspension was adjusted to 200 g with ultrapure water. The pH of extracts was adjusted to 3.6 with NaOH or HCl. Extracts were macerated for 2 h at 25 °C using an orbital shaker at 200 rpm and then centrifuged for 15 min at 2038*g*.

Spectrophotometric Characterization. Total phenols were determined by direct reading of the absorbance of the samples at 280 nm.[17] Total phenols were expressed as milligrams of gallic acid equivalents per gram of sample (GAE, mg/g). Total anthocyanins were measured by diluting the extract with 2% hydrochloric acid in ethanol and by comparing spectrophotometric readings at 520 nm of single aliquots treated with either sodium metabisulfite or water.[17] Total anthocyanins were expressed as milligrams of malvidin-3-glucoside per gram of sample. For total proanthocyanidins, the analytical method applied was the acid butanol assay.[18] This method is based on the acid-catalyzed oxidative cleavage of the C−C interflavanic bond of proanthocyanidins in butanol−HCl. Total proanthocyanidins were expressed as milligrams of (+)-catechin per gram of sample. Other chemical parameters measured in the samples were flavanol reagents

Table 1. Monthly Rainfall, Growing Degree Days, and Maximum and Minimum Air Temperatures at Altamira in the 2008 and 2009 Seasons[a]

	2008				2009			
month	rain (mm)	GDD (°C)	T_{max} (°C)	T_{min} (°C)	rain (mm)	GDD (°C)	T_{max} (°C)	T_{min} (°C)
July	6	0	23.0	−14.5	0	0	22.5	−4.0
Aug	24	0	20.0	−7.5	41	0	29.0	−4.5
Sep	31	45	28.0	−1.5	17	60	27.0	−5.0
Oct	65	171	32.5	3.0	4	155	30.0	1.5
Nov	6	225	33.5	0.0	7	360	39.0	8.5
Dec	54	341	34.5	6.5	53	372	37.5	8.5
Jan	46	357	35.0	6.5	24	341	37.0	8.0
Feb	59	290	35.0	8.0	0	336	35.5	9.0
March	47	248	30.5	9.0	0	341	35.0	7.0
April	51	105	29.5	−3.0	0	210	30.0	2.0
May	0	nd	nd	nd	0	nd	nd	nd
June	0	nd	nd	nd	0	nd	nd	nd
total	389	1782			146	2175		

[a] Season 2008 includes July 2007−June 2008. Season 2009 includes July 2008−June 2009. GDD, growing degree days; T_{max}, maximum air temperature; T_{min}, minimum air temperature; nd, data not available.

Table 2. Yield Components of Malbec Vines from Altamira (2008−2009)[a]

		parameter, mean ± SE (n = 3)				
year	treatment	cluster number/vine	cluster weight (g)	yield/vine (kg/vine)	crop yield (t/ha)	canopy surface area/yield (m^2/kg)
2008	C	24.2 ± 0.4 b	151.3 ± 11.0 a	3.6 ± 0.1 b	14.7 ± 0.3 b	0.5 ± 0.1 a
	T1	13.5 ± 0.1 a	161.9 ± 8.4 a	2.2 ± 0.1 a	9.4 ± 0.1 a	0.9 ± 0.1 b
	T2	13.7 ± 0.1 a	162.0 ± 6.7 a	2.3 ± 0.1 a	9.6 ± 0.1 a	0.9 ± 0.1 b
	T3	13.3 ± 0.1 a	164.4 ± 8.3 a	2.2 ± 0.1 a	9.6 ± 0.1 a	0.9 ± 0.1 b
2009	C	20.9 ± 0.5 b	80.2 ± 4.0 a	1.6 ± 0.1 b	6.8 ± 0.1 b	1.0 ± 0.1 a
	T1	10.8 ± 0.4 a	91.4 ± 12.2 a	0.9 ± 0.1 a	4.1 ± 0.1 a	1.6 ± 0.1 b
	T2	12.1 ± 0.4 a	76.1 ± 10.3 a	0.9 ± 0.1 a	3.9 ± 0.1 a	1.6 ± 0.1 b
	T3	11.2 ± 0.5 a	93.9 ± 11.5 a	1.0 ± 0.1 a	4.3 ± 0.2 a	1.5 ± 0.1 b

[a] In each column, within the same year, mean values followed by different letters indicate significant differences (Tukey, p < 0,05). SE, standard error. Cluster thinning treatments: C, control; T1, early thinning; T2, veraison thinning; T3, late thinning.

with *p*-dimethylaminocinnamaldehyde (flavanols DMACH), expressed as milligrams of (+)-catechin per gram of sample,[19] and color intensity.[20]

HPLC Analysis of Anthocyanins. Two milliliters of skin extracts per experimental unit was filtered through a 0.45 μm pore size nylon membrane, and then 100 μL was injected in the HPLC-DAD system. Separation was performed at 25 °C. A gradient consisting of solvent A (water/formic acid, 90:10, v/v) and solvent B (acetonitrile) was applied at a flow rate of 1.1 mL/min from 0 to 22 min and at flow rate of 1.5 mL/min from 22 to 35 min as follows: 96−85% A and 4−15% B from 0 to 12 min, 85−85% A and 15−15% B from 12 to 22 min, 85−70% A and 15−30% B from 22 to 35 min. This was followed by a final wash with 100% methanol and re-equilibration of the column. Photodiode array detection was performed from 210 to 600 nm, and the quantification was carried out by peak area measurements at 520 nm, according to the method of Fanzone et al.[11] Anthocyanin amount was expressed by using malvidin-3-glucoside chloride as standard for a calibration curve (R^2 = 0.98). Identification and confirmation of anthocyanic pigments was performed by HPLC-DAD/ESI-MS as described by Monagas et al.[21]

HPLC Analysis of Low Molecular Weight Phenolic Compounds. Sodium chloride (1 g) was added to 50 mL of skin and seed extract and extracted three times with 20 mL of ethyl ether and three times with 20 mL of ethyl acetate. The organic fractions were combined, dehydrated with 2.5 g of sodium sulfate anhydrous, filtered through a 3 μm pore size cellulose filter, and evaporated to dryness under a gentle nitrogen gas stream at 35 °C. The solid residue was dissolved in 2 mL of methanol/water (1:1, v/v) and filtered through a 0.45 μm pore size nylon membrane, and then 30 μL was injected in the HPLC-DAD system according to the conditions described previously.[11,22] Separation was performed at 25 °C. Two mobile phases were employed for elution: A (water/acetic acid, 98:2, v/v) and B (water/acetonitrile/acetic acid, 78:20:2, v/v/v). The gradient profile was 0−55 min, 100−20% A and 0−80% B; 55−57 min, 20−10% A and 80−90% B; 57−70 min, 10% A and 90% B isocratic; 70−80 min, 10−0% A and 90−100% B; 80−125 min, 100% B isocratic; followed by a 100% methanol washing and re-equilibration of the column. The flow rate was 0.9 mL/min from 0 to 55 min and 1.0 mL/min from 55 to 125 min. Detection was performed by scanning from 210 to 360 nm

Table 3. General Physical and Chemical Analyses of Malbec Grapes from Altamira during Ripening (2008−2009)[a]

			mean ± SE (n = 3) for treatment			
parameter	year	DAF	C	T1	T2	T3
av wt per 100 berries (g)	2008	85[b]	139.6 ± 0.7 a	139.8 ± 2.6 a	148.4 ± 7.5 a	143.1 ± 1.1 a
		113	184.3 ± 6.5 a	182.1 ± 2.6 a	189.5 ± 3.6 a	188.2 ± 2.5 a
		154[c]	191.7 ± 3.0 a	189.4 ± 4.6 a	188.4 ± 5.6 a	191.6 ± 3.5 a
	2009	67[b]	133.3 ± 0.8 a	141.1 ± 2.7 a	129.5 ± 5.2 a	139.4 ± 2.3 a
		98	172.3 ± 3.0 a	173.1 ± 3.7 a	163.2 ± 3.9 a	170.0 ± 2.0 a
		121[c]	180.4 ± 6.7 a	182.9 ± 7.0 a	175.3 ± 3.2 a	187.3 ± 5.0 a
av wt per 100 skins (g)	2008	85[b]	19.7 ± 1.7 a	15.5 ± 0.8 a	19.8 ± 0.7 a	17.3 ± 0.6 a
		113	22.6 ± 1.6 a	21.6 ± 2.2 a	20.0 ± 1.1 a	17.6 ± 1.1 a
		154[c]	22.4 ± 2.1 a	20.6 ± 0.7 a	22.2 ± 1.2 a	24.0 ± 0.5 a
	2009	67[b]	16.5 ± 1.0 a	17.1 ± 0.6 a	15.9 ± 0.6 a	17.0 ± 0.5 a
		98	17.1 ± 1.7 a	15.9 ± 0.3 a	14.6 ± 0.1 a	15.7 ± 0.7 a
		121[c]	16.2 ± 0.3 a	17.5 ± 0.7 a	16.4 ± 0.6 a	17.7 ± 0.8 a
av wt per 100 seeds (g)	2008	85[b]	6.6 ± 0.2 ab	5.9 ± 0.3 a	6.9 ± 0.2 b	6.3 ± 0.1 ab
		113	5.3 ± 0.3 a	5.2 ± 0.1 a	5.4 ± 0.1 a	5.4 ± 0.2 a
		154[c]	5.7 ± 0.2 a	5.5 ± 0.1 a	5.3 ± 0.3 a	5.9 ± 0.2 a
	2009	67[b]	5.4 ± 0.1 a	5.8 ± 0.3 a	5.3 ± 0.2 a	5.6 ± 0.2 a
		98	5.4 ± 0.1 a	5.5 ± 0.2 a	5.0 ± 0.1 a	5.1 ± 0.1 a
		121[c]	5.4 ± 0.2 a	5.5 ± 0.6 a	5.2 ± 0.3 a	6.0 ± 0.3 a
soluble solids (°Brix)	2008	85[b]	13.6 ± 0.1 a	15.5 ± 0.2 b	13.8 ± 0.3 a	14.1 ± 0.2 a
		113	21.3 ± 0.7 a	23.1 ± 0.3 a	22.9 ± 0.7 a	22.0 ± 0.4 a
		154[c]	25.0 ± 0.4 a	26.7 ± 0.1 b	26.5 ± 0.3 b	26.3 ± 0.3 ab
	2009	67[b]	14.5 ± 0.3 ab	15.1 ± 0.1 b	15.0 ± 0.2 ab	13.8 ± 0.4 a
		98	23.1 ± 0.4 ab	24.6 ± 0.3 b	23.9 ± 0.1 ab	22.8 ± 0.1 a
		121[c]	24.5 ± 0.1 a	25.5 ± 0.1 c	25.0 ± 0.1 ab	24.9 ± 0.2 ab
titratable acidity (tartaric acid, g/L)	2008	85[b]	19.3 ± 0.4 b	18.0 ± 0.6 a	19.6 ± 0.1 b	19.1 ± 0.5 ab
		113	6.5 ± 0.1 c	5.2 ± 0.1 a	5.7 ± 0.1 ab	5.8 ± 0.3 b
		154[c]	4.1 ± 0.1 a	3.9 ± 0.3 a	4.0 ± 0.2 a	3.9 ± 0.1 a
	2009	67[b]	17.3 ± 0.3 a	17.9 ± 0.5 a	17.5 ± 0.4 a	17.9 ± 0.1 a
		98	5.0 ± 0.1 a	4.9 ± 0.1 a	4.8 ± 0.3 a	5.2 ± 0.2 a
		121[c]	3.3 ± 0.1 a	3.6 ± 0.1 a	3.4 ± 0.2 a	3.8 ± 0.2 a
pH	2008	85[b]	2.86 ± 0.03 a	2.86 ± 0.02 a	2.86 ± 0.02 a	2.80 ± 0.01 a
		113	3.24 ± 0.04 a	3.39 ± 0.02 b	3.30 ± 0.04 ab	3.22 ± 0.03 a
		154[c]	3.60 ± 0.05 a	3.71 ± 0.06 a	3.61 ± 0.01 a	3.63 ± 0.03 a
	2009	67[b]	2.89 ± 0.02 a	2.90 ± 0.01 a	2.90 ± 0.01 a	2.86 ± 0.01 a
		98	3.52 ± 0.03 a	3.59 ± 0.01 a	3.58 ± 0.03 a	3.53 ± 0.01 a
		121[c]	3.86 ± 0.04 a	3.92 ± 0.01 a	3.93 ± 0.01 a	3.89 ± 0.01 a

[a] Mean values followed by different letters in the same row indicate significant differences between yield treatments for the same sampling date (Tukey, p < 0,05). SE, standard error. DAF, days after flowering. Cluster thinning treatments: C, control; T1, early thinning; T2, veraison thinning; T3, late thinning. [b] Veraison. [c] Harvest time.

with an acquisition speed of 1 s. The identification of specific compounds was carried out by comparison of their spectra and retention times with those of standards. All of the individual phenolic compounds were confirmed by HPLC-DAD/ESI-MS as described by Monagas et al.[22] Quantitative determinations were made by using the external standard method with the commercial standards. The calibration curves were obtained by injection of standard solutions, under the same conditions as for the samples analyzed, over the range of concentrations observed ($R^2 \geq 0.94$). The compounds for which no standards were available were quantified with the curves of quercetin (dihydroflavonols), quercetin-3-glucoside (quercetin and flavonol glycosides), myricetin (myricetin glycosides), kaempferol-3-glucoside (kaempferol-3-galactoside), resveratrol (*trans*- and *cis*-resveratrol glucoside), caffeic acid (*trans*-fertaric acid), ethyl gallate (methyl gallate), and (+)-catechin (procyanidins). All of the analyses (including extraction) were performed in triplicate.

Statistical Analysis. Statistical analysis was carried out with Statgraphics Plus version 4.0 software (copyright 1994−1999, Statistical Graphics Corp., Warranton, VA). All of the results were tested for homogeneity of variance using Cochran's test and analyzed by one-way or multifactorial analysis of variance (ANOVA) and Tukey's multiple-range tests (TMRT). A $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

To our best knowledge, there is very scarce information in the literature about the phenolic composition of Malbec grapes. Therefore, our discussion will be mainly focused on a comparison between Malbec and other international red varieties.

Vine Vegetative Parameters. The effectiveness in yield reduction by thinning treatments in 2008 and 2009 is shown in Table 2. Yield components of Malbec vines, with the exception of cluster weight, were significantly affected by CT in both seasons, but we found no statistically significant differences among treatments T1, T2, and T3. In 2008, thinned vines averaged 38% less yield, with 44.2% fewer clusters per vine as compared to the control plants. Similar results were observed in 2009, with a 41.7 and 45.6% reductions of yield and number of clusters, respectively, in thinned plants. With respect to canopy surface area/yield ratio, CT caused decreases of 44.4 and 36.1% in 2008 and 2009, respectively, as compared to the control. However, in 2009 the annual yield was significantly lower and the canopy surface area/yield ratio was significantly higher than in 2008, possibly due to climatic differences between seasons, especially higher temperatures at flowering in November 2009 (Table 1).

General Analytical Parameters. The analysis of 100 berries of Malbec grapes during ripening showed no significant differences among treatments, with respect to the total weight, skin weight, and seed weight (Table 3). These results, with the absence of differences in the cluster weight (Table 2), indicate that no yield compensation occurred.[10,15] In general, for both seasons, the weight of the berries as well as that of the skins increased from veraison to harvest, whereas seeds showed a slight decrease in weight at the second sampling without continuing the trend toward harvesting. The pooled data of the grapes for the two years of study are shown in Table 4, which represents a two-way ANOVA using CT treatments and year as factors. When the factors "CT and year" were jointly analyzed, there was a significant effect of "year" for these evaluated general physical parameters.

In terms of berry composition, CT treatments led to significantly higher soluble solids concentration (°Brix) in both seasons, with a similar evolution among all treatments until harvest (Table 3). The enhancement of sugar accumulation could be linked to the increase of canopy surface area/yield ratio. Similar results are described for other grape varieties in other studies.[7,23] According to Petrie and Clingeleffer,[23] the observed increase in soluble solids caused by thinning treatments would be mostly due to the advancement of berry maturity rather than to the variation of the sugar accumulation rate. Other must parameters (pH, titratable acidity) were unaffected by CT in both seasons (Table 3), but there was a significant effect of factor "year" for pH (Table 4), with values slightly higher in 2009.

Phenolic Composition of Skins during Ripening. *Total Phenolic Composition.* Table 5 shows the results of total phenolic parameters for Malbec grape skins and seeds during ripening. At harvest time, there were no significant differences among

Table 4. Probability Values for Year and Cluster Thinning Treatments to the General Analytical Parameters and Total Phenolic Compounds in Malbec Grapes from Altamira (Pooled Data for 2008 and 2009 Seasons)

	p value[a] for factor		
parameter	year	CT treatment	year × CT[b]
berries			
wt per 100 berries	0.0486	0.9523	0.7971
soluble solids	0.8919	0.8495	0.9920
titratable acidity	0.5874	0.9985	0.9940
pH	0.0449	0.9412	0.9932
skins			
wt per 100 skins	<0.0001	0.5774	0.1803
total anthocyanins	0.0172	0.4832	0.9491
proanthocyanidins	0.7582	0.9175	0.9813
flavanols DMACH	0.0116	0.3053	0.8547
total phenols	0.0913	0.2661	0.6852
color intensity	0.3782	0.3782	0.9994
seeds			
wt per 100 seeds	0.0055	0.6800	0.1928
proanthocyanidins	0.0892	0.0055	0.2093
flavanols DMACH	0.4386	0.7481	0.8024
total phenols	0.8317	0.9945	0.9578

[a] Considered to be significant when $p < 0.05$. [b] Interaction effect between year and cluster thinning treatments.

treatments relating to total anthocyanins, proanthocyanidins, flavanols DMACH, total phenols, and color intensity. These results are in agreement with Da Silva et al.,[24] when yield of Malbec grapes from Brazil was reduced by 45%. However, in both seasons T1 resulted in a higher total anthocyanin concentration at veraison, as compared to the other treatments. This could possibly be the result of an increased availability of sugars (Table 3), which can promote enzyme activity.[25,26] Total anthocyanin content showed biosynthesis, for both growing seasons evaluated, from the first sampling date until reaching a maximum value at the second sampling, and then diminished toward harvest. A similar decline has been reported by different authors.[4,14,24,25] Fournand et al.[27] suggested that this decrease could be caused by the conversion of free anthocyanins into polymeric pigments. At harvest, the values for this parameter ranged from 3.9 to 4.6 mg/g of skins and from 3.2 to 4.5 mg/g of skins in 2008 and 2009, respectively, indicating a significant effect of the factor "year" on the anthocyanin content (Tables 4 and 5). The lower contents detected in 2009, particularly in control vines, could indicate that climatic conditions with high temperatures (>33 °C) between January and March might affect anthocyanin accumulation, through biosynthetic inhibition and degradation phenomena.[8,28] These concentrations are similar to those observed by Da Silva et al.[24] in Malbec berry skins from Brazil and higher than those reported by other authors in Cabernet Sauvignon and Carménère berry skins from Chile.[14,29]

Skin proanthocyanidins have been reported to increase in size during the later stages of ripening and react with pectins and anthocyanins, which can affect the mouthfeel and texture of red wines as well as color stability.[30] Due to the analytical method used, the results of this study reflect only quantitative changes without power assessed for the qualitative changes. In 2008, the

Table 5. Total Phenolic Analyses of Malbec Berry Skins and Seeds from Altamira during Ripening (2008−2009)[a]

parameter	year	DAF	mean ± SE (n = 3) for treatment C	T1	T2	T3
			Skins			
total anthocyanins (M3Gl, mg/g)	2008	85[b]	1.3 ± 0.1 ab	1.5 ± 0.1 b	1.1 ± 0.1 ab	0.9 ± 0.1 a
		113	4.6 ± 0.4 a	5.7 ± 0.2 ab	6.0 ± 0.2 b	4.9 ± 0.4 ab
		154[c]	4.3 ± 0.6 a	4.6 ± 0.2 a	4.6 ± 0.1 a	3.9 ± 0.2 a
	2009	67[b]	0.9 ± 0.1 a	1.6 ± 0.1 b	0.9 ± 0.1 a	1.0 ± 0.1 a
		98	2.5 ± 0.1 a	3.3 ± 0.5 a	3.8 ± 0.4 a	3.4 ± 0.1 a
		121[c]	3.3 ± 0.4 a	4.5 ± 0.5 a	3.5 ± 0.4 a	3.2 ± 0.2 a
proanthocyanidins (catechin, mg/g)	2008	85[b]	20.9 ± 2.7 a	18.4 ± 0.2 a	20.0 ± 2.6 a	15.6 ± 0.6 a
		113	9.5 ± 0.8 a	11.7 ± 2.3 a	11.8 ± 0.1 a	13.5 ± 0.5 a
		154[c]	7.7 ± 0.7 a	10.7 ± 0.9 a	8.5 ± 0.8 a	8.1 ± 0.6 a
	2009	67[b]	13.9 ± 1.3 a	10.3 ± 2.4 a	12.8 ± 2.9 a	12.5 ± 2.6 a
		98	13.0 ± 1.2 a	14.8 ± 0.7 a	16.7 ± 0.7 a	14.2 ± 1.4 a
		121[c]	10.8 ± 1.1 a	12.6 ± 0.5 a	10.4 ± 0.2 a	10.8 ± 1.4 a
flavanols DMACH (catechin, mg/g)	2008	85[b]	1.2 ± 0.1 a	1.4 ± 0.1 b	1.1 ± 0.1 a	1.2 ± 0.1 a
		113	1.2 ± 0.1 a	1.3 ± 0.3 a	1.3 ± 0.1 a	1.5 ± 0.3 a
		154[c]	1.0 ± 0.1 a	1.4 ± 0.1 a	0.9 ± 0.2 a	0.9 ± 0.1 a
	2009	67[b]	1.2 ± 0.1 a	1.2 ± 0.1 a	1.0 ± 0.1 a	1.0 ± 0.1 a
		98	1.6 ± 0.1 a	1.9 ± 0.1 a	2.0 ± 0.1 a	1.7 ± 0.2 a
		121[c]	1.2 ± 0.1 a	1.4 ± 0.2 a	1.2 ± 0.1 a	1.2 ± 0.1 a
total phenols (GAE, mg/g)	2008	85[b]	6.0 ± 0.1 a	8.3 ± 0.1 b	6.0 ± 0.3 a	6.8 ± 0.4 a
		113	9.1 ± 0.5 a	11.4 ± 2.2 a	11.6 ± 1.3 a	12.0 ± 1.2 a
		154[c]	10.5 ± 1.5 a	14.3 ± 0.8 a	11.2 ± 1.2 a	10.6 ± 0.4 a
	2009	67[b]	5.7 ± 0.3 a	5.7 ± 0.3 a	5.4 ± 0.2 a	5.2 ± 0.1 a
		98	9.8 ± 0.7 a	12.0 ± 0.5 a	12.4 ± 0.6 a	11.0 ± 0.9 a
		121[c]	9.3 ± 0.8 a	9.5 ± 0.6 a	9.6 ± 0.2 a	8.6 ± 0.6 a
color intensity ($A_{420} + A_{520} + A_{620}$) × 10	2008	85[b]	3.0 ± 0.1 a	3.2 ± 0.3 a	2.8 ± 0.1 a	2.7 ± 0.1 a
		113	6.2 ± 0.4 a	8.0 ± 0.4 a	8.0 ± 0.8 a	6.8 ± 0.7 a
		154[c]	6.8 ± 0.4 a	8.7 ± 1.0 a	8.1 ± 0.7 a	7.9 ± 0.1 a
	2009	67[b]	2.4 ± 0.2 ab	2.8 ± 0.2 b	2.2 ± 0.1 ab	2.4 ± 0.1 a
		98	6.4 ± 0.4 a	8.7 ± 0.7 b	8.0 ± 0.2 ab	7.1 ± 0.4 ab
		121[c]	5.5 ± 0.5 a	7.2 ± 0.1 a	7.1 ± 0.4 a	6.4 ± 0.6 a
			Seeds			
proanthocyanidins (catechin, mg/g)	2008	85[b]	123.0 ± 14.2 a	106.4 ± 9.0 a	134.7 ± 9.0 a	95.2 ± 2.2 a
		113	109.5 ± 2.0 a	113.2 ± 3.7 a	96.9 ± 9.0 a	101.3 ± 4.7 a
		154[c]	125.2 ± 3.6 a	120.2 ± 16.8 a	125.1 ± 7.5 a	96.8 ± 6.1 a
	2009	67[b]	116.9 ± 1.8 a	116.1 ± 4.9 a	116.1 ± 9.9 a	108.8 ± 3.6 a
		98	102.3 ± 3.4 a	105.3 ± 1.9 a	105.7 ± 2.4 a	98.7 ± 1.7 a
		121[c]	99.2 ± 3.0 a	102.3 ± 0.4 ab	112.5 ± 2.4 b	100.8 ± 3.5 ab
flavanols DMACH (catechin, mg/g)	2008	85[b]	17.1 ± 0.9 ab	17.5 ± 0.4 ab	19.8 ± 0.9 b	15.2 ± 0.6 a
		113	13.0 ± 1.5 a	13.9 ± 0.3 a	12.2 ± 0.3 a	12.3 ± 0.3 a
		154[c]	10.8 ± 0.6 ab	12.4 ± 0.5 b	10.9 ± 0.2 ab	10.1 ± 0.2 a
	2009	67[b]	17.5 ± 1.5 a	20.4 ± 0.2 a	20.1 ± 0.3 a	20.3 ± 1.1 a
		98	11.7 ± 0.2 a	12.7 ± 0.2 a	12.4 ± 0.2 a	12.5 ± 0.8 a
		121[c]	11.9 ± 0.2 a	11.3 ± 0.5 a	11.2 ± 0.6 a	11.1 ± 1.5 a

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Table 5. Continued

parameter	year	DAF	mean ± SE (n = 3) for treatment C	T1	T2	T3
total phenols (GAE, mg/g)	2008	85[b]	38.3 ± 0.7 a	40.0 ± 3.1 a	37.9 ± 2.5 a	43.2 ± 6.0 a
		113	34.4 ± 1.9 a	31.1 ± 1.7 a	30.0 ± 2.6 a	30.8 ± 0.8 a
		154[c]	26.7 ± 2.5 a	26.9 ± 1.7 a	29.9 ± 0.7 a	25.7 ± 2.3 a
	2009	67[b]	36.4 ± 0.5 a	35.6 ± 0.3 a	35.7 ± 1.6 a	35.4 ± 1.5 a
		98	31.2 ± 1.1 a	32.2 ± 0.4 a	32.0 ± 0.4 a	32.3 ± 0.4 a
		121[c]	28.6 ± 1.2 a	29.6 ± 0.5 ab	32.5 ± 1.0 b	29.8 ± 0.3 ab

[a] Mean values followed by different letters in the same row indicate significant differences between yield treatments for the same sampling date (Tukey, $p < 0.05$). SE, standard error. DAF, days after flowering. Cluster thinning treatments: C, control; T1, early thinning; T2, veraison thinning; T3, late thinning. [b] Veraison. [c] Harvest time.

Table 6. Individual Anthocyanins Quantified in 2008 Malbec Berry Skins from Altamira during Ripening[a]

compound	DAF	concentration (mg/kg of skins, mean ± SE) for treatment C	T1	T2	T3
delphinidin-3-glucoside	85[b]	175.5 ± 8.9 a	244.2 ± 16.3 b	172.0 ± 4.3 a	180.6 ± 8.8 a
	113	282.3 ± 6.9 a	455.0 ± 44.1 b	379.7 ± 17.2 ab	300.3 ± 32.2 a
	154[c]	197.4 ± 29.9 a	351.4 ± 46.0 b	309.6 ± 7.6 ab	257.5 ± 6.6 ab
cyanidin-3-glucoside	85[b]	22.9 ± 3.1 a	52.6 ± 4.1 b	22.3 ± 2.4 a	27.7 ± 1.7 a
	113	24.1 ± 0.9 a	43.4 ± 6.6 a	37.3 ± 0.9 a	28.3 ± 6.0 a
	154[c]	25.9 ± 2.9 a	47.7 ± 4.2 c	39.2 ± 1.8 bc	33.8 ± 0.8 ab
petunidin-3-glucoside	85[b]	159.6 ± 7.8 a	207.6 ± 12.2 b	156.9 ± 4.1 a	161.7 ± 5.9 a
	113	316.0 ± 5.0 a	483.0 ± 41.7 b	412.5 ± 16.1 ab	335.4 ± 32.4 a
	154[c]	230.9 ± 32.2 a	393.5 ± 45.5 b	348.2 ± 5.4 ab	290.6 ± 5.6 ab
peonidin-3-glucoside	85[b]	67.3 ± 7.1 a	125.0 ± 7.4 b	65.1 ± 5.9 a	75.4 ± 1.6 a
	113	119.1 ± 3.8 a	208.7 ± 26.7 b	175.0 ± 11.1 ab	134.7 ± 20.3 ab
	154[c]	163.5 ± 14.0 a	246.1 ± 17.8 b	218.1 ± 6.1 ab	194.5 ± 12.5 ab
malvidin-3-glucoside	85[b]	592.2 ± 31.5 a	663.9 ± 32.4 a	587.5 ± 12.0 a	561.2 ± 15.7 a
	113	1668.8 ± 46.8 a	2054.0 ± 70.4 b	1695.9 ± 60.1 a	1617.5 ± 68.7 a
	154[c]	1276.7 ± 103.0 a	1688.3 ± 126.1 b	1569.2 ± 41.3 ab	1419.2 ± 29.5 ab
total glucosylated	85[b]	1017.5 ± 54.4 a (79.8[d])	1293.4 ± 70.9 b (80.4)	1003.7 ± 25.5 a (79.3)	1006.6 ± 27.0 a (80.4)
	113	2410.3 ± 33.4 a (76.4)	3244.2 ± 184.2 b (75.3)	2700.3 ± 104.4 ab (74.8)	2416.2 ± 158.1 a (75.0)
	154[c]	1894.4 ± 181.4 a (75.3)	2727.0 ± 239.0 b (76.8)	2484.4 ± 56.3 ab (76.0)	2195.6 ± 30.9 ab (75.4)
delphinidin-3-(6″-acetyl)glucoside	85[b]	25.3 ± 2.9 a	40.0 ± 2.5 b	26.2 ± 1.0 a	27.0 ± 1.2 a
	113	37.2 ± 0.4 a	65.8 ± 7.7 b	59.2 ± 2.4 ab	44.8 ± 7.4 ab
	154[c]	30.3 ± 4.9 a	55.2 ± 6.5 b	48.1 ± 1.0 ab	39.2 ± 1.3 ab
cyanidin-3-(6″-acetyl)glucoside	85[b]	3.7 ± 0.6 a	7.8 ± 0.4 b	3.8 ± 0.2 a	3.9 ± 0.2 a
	113	8.5 ± 1.4 a	13.5 ± 2.9 a	14.1 ± 0.4 a	12.7 ± 3.1 a
	154[c]	9.5 ± 3.0 a	17.8 ± 1.4 b	15.7 ± 0.9 ab	13.9 ± 0.5 ab
petunidin-3-(6″-acetyl)glucoside	85[b]	27.7 ± 2.6 a	41.3 ± 2.0 b	29.0 ± 0.7 a	29.0 ± 0.7 a
	113	49.7 ± 0.4 a	81.5 ± 7.4 b	73.4 ± 3.3 ab	58.0 ± 8.7 ab
	154[c]	35.4 ± 5.7 a	61.9 ± 7.5 b	55.7 ± 1.2 ab	47.1 ± 2.1 ab
peonidin-3-(6″-acetyl)glucoside	85[b]	23.0 ± 1.5 a	33.4 ± 2.3 b	23.3 ± 0.4 a	23.3 ± 0.9 a
	113	40.3 ± 1.5 a	68.1 ± 5.3 b	59.5 ± 4.9 ab	47.5 ± 6.4 ab
	154[c]	38.1 ± 6.1 a	53.7 ± 4.8 a	48.5 ± 1.4 a	44.3 ± 1.2 a
malvidin-3-(6″-acetyl)glucoside	85[b]	93.8 ± 6.8 ab	111.8 ± 4.4 b	98.3 ± 2.0 ab	89.5 ± 2.9 a
	113	331.4 ± 14.4 a	450.7 ± 7.4 b	378.2 ± 21.6 ab	340.6 ± 18.1 a
	154[c]	264.7 ± 27.5 a	339.2 ± 27.6 a	320.8 ± 1.8 a	300.4 ± 5.5 a
total acetylated	85[b]	173.4 ± 14.2 a (13.6)	234.3 ± 11.1 b (14.6)	180.7 ± 3.5 a (14.3)	172.8 ± 3.3 a (13.8)
	113	467.1 ± 17.0 a (14.8)	679.6 ± 30.1 b (15.8)	584.5 ± 32.1 ab (16.2)	503.8 ± 43.2 a (15.6)
	154[c]	378.0 ± 45.3 a (15.0)	527.7 ± 47.7 b (14.9)	488.8 ± 3.8 ab (15.0)	444.8 ± 4.1 ab (15.3)

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Table 6. Continued

compound	DAF	concentration (mg/kg of skins, mean ± SE) for treatment C	T1	T2	T3
cyanidin-3-(6″-*p*-coumaroyl)glucoside	85[b]	2.2 ± 0.2 a	4.0 ± 0.4 b	2.1 ± 0.2 a	2.5 ± 0.2 a
	113	2.0 ± 0.1 a	4.0 ± 0.6 b	3.6 ± 0.3 ab	2.5 ± 0.4 ab
	154[c]	1.7 ± 0.3 a	3.1 ± 0.4 b	2.8 ± 0.2 ab	2.26 ± 0.02 ab
malvidin-3-(6″-caffeoyl)glucoside	85[b]	0.7 ± 0.1 a	0.8 ± 0.1 a	0.47 ± 0.02 a	0.6 ± 0.1 a
	113	4.3 ± 0.3 a	5.8 ± 0.3 b	5.0 ± 0.4 ab	4.7 ± 0.2 ab
	154[c]	5.8 ± 1.1 a	6.4 ± 0.3 a	7.1 ± 0.6 a	5.8 ± 0.6 a
petunidin-3-(6″-*p*-coumaroyl)glucoside	85[b]	13.9 ± 0.3 a	15.2 ± 1.2 a	13.6 ± 0.2 a	13.0 ± 0.5 a
	113	27.6 ± 1.9 a	42.8 ± 1.9 b	38.2 ± 3.7 ab	31.1 ± 2.7 ab
	154[c]	19.5 ± 3.1 a	28.4 ± 4.1 a	27.2 ± 0.9 a	23.6 ± 0.4 a
malvidin-3-(6″-*p*-coumaroyl)glucoside cis	85[b]	4.6 ± 0.2 a	3.4 ± 0.3 a	4.3 ± 0.4 a	3.6 ± 0.1 a
	113	10.4 ± 1.1 a	10.3 ± 1.2 a	8.3 ± 0.6 a	10.3 ± 0.4 a
	154[c]	5.4 ± 0.4 a	5.4 ± 0.5 a	5.2 ± 0.1 a	5.6 ± 0.1 a
peonidin-3-(6″-*p*-coumaroyl)glucoside	85[b]	6.0 ± 0.3 a	8.4 ± 0.6 b	5.9 ± 0.3 a	6.0 ± 0.1 a
	113	14.9 ± 0.9 a	25.3 ± 2.0 b	22.1 ± 2.1 ab	17.8 ± 2.0 ab
	154[c]	23.2 ± 2.5 a	29.4 ± 2.6 a	28.3 ± 0.9 a	25.7 ± 2.0 a
malvidin-3-(6″-*p*-coumaroyl)glucoside trans	85[b]	56.1 ± 2.2 a	48.8 ± 4.1 a	55.5 ± 2.1 a	47.1 ± 1.1 a
	113	219.8 ± 18.9 a	295.6 ± 4.3 b	249.5 ± 20.0 ab	236.6 ± 12.9 ab
	154[c]	187.9 ± 23.2 a	225.4 ± 24.4 a	224.2 ± 4.1 a	209.3 ± 5.4 a
total coumaroylated	85[b]	82.8 ± 2.8 a (6.5)	79.7 ± 6.3 a (5.0)	81.4 ± 2.7 a (6.4)	72.2 ± 1.5 a (5.8)
	113	274.7 ± 22.7 a (8.7)	378.1 ± 4.3 b (8.8)	321.7 ± 26.6 ab (8.9)	298.4 ± 18.1 ab (9.3)
	154[c]	237.7 ± 29.4 a (9.4)	291.7 ± 31.9 a (8.2)	287.7 ± 6.0 a (8.8)	266.4 ± 7.1 a (9.1)
total cinnamoylated	85[b]	83.5 ± 2.8 a (6.6)	80.5 ± 6.4 a (5.0)	81.9 ± 2.7 a (6.5)	72.8 ± 1.5 a (5.8)
	113	279.0 ± 23.1 a (8.8)	383.9 ± 4.4 b (8.9)	326.7 ± 26.7 ab (9.0)	303.1 ± 18.2 ab (9.4)
	154[c]	243.5 ± 30.5 a (9.7)	298.1 ± 32.1 a (8.4)	294.8 ± 6.4 a (9.0)	272.2 ± 7.2 a (9.3)
total anthocyanins	85[b]	1274.4 ± 69.8 a	1608.2 ± 88.2 b	1266.3 ± 29.7 a	1252.3 ± 31.5 a
	113	3156.4 ± 70.1 a	4307.7 ± 216.7 b	3611.5 ± 162.8 ab	3223.1 ± 218.0 a
	154[c]	2516.0 ± 254.6 a	3552.8 ± 318.6 b	3268.0 ± 53.6 ab	2912.6 ± 41.9 ab

[a] Average of three replicates followed by different letters in the same row indicate significant differences between treatments for each compound and date (Tukey, $p < 0,05$). SE, standard error. DAF, days after flowering. Cluster thinning treatments: C, control; T1, early thinning; T2, veraison thinning; T3, late thinning. [b] Veraison. [c] Harvest time. [d] Relatioship (%) between anthocyanin derivatives by acylation and total anthocyanins.

results showed a decrease during ripening, whereas in 2009 there was a slight increase toward the second sampling and then a decrease until harvest. Some authors have suggested that this decrease is caused by reduced extractability as a result of tannin being bound to other cellular components.[30] At harvest, total proanthocyanidin content ranged between 7.7 and 10.7 mg/g in 2008 and between 10.4 and 12.6 mg/g in 2009 (Table 5). These results are in agreement with those determined by others authors[14] in Cabernet Sauvignon and Carménère berry skins.

The remaining parameters (total phenols, flavanols DMACH, and color intensity) followed the same trend observed for anthocyanins in both seasons. In the particular case of flavanols DMACH, there was a significant effect of the factor "year" (Table 4), with values slightly higher in 2009.

HPLC Anthocyanin Profile of Grape Skins. The identified and quantified compounds in Malbec skins were grouped according to acylation (nonacylated glucosides, acetyl-glucosides, and cinnamoyl-glucosides) and anthocyanidin (delphinidins, cyanidins, petunidins, peonidins, and malvidins) characteristics. Cinnamoyl-glucosides included both *p*-coumaroyl and caffeoyl anthocyanins. The results for these compounds in both seasons are presented in Tables 6 and 7. During the ripening period, independent of treatment, cyanidin-3-glucoside, cyanidin-3-(6″-acetyl)glucoside, and peonidin-3-(6″-*p*-coumaroyl)glucoside in 2008 and only cyanidin-3-(6″-acetyl)glucoside in 2009 increased from veraison to harvest, whereas the remaining anthocyanins showed the same pattern of evolution observed for total anthocyanins. Nonacylated glucosides were the most abundant group as compared with the acylated forms, and malvidin was the main anthocyanidin, in accordance with many other cultivars that had prevalence of 3′,5′-substituted anthocyanins,[31] including Malbec wines.[11] Predominance of 3′,5′-substituted anthocyanins was associated with higher ratios of flavonoid 3′,5′-hydroxylase (F3′5′H) to flavonoid 3′-hydroxylase (F3′H) transcription activity, and higher levels of *O*-methyltransferase (OMT) transcripts were observed in berries that accumulated methoxylated forms of anthocyanins more abundantly.[31,32] Considering the acylated derivatives, Malbec skins presented a higher proportion of acetyl-glucosides than of coumaroyl-glucosides (Tables 6 and 7), a pattern similar to that observed in Malbec and Cabernet Sauvignon wines.[11,21] When analyzing the distribution of anthocyanidins, we observed the same profile previously found in Malbec wines.[11]

At harvest, the berry skins from CT treatments had higher contents of nonacylated glucosides and acetylated and cinnamoylated derivatives in 2008 compared with 2009. In 2008, there was a significant effect of CT on the anthocyanin composition.

Table 7. Individual Anthocyanins Quantified in 2009 Malbec Berry Skins from Altamira during Ripening[a]

		concentration (mg/kg of skins, mean ± SE) for treatment			
compound	DAF	C	T1	T2	T3
delphinidin-3-glucoside	67[b]	109.2 ± 13.6 a	116.5 ± 10.9 a	88.2 ± 7.2 a	87.2 ± 4.2 a
	98	192.9 ± 13.9 ab	278.1 ± 26.4 ab	280.7 ± 25.1 b	174.0 ± 25.4 a
	121[c]	147.0 ± 22.7 a	176.9 ± 7.2 a	156.4 ± 13.6 a	124.1 ± 13.9 a
cyanidin-3-glucoside	67[b]	14.5 ± 3.3 a	19.5 ± 2.9 a	10.5 ± 1.7 a	10.5 ± 1.0 a
	98	15.5 ± 2.2 a	30.6 ± 3.4 b	28.9 ± 3.4 ab	16.9 ± 2.9 ab
	121[c]	12.4 ± 2.7 a	20.0 ± 0.5 b	17.4 ± 1.2 ab	12.4 ± 1.3 a
petunidin-3-glucoside	67[b]	119.4 ± 13.4 a	123.5 ± 10.1 a	100.6 ± 6.8 a	100.1 ± 2.3 a
	98	236.4 ± 17.9 ab	333.0 ± 28.0 ab	334.9 ± 25.7 b	221.1 ± 27.1 a
	121[c]	196.0 ± 27.4 a	230.3 ± 7.6 a	211.9 ± 13.6 a	169.2 ± 15.9 a
peonidin-3-glucoside	67[b]	58.6 ± 7.7 a	67.1 ± 6.5 a	45.0 ± 5.5 a	45.4 ± 0.9 a
	98	102.1 ± 11.9 ab	167.9 ± 14.5 c	163.1 ± 12.4 bc	95.1 ± 15.0 a
	121[c]	81.6 ± 16.7 ab	120.0 ± 1.9 b	107.6 ± 5.1 ab	75.1 ± 6.7 a
malvidin-3-glucoside	67[b]	567.6 ± 40.4 a	525.9 ± 30.3 a	470.3 ± 22.9 a	483.1 ± 12.6 a
	98	1465.8 ± 137.1 a	1764.4 ± 77.5 a	1842.9 ± 58.0 a	1405.3 ± 119.3 a
	121[c]	1457.0 ± 115.5 a	1363.1 ± 38.6 a	1417.9 ± 42.8 a	1233.0 ± 49.8 a
total glucosylated	67[b]	869.3 ± 78.3 a (79.6[d])	852.5 ± 57.7 a (79.9)	714.5 ± 43.8 a (78.9)	726.4 ± 4.8 a (79.4)
	98	2012.7 ± 174.3 ab (76.4)	2574.0 ± 148.5 ab (76.1)	2650.6 ± 121.7 b (76.8)	1912.4 ± 185.1 a (78.6)
	121[c]	1894.0 ± 184.1 a (73.9)	1910.2 ± 45.2 a (75.3)	1911.2 ± 69.7 a (75.1)	1613.8 ± 82.8 a (74.2)
delphinidin-3-(6″-acetyl)glucoside	67[b]	17.4 ± 2.6 a	19.6 ± 1.8 a	14.7 ± 1.5 a	12.1 ± 1.8 a
	98	24.6 ± 4.4 a	43.1 ± 4.0 b	40.3 ± 4.5 ab	24.7 ± 3.2 ab
	121[c]	22.7 ± 4.0 a	30.8 ± 2.1 a	26.6 ± 1.9 a	21.7 ± 2.3 a
cyanidin-3-(6″-acetyl)glucoside	67[b]	5.1 ± 0.9 a	5.9 ± 0.6 a	4.5 ± 0.6 a	3.3 ± 0.7 a
	98	8.3 ± 2.5 a	15.2 ± 0.4 a	12.9 ± 0.8 a	10.0 ± 1.4 a
	121[c]	14.1 ± 1.5 a	15.7 ± 1.7 a	15.6 ± 0.4 a	14.0 ± 1.1 a
petunidin-3-(6″-acetyl)glucoside	67[b]	22.0 ± 2.6 a	23.8 ± 1.9 a	19.4 ± 1.6 a	17.6 ± 0.9 a
	98	37.3 ± 3.8 a	59.7 ± 3.3 b	53.1 ± 5.5 ab	34.3 ± 4.2 a
	121[c]	33.4 ± 5.4 a	42.8 ± 3.6 a	38.6 ± 3.0 a	32.9 ± 3.3 a
peonidin-3-(6″-acetyl)glucoside	67[b]	16.1 ± 1.4 a	17.0 ± 1.2 a	13.4 ± 0.6 a	13.5 ± 0.8 a
	98	31.9 ± 2.8 ab	48.2 ± 2.9 c	45.0 ± 3.0 bc	25.6 ± 3.6 a
	121[c]	28.5 ± 3.4 a	35.0 ± 2.9 a	31.2 ± 2.9 a	26.5 ± 1.9 a
malvidin-3-(6″-acetyl)glucoside	67[b]	98.3 ± 5.7 a	90.5 ± 5.3 a	83.4 ± 3.2 a	83.6 ± 2.2 a
	98	293.5 ± 27.8 ab	363.1 ± 8.7 b	369.5 ± 8.4 b	260.4 ± 19.0 a
	121[c]	310.4 ± 24.1 a	281.5 ± 19.0 a	287.8 ± 10.5 a	254.2 ± 9.0 a
total acetylated	67[b]	159.0 ± 13.0 a (14.5)	156.7 ± 10.0 a (14.7)	135.3 ± 7.4 a (15.0)	130.2 ± 3.2 a (14.2)
	98	395.6 ± 36.1 a (15.0)	529.3 ± 18.7 b (15.7)	520.7 ± 22.1 b (15.1)	354.9 ± 30.3 a (14.6)
	121[c]	409.2 ± 37.9 a (16.0)	405.8 ± 28.0 a (16.0)	399.9 ± 14.3 a (15.7)	349.3 ± 17.1 a (16.1)
cyanidin-3-(6″-*p*-coumaroyl)glucoside	67[b]	1.1 ± 0.2 a	1.5 ± 0.2 a	1.1 ± 0.1 a	1.2 ± 0.2 a
	98	1.2 ± 0.1 ab	2.3 ± 0.3 c	2.1 ± 0.2 bc	0.8 ± 0.1 a
	121[c]	1.0 ± 0.2 ab	1.6 ± 0.1 b	1.1 ± 0.1 ab	0.8 ± 0.1 a
malvidin-3-(6″-caffeoyl)glucoside	67[b]	1.30 ± 0.03 a	1.12 ± 0.03 a	1.2 ± 0.1 a	1.2 ± 0.2 a
	98	9.2 ± 2.7 a	8.4 ± 1.4 a	7.8 ± 1.0 a	5.3 ± 0.3 a
	121[c]	9.3 ± 0.6 a	7.2 ± 0.5 a	11.1 ± 1.6 a	7.8 ± 0.4 a
petunidin-3-(6″-*p*-coumaroyl)glucoside	67[b]	9.3 ± 0.4 a	9.0 ± 0.6 a	8.1 ± 0.4 a	8.5 ± 0.6 a
	98	20.1 ± 1.8 ab	26.8 ± 2.1 b	26.8 ± 1.4 b	14.6 ± 1.6 a
	121[c]	19.5 ± 1.9 a	18.6 ± 1.0 a	17.8 ± 1.3 a	15.5 ± 1.2 a
malvidin-3-(6″-*p*-coumaroyl)glucoside *cis*	67[b]	4.2 ± 0.1 a	3.2 ± 0.2 a	3.6 ± 0.5 a	3.7 ± 0.1 a
	98	8.5 ± 1.2 a	8.0 ± 0.6 a	8.8 ± 0.5 a	6.3 ± 0.7 a
	121[c]	8.3 ± 0.8 b	5.0 ± 0.6 a	6.1 ± 0.6 ab	6.3 ± 0.2 ab
peonidin-3-(6″-*p*-coumaroyl)glucoside	67[b]	4.6 ± 0.4 a	4.7 ± 0.3 a	3.6 ± 0.2 a	3.9 ± 0.2 a
	98	11.1 ± 1.2 a	18.3 ± 0.6 b	17.5 ± 1.2 b	8.0 ± 1.3 a
	121[c]	12.9 ± 2.3 a	16.2 ± 1.4 a	15.5 ± 0.7 a	11.2 ± 0.5 a

Table 7. Continued

compound	DAF	concentration (mg/kg of skins, mean ± SE) for treatment C	T1	T2	T3
malvidin-3-(6″-*p*-coumaroyl)glucoside *trans*	67[b]	44.0 ± 1.7 a	37.7 ± 1.8 a	37.7 ± 3.1 a	39.3 ± 0.5 a
	98	175.4 ± 15.8 ab	213.8 ± 8.2 b	219.1 ± 4.3 b	129.8 ± 14.4 a
	121[c]	209.1 ± 13.9 a	172.8 ± 9.6 a	183.9 ± 5.7 a	169.2 ± 5.1 a
total coumaroylated	67[b]	63.2 ± 2.5 a (5.8)	56.2 ± 2.8 a (5.3)	54.1 ± 4.0 a (6.0)	56.6 ± 1.0 a (6.2)
	98	216.4 ± 19.2 ab (8.2)	269.3 ± 11.7 b (8.0)	274.3 ± 6.1 b (7.9)	159.6 ± 17.7 a (6.6)
	121[c]	250.8 ± 17.4 a (9.8)	214.2 ± 12.3 a (8.4)	224.4 ± 7.4 a (8.8)	203.0 ± 6.9 a (9.3)
total cinnamoylated	67[b]	64.5 ± 2.5 a (5.9)	57.3 ± 2.8 a (5.4)	55.3 ± 4.0 a (6.1)	57.8 ± 0.9 a (6.3)
	98	225.6 ± 21.7 ab (8.6)	277.7 ± 12.6 b (8.2)	282.1 ± 6.4 b (8.2)	164.9 ± 17.5 a (6.8)
	121[c]	260.1 ± 17.9 a (10.1)	221.4 ± 12.8 a (8.7)	235.5 ± 7.0 a (9.2)	210.8 ± 7.3 a (9.7)
total anthocyanins	67[b]	1092.7 ± 93.4 a	1066.6 ± 69.5 a	905.2 ± 48.1 a	914.3 ± 6.2 a
	98	2633.9 ± 231.1 ab	3381.0 ± 179.0 b	3453.4 ± 149.2 b	2432.3 ± 226.5 a
	121[c]	2563.2 ± 238.2 a	2537.4 ± 79.0 a	2546.6 ± 90.5 a	2173.9 ± 107.1 a

[a] Average of three replicates followed by different letters in the same row indicate significant differences between treatments for each compound and date (Tukey, $p < 0,05$). SE, standard error. DAF, days after flowering. Cluster thinning treatments: C, control; T1, early thinning; T2, veraison thinning; T3, late thinning. [b] Veraison. [c] Harvest time. [d] Relatioship (%) between anthocyanin derivatives by acylation and total anthocyanins.

Early thinning (T1) was the most significant, generating 44.0, 39.6, and 41.2% more glucosylated, acetylated, and total anthocyanins, respectively, as compared with the control (C). In the treatments at the most advanced stages of the ripening period (T2 and T3) the effect on the anthocyanin composition was lower. It has to be emphasized that this might be of importance because acetylated anthocyanins confer high stability to color intensity and threshold for visual detection in red wines and, therefore, better color quality.[15] Also of importance for color is the proportion of coumaroylated derivatives, but data from Tables 6 and 7 suggest that these compounds are not affected by yield. With respect to individual compounds, we observed a significant effect of CT for 2008 on monoglucosides, acetylglucosides of delphinidin, cyanidin, and petunidin, and cyanidin-3-(6″-*p*-coumaroyl)glucoside, whereas in 2009 CT produced a significant effect only on the cyanidin-3-glucoside, peonidin-3-glucoside, and cyanidin-3-(6″-acetyl)glucoside contents (Tables 6 and 7). Increases in individual anthocyanin contents of berries following cluster removal have also been reported in other grape varieties by several authors.[8,15]

The pooled data of individual phenolic compounds of skins and seeds for the two years of study are shown in Table 8, which represents a two-way ANOVA using "CT treatments and year" as factors. The differences observed in the composition of anthocyanins for both seasons could be explained by a differential content of primary metabolites (mainly sugars) required for anthocyanin biosynthesis. In 2008, with a lower canopy surface area/yield ratio in control vines, there may be some sugar limitation, and therefore the CT would alter the sink-source relationship affecting the anthocyanin-related gene expression,[25,26,29] whereas in 2009 with an increased canopy surface area/yield ratio, and thus no apparent sugar limitation, thinning did not affect the biosynthesis of anthocyanins. There was even a decline of its content, possibly due to a decrease in the expression of dihydroflavonol reductase gene.[33] Moreover, regardless of the thinning treatments, sun exposure may differentially affect the biosynthesis of anthocyanins in berries. The light significantly increases their accumulation and expression of biosynthetic genes.[29] Conversely, the temperature has a negative influence.

Table 8. Probability Values for Year and Cluster Thinning Treatments to the Phenolic Compounds in Malbec Berries Skins and Seeds from Altamira (Pooled Data for 2008 and 2009 Seasons)

compound	*p* value[a] for factor year	CT treatment	year × CT[b]
skins			
anthocyanins glucosylated	0.0256	0.2227	0.8043
anthocyanins acetylated	0.0548	0.2269	0.8135
anthocyanins cinnamoylated	0.0457	0.6603	0.8622
delphinidins	<0.0001	0.0014	0.3443
cyanidins	<0.0001	<0.0001	0.0844
petunidins	0.0001	0.0196	0.6402
peonidins	<0.0001	0.0050	0.6839
malvidins	0.3091	0.6211	0.9009
total anthocyanins	0.0303	0.2577	0.8162
hydroxybenzoic acids/derivatives	0.1914	0.7050	0.9457
hydroxycinnamic acids/derivatives	<0.0001	0.0208	0.0215
stilbenes	0.0002	0.8856	0.9441
flavanols	0.0323	0.5862	0.9883
flavonols	0.0673	0.4590	0.9257
dihydroflavonols	<0.0001	0.9032	0.9592
total phenolic compounds	0.0023	0.7441	0.9625
seeds			
total phenolic compounds	0.3947	0.7972	0.7787

[a] Considered to be significant when $p < 0.05$. [b] Interaction effect between year and cluster thinning treatments.

Mori et al.[28] demonstrated that high temperature increases anthocyanin degradation in grape skin, together with a decrease in expression of flavonoid biosynthetic and *MYBA* genes.

HPLC Nonanthocyanin Profile of Grape Skins. The identified and quantified low molecular weight phenolic compounds in Malbec skins during ripening (2008−2009) were grouped in nonflavonoids (hydroxybenzoic and hydroxycinnamic acids/derivatives, stilbenes) and flavonoids (flavanols, flavonols, and

Table 9. Low Molecular Weight Phenolic Compounds Quantified in Malbec Berry Skins, from Altamira, during Ripening (2008−2009)[a]

			concentration (mg/kg of skins, mean ± SE) for treatment			
compound	year	DAF	C	T1	T2	T3
total hydroxybenzoic acids/derivatives	2008	85[b]	13.8 ± 1.1 ab (4.0[d])	17.3 ± 0.9 b (3.3)	11.9 ± 0.3 a (4.4)	14.0 ± 1.0 ab (3.9)
		113	21.4 ± 1.8 a (2.8)	25.1 ± 4.5 a (2.9)	27.5 ± 3.2 a (2.9)	28.0 ± 3.4 a (3.0)
		154[c]	25.4 ± 2.1 a (2.5)	26.8 ± 1.7 a (2.4)	25.8 ± 2.1 a (2.4)	23.2 ± 1.6 a (2.3)
	2009	67[b]	17.7 ± 0.9 a (2.8)	17.0 ± 1.0 a (2.6)	17.1 ± 0.6 a (3.0)	16.2 ± 0.6 a (2.8)
		98	21.7 ± 1.0 a (1.7)	26.8 ± 1.8 ab (1.7)	28.8 ± 1.1 b (1.8)	25.7 ± 2.0 ab (1.9)
		121[c]	16.8 ± 0.6 a (1.6)	16.5 ± 0.7 a (1.4)	17.2 ± 0.2 a (1.7)	16.5 ± 0.6 a (1.8)
total hydroxycinnamic acids/derivatives	2008	85[b]	7.1 ± 0.7 a (2.1)	10.1 ± 0.7 b (1.9)	6.6 ± 0.4 a (2.4)	7.8 ± 0.2 ab (2.2)
		113	5.8 ± 0.1 a (0.8)	7.6 ± 1.1 a (0.9)	7.5 ± 0.7 a (0.8)	7.8 ± 0.9 a (0.8)
		154[c]	8.1 ± 0.8 a (0.8)	9.5 ± 0.6 a (0.9)	9.0 ± 0.6 a (0.9)	8.2 ± 0.5 a (0.8)
	2009	67[b]	2.9 ± 0.1 a (0.5)	2.9 ± 0.2 a (0.4)	3.2 ± 0.1 a (0.6)	3.0 ± 0.1 a (0.5)
		98	3.1 ± 0.2 a (0.2)	3.5 ± 0.1 ab (0.2)	3.8 ± 0.1 b (0.2)	3.5 ± 0.2 ab (0.3)
		121[c]	3.3 ± 0.1 a (0.3)	3.0 ± 0.1 a (0.3)	3.2 ± 0.1 a (0.3)	2.9 ± 0.1 a (0.3)
total stilbenes	2008	85[b]	1.1 ± 0.1 a (0.3)	1.7 ± 0.1 b (0.3)	1.0 ± 0.1 a (0.4)	1.4 ± 0.1 ab (0.4)
		113	4.1 ± 0.3 a (0.5)	4.8 ± 0.5 a (0.6)	4.6 ± 0.6 a (0.5)	5.0 ± 0.4 a (0.5)
		154[c]	7.2 ± 0.7 a (0.7)	9.4 ± 1.5 a (0.9)	9.3 ± 1.2 a (0.9)	9.0 ± 0.7 a (0.9)
	2009	67[b]	3.5 ± 0.1 a (0.6)	3.6 ± 0.3 a (0.5)	3.3 ± 0.2 a (0.6)	3.2 ± 0.2 a (0.6)
		98	12.2 ± 1.2 a (0.9)	13.1 ± 0.5 a (0.8)	13.7 ± 0.7 a (0.9)	11.5 ± 1.1 a (0.8)
		121[c]	9.1 ± 0.6 a (0.8)	10.2 ± 1.0 a (0.9)	9.1 ± 0.4 a (0.9)	8.6 ± 0.1 a (0.9)
total flavanols	2008	85[b]	71.2 ± 5.7 ab (20.8)	95.9 ± 3.3 b (18.2)	54.4 ± 2.3 a (19.9)	72.5 ± 0.8 ab (20.2)
		113	154.6 ± 4.7 a (20.2)	194.7 ± 26.5 a (22.9)	191.5 ± 16.2 a (20.3)	202.5 ± 22.1 a (21.6)
		154[c]	228.8 ± 16.2 a (22.4)	246.7 ± 7.8 a (22.4)	248.0 ± 25.4 a (23.3)	211.8 ± 20.3 a (21.0)
	2009	67[b]	114.0 ± 6.8 a (18.2)	123.6 ± 12.3 a (18.6)	104.1 ± 6.0 a (18.4)	103.2 ± 6.9 a (18.0)
		98	152.3 ± 4.9 a (11.7)	189.5 ± 4.3 bc (12.2)	198.8 ± 4.8 c (12.4)	166.1 ± 10.1 ab (12.0)
		121[c]	112.1 ± 5.1 a (10.4)	136.6 ± 6.6 b (11.9)	103.5 ± 5.3 a (10.5)	100.8 ± 2.4 a (11.1)
total flavonols	2008	85[b]	148.0 ± 16.3 ab (43.3)	218.2 ± 18.6 b (41.4)	116.3 ± 2.6 a (42.4)	156.6 ± 19.4 ab (43.7)
		113	215.7 ± 21.0 a (28.2)	245.9 ± 34.5 a (28.9)	276.2 ± 37.3 a (29.2)	251.2 ± 37.5 a (26.8)
		154[c]	288.6 ± 22.3 a (28.3)	318.0 ± 10.9 a (28.9)	302.4 ± 24.3 a (28.5)	292.6 ± 15.2 a (29.0)
	2009	67[b]	180.7 ± 9.1 ab (28.8)	200.5 ± 10.0 b (30.2)	165.2 ± 4.1 a (29.2)	171.3 ± 2.2 ab (29.9)
		98	355.8 ± 30.7 a (27.2)	410.0 ± 25.7 a (26.4)	442.5 ± 33.0 a (27.6)	371.2 ± 24.2 a (26.8)
		121[c]	240.8 ± 6.7 ab (22.3)	291.9 ± 10.5 c (25.5)	257.2 ± 12.4 bc (26.0)	208.3 ± 4.7 a (22.8)
total dihydroflavonols	2008	85[b]	100.7 ± 7.6 a (29.4)	184.3 ± 23.3 b (34.9)	83.7 ± 9.8 a (30.5)	106.0 ± 13.8 a (29.6)
		113	363.1 ± 45.4 a (47.5)	373.9 ± 59.8 a (43.9)	438.1 ± 62.4 a (46.3)	442.5 ± 62.8 a (47.2)
		154[c]	461.9 ± 30.0 a (45.3)	490.3 ± 6.0 a (44.5)	468.1 ± 29.5 a (44.0)	463.2 ± 16.5 a (46.0)
	2009	67[b]	308.9 ± 20.6 a (49.2)	316.5 ± 20.8 a (47.7)	272.0 ± 4.4 a (48.2)	276.0 ± 13.5 a (48.2)
		98	760.7 ± 37.1 a (58.3)	910.6 ± 38.3 a (58.6)	916.8 ± 44.4 a (57.1)	809.7 ± 50.7 a (58.3)
		121[c]	695.6 ± 21.6 b (64.5)	687.3 ± 39.3 ab (60.0)	597.5 ± 13.3 ab (60.5)	574.6 ± 23.0 a (63.0)
total nonanthocyanin phenolic compounds	2008	85[b]	341.9 ± 28.4 a	527.7 ± 38.9 b	274.0 ± 5.1 a	358.3 ± 46.1 a
		113	764.7 ± 71.0 a	852.0 ± 126.8 a	945.3 ± 112.9 a	937.0 ± 125.6 a
		154[c]	1020.0 ± 67.5 a	1100.8 ± 18.8 a	1062.6 ± 28.3 a	1008.1 ± 40.3 a
	2009	67[b]	627.6 ± 36.0 a	664.1 ± 43.8 a	564.8 ± 5.2 a	572.9 ± 22.4 a
		98	1305.8 ± 69.6 a	1553.5 ± 67.5 a	1604.4 ± 82.2 a	1387.7 ± 86.9 a
		121[c]	1077.7 ± 28.9 b	1145.6 ± 55.9 b	987.6 ± 28.4 ab	911.8 ± 20.3 a

[a] Average of three replicates followed by different letters in the same row indicate significant differences between treatments for each compounds group and date (Tukey, $p < 0.05$). SE, standard error. DAF, days after flowering. Cluster thinning treatments: C, control; T1, early thinning; T2, veraison thinning; T3, late thinning. [b] Veraison. [c] Harvest time. [d] Relative abundance (%) between phenolic groups and total phenolics.

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Table 10. Dihydroflavonols Identified by HPLC-DAD/ESI-MS in Malbec Berry Skins

compound	λ_{max} (nm)	molecular ion $[M - H]^-$ (m/z)	fragment ions (m/z)
dihydroquercetin-3-glucoside	336 (sh),[a] 292	465	303
dihydroquercetin-3-rhamnoside	336 (sh), 292	449	303
dihydrokaempferol-3-glucoside	340 (sh), 292	449	287

[a] sh, shoulder.

dihydroflavonols). Among nonflavonoids, we identified gallic, protocatechuic, syringic, *trans*-fertaric, and *trans*-caffeic acids, methyl and ethyl gallates, and *trans* and *cis*-resveratrol-3-glucosides. Among nonanthocyanin flavonoids, we found 7 flavanols [(+)-catechin, (−)-epicatechin, 3 procyanidin dimers, and 2 procyanidin trimers], 10 flavonols (myricetin-3-galactoside, myricetin-3-glucoside, kaempferol-3-galactoside, quercetin-3-glucuronide, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, isorhamnetin-3-glucoside, laricitrin-3-glucoside, and naringenin), and 4 dihydroflavonols (dihydroquercetin-3-rhamnoside, dihydroquercetin-3-glucoside, dihydrokaempferol-3-glucoside, and an unknown dihydroflavonol).

During the 2008 ripening period, independent of the treatment, the phenolic profile changed remarkably from 85 to 154 DAF (Table 9). There was a decrease in the relative abundance (percent) of hydroxybenzoic and hydroxycinnamic acids/derivatives and flavonols with respect to the total, representing 3.9, 2.1, and 42.7% at 85 DAF and 2.4, 0.8, and 28.7% at 154 DAF, respectively, whereas stilbenes, flavanols, and dihydroflavonols increased from 0.3, 19.8, and 31.1% to 0.8, 22.3, and 45.0%, respectively. In 2009, these compounds showed the same pattern of evolution as observed in 2008, except for flavanols, the relative abundance of which decreased until harvest. At this moment their general distribution was 1.6% of hydroxybenzoic acids, 0.3% of hydroxycinnamic acids, 0.9% of stilbenes, 11.0% of flavanols, 24.2% of flavonols, and 62.0% of dihydroflavonols. All of the phenolic groups (nonflavonoids and flavonoids) increased progressively from veraison to harvest in 2008, whereas 2009 showed a considerable increase toward the second sampling and then a decrease until harvest (Table 9).

At harvest, the nonanthocyanin phenolic composition of skins was differentially affected by CT treatments. With regard to the nonflavonoids, the yield treatments did not modify their content, with significant higher values for phenolic acids in 2008 than 2009 and similar contents of stilbenes in both seasons (Tables 8 and 9). These results are in agreement with those obtained by other authors in different grape varieties.[14,34] Stilbenes are important compounds of interest in human health due to their putative protective effects against cardiovascular diseases. In grape cluster, stilbenes are considered to be located essentially in skins and mainly in glucosylated form. The levels determined in this study, ranging from 7.2 to 10.2 mg/kg of skins, are coincident with previous results.[35]

Considering the flavanols, we observed a significant effect of T1 only in 2009 berry skins with respect to the rest of the treatments, and the total content was lower compared to 2008 (Tables 8 and 9). The main monomer quantified in the skins was (+)-catechin (6.4−8.1 mg/kg of skins), followed by (−)-epicatechin (5.2−6.9 mg/kg of skins). Considering the ratio (+)-catechin/(−)-epicatechin (average of 1.2 among samples), it would be possible to suggest that in Malbec grapes, the leucoanthocyanidin reductase enzyme is more active than the anthocyanidin reductase, as shown in Fanzone et al.[11] The highest levels of procyanidin dimers and trimers found in the skins of 2008, together with the higher anthocyanins content, represent their ability to obtain quality wines with potential for aging, because these compounds are related to color stability and body through reactions of copigmentation and polymerization.[36]

Flavonols were the second most abundant group found in Malbec berry skins at harvest. The total contents ranged from 288.6 mg/kg of skins to 318.0 and from 208.3 to 291.9 mg/kg of skins in 2008 and 2009, respectively (Table 9). These concentrations are lower than those found in Syrah grapes with thinning management,[15] higher with respect to Carménère,[14] and similar to those determined by other authors.[34] In the pattern of 2008, the main flavonol was myricetin (mean = 29.4%), followed by isorhamnetin (22.1%), quercetin (18.9%), kaempferol (13.4%), laricitrin (8.3%), and naringenin (8%), whereas in 2009, a different profile was observed, with flavonol myricetin (33.1%) being the main flavonol, followed by kaempferol (28.0%), quercetin (13.9%), naringenin (11.0%), laricitrin (7.4%), and isorhamnetin (6.7%). These results differ from those presented by Mattivi et al.[37] for other red grape varieties, indicating a distinctive flavonol profile of Malbec grapes. To analyze the effect of CT on these compounds, in 2008 there were no differences between them with respect to the control, whereas in 2009, T1 and T2 positively affected the contents of myricetin-3-glucoside, kaempferol-3-galactoside, quercetin-3-galactoside, quercetin-3-glucoside, laricitrin-3-glucoside, and naringenin, increasing the level of total flavonols by 21.2 and 6.8%, respectively. This could be mainly explained by climatic differences between seasons. First, regardless of thinning treatments, higher temperatures and radiation existing in 2009 induced a higher expression and regulation of flavonol synthesis in berries[29] between the first and second samplings. Then toward the harvest, there was a decrease in the rate of these compounds, probably due to degradation and inhibition by high temperatures, as has been described for other flavonoids such as anthocyanins.[28] However, the thinning treatments applied to pea size (T1) and veraison (T2) would allow greater availability of photoassimilates (sugars) in leaves at early stages before berry fillling. Thus, the higher rate of flavonols might be explained by an inductive effect of sugars on the expression of genes involved in biosynthesis, as in the case of anthocyanins.[25,26]

Another important 2-phenylbenzopyran subclass found in some fruits is the dihydroflavonols. These compounds contribute to a smaller fraction of total wine flavonoids, and they play functional roles in grape berries. Data on dihydroflavonols in grapes are rather scarce and, as far as we know, have been reported especially in white varieties.[38,39] Dihydroflavonols such as astilbin (dihydroquercetin-3-rhamnoside) most likely function in plants to fight *Botrytis* infection. It is also considered as a bioactive compound that would provide antimicrobial, antibacterial, cardiopreventive, and possibly chemopreventive effects in humans. In our experiment, these compounds were characterized by HPLC-DAD/ESI-MS and using the UV spectral information (Table 10). The total content, independent of treatment, ranged from 461.9 to 490.3 mg/kg of skins and from 574.6 to 695.6 mg/kg of skins in 2008 and 2009, respectively (Table 9). Analyzing the effect of CT on these compounds, in 2008 we found no differences between the treatments with respect to the control,

Table 11. Low Molecular Weight Phenolic Compounds Quantified in Malbec Berry Seeds, from Altamira, during Ripening (2008−2009)[a]

			concentration (mg/kg of seeds, mean ± SE) for treatment			
compound	year	DAF	C	T1	T2	T3
gallic acid	2008	85[b]	140.4 ± 12.1 a	143.5 ± 0.2 a	139.3 ± 3.7 a	136.1 ± 11.1 a
		113	30.3 ± 0.9 a	35.0 ± 1.1 a	26.0 ± 3.0 a	29.7 ± 5.3 a
		154[c]	151.6 ± 9.6 a	144.3 ± 12.3 a	102.4 ± 12.2 a	101.9 ± 9.0 a
	2009	67[b]	108.2 ± 4.8 a	120.7 ± 0.4 a	103.1 ± 1.6 a	108.4 ± 5.8 a
		98	64.8 ± 2.0 a	70.4 ± 3.4 a	74.4 ± 4.3 a	74.9 ± 8.6 a
		121[c]	80.7 ± 3.8 a	83.6 ± 4.2 a	73.5 ± 2.2 a	77.1 ± 3.2 a
(+)-catechin	2008	85[b]	2649.7 ± 103.4 a	3095.1 ± 199.8 a	2860.5 ± 214.7 a	3144.7 ± 401.5 a
		113	2565.0 ± 280.6 a	2411.6 ± 220.8 a	2413.5 ± 168.2 a	2863.2 ± 227.3 a
		154[c]	2061.0 ± 145.5 a	2002.8 ± 14.3 a	2754.5 ± 251.5 b	2046.6 ± 99.1 a
	2009	67[b]	2717.0 ± 162.7 a	5345.2 ± 226.7 b	4226.4 ± 633.3 ab	3257.6 ± 261.7 ab
		98	2099.8 ± 116.7 a	2659.1 ± 307.8 a	2888.7 ± 280.7 a	1874.4 ± 106.6 a
		121[c]	1806.4 ± 56.2 ab	2025.3 ± 2.6 b	1662.9 ± 16.9 a	1682.0 ± 88.2 a
(−)-epicatechin	2008	85[b]	1532.3 ± 246.2 a	1824.3 ± 101.0 a	1750.8 ± 94.8 a	1650.7 ± 128.4 a
		113	1324.8 ± 50.5 a	1118.9 ± 72.6 a	1725.7 ± 35.9 b	1239.2 ± 17.7 a
		154[c]	1221.3 ± 97.6 a	835.9 ± 154.8 a	1061.9 ± 140.6 a	1183.6 ± 188.5 a
	2009	67[b]	1548.2 ± 61.6 a	3187.4 ± 135.5 b	2372.0 ± 228.1 ab	1932.7 ± 137.8 a
		98	1322.5 ± 141.7 a	1332.7 ± 131.3 a	1433.5 ± 139.3 a	975.1 ± 81.7 a
		121[c]	1090.7 ± 53.6 a	1219.6 ± 118.0 a	1048.8 ± 39.9 a	941.6 ± 66.5 a
epicatechin-3-gallate	2008	85[b]	115.9 ± 18.4 a	92.6 ± 5.2 a	142.5 ± 16.2 a	163.0 ± 0.4 a
		113	61.4 ± 2.3 a	83.7 ± 4.0 b	57.3 ± 2.7 a	62.1 ± 2.0 a
		154[c]	27.8 ± 2.7 a	54.4 ± 1.0 c	44.3 ± 3.8 ab	36.8 ± 2.1 ab
	2009	67[b]	145.9 ± 12.9 a	202.1 ± 26.4 a	185.2 ± 16.3 a	173.5 ± 5.3 a
		98	77.8 ± 4.1 a	123.0 ± 15.1 a	97.8 ± 3.1 a	88.4 ± 8.9 a
		121[c]	74.7 ± 9.9 a	86.8 ± 5.0 a	86.7 ± 2.5 a	83.9 ± 2.9 a
procyanidin B1	2008	85[b]	86.7 ± 6.4 a	80.8 ± 4.8 a	105.3 ± 15.3 a	86.2 ± 3.7 a
		113	30.4 ± 7.4 a	60.1 ± 9.9 a	39.5 ± 2.4 a	46.7 ± 4.6 a
		154[c]	14.3 ± 1.8 a	14.1 ± 1.0 a	14.4 ± 1.6 a	19.3 ± 2.5 a
	2009	67[b]	102.4 ± 4.4 a	207.0 ± 21.2 b	165.3 ± 14.7 ab	137.7 ± 13.2 ab
		98	34.4 ± 2.7 a	36.4 ± 2.0 a	46.0 ± 5.7 a	27.5 ± 1.9 a
		121[c]	27.9 ± 4.3 a	27.0 ± 3.2 a	24.5 ± 0.1 a	27.3 ± 1.5 a
procyanidin B2	2008	85[b]	298.9 ± 18.1 a	223.8 ± 18.8 a	272.7 ± 18.1 a	286.7 ± 11.9 a
		113	155.3 ± 25.2 a	216.8 ± 36.1 a	144.3 ± 10.9 a	169.0 ± 21.9 a
		154[c]	70.9 ± 8.4 a	44.0 ± 7.8 a	40.9 ± 0.9 a	126.0 ± 9.9 b
	2009	67[b]	222.7 ± 5.4 a	454.1 ± 2.2 b	350.3 ± 48.4 ab	304.7 ± 49.7 ab
		98	120.8 ± 15.4 a	129.0 ± 19.1 a	152.4 ± 23.5 a	85.0 ± 11.3 a
		121[c]	108.6 ± 13.6 a	105.5 ± 3.0 **a**	113.2 ± 1.1 **a**	106.9 ± 10.9 **a**
procyanidin B3	2008	85[b]	141.7 ± 23.3 a	133.9 ± 5.1 a	191.4 ± 0.5 a	153.6 ± 17.3 a
		113	138.2 ± 8.4 a	107.5 ± 16.7 a	195.7 ± 2.5 b	126.1 ± 9.5 a
		154[c]	93.7 ± 9.8 a	63.9 ± 4.0 a	68.7 ± 11.1 a	114.7 ± 19.1 a
	2009	67[b]	187.3 ± 23.1 a	366.9 ± 10.9 b	301.5 ± 33.0 ab	228.6 ± 11.6 a
		98	84.6 ± 3.1 a	95.0 ± 3.8 a	105.2 ± 13.0 a	76.9 ± 3.9 a
		121[c]	58.2 ± 6.9 a	61.5 ± 4.7 a	58.6 ± 0.1 a	53.3 ± 6.8 a
procyanidin B4	2008	85[b]	250.7 ± 14.5 a	294.1 ± 19.5 a	308.0 ± 22.3 a	308.6 ± 16.4 a
		113	235.7 ± 18.7 a	170.1 ± 1.8 a	265.6 ± 2.2 a	260.3 ± 35.8 a
		154[c]	205.9 ± 23.2 ab	115.8 ± 13.8 a	251.3 ± 11.0 b	199.6 ± 19.0 ab
	2009	67[b]	295.1 ± 27.0 a	496.7 ± 30.5 a	444.6 ± 34.0 a	386.6 ± 45.9 a
		98	185.5 ± 5.0 ab	215.6 ± 3.0 b	158.9 ± 5.6 a	175.3 ± 6.1 a
		121[c]	86.5 ± 2.8 a	217.2 ± 24.8 b	126.3 ± 22.9 ab	109.9 ± 12.0 a

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Table 11. Continued

compound	year	DAF	concentration (mg/kg of seeds, mean ± SE) for treatment C	T1	T2	T3
procyanidin B6	2008	85[b]	30.1 ± 2.7 a	38.6 ± 2.2 a	30.5 ± 4.0 a	57.7 ± 0.1 b
		113	36.3 ± 0.5 a	43.0 ± 4.1 a	33.5 ± 0.3 a	46.3 ± 4.2 a
		154[c]	29.5 ± 0.1 a	27.9 ± 0.2 a	28.4 ± 0.4 a	28.1 ± 0.5 a
	2009	67[b]	49.9 ± 1.4 a	75.9 ± 2.2 b	59.6 ± 5.9 ab	55.3 ± 4.5 ab
		98	54.0 ± 1.8 a	44.6 ± 1.8 a	49.3 ± 6.1 a	51.3 ± 1.1 a
		121[c]	29.1 ± 3.3 a	43.8 ± 4.5 ab	51.0 ± 6.2 b	31.7 ± 3.5 ab
procyanidin C1	2008	85[b]	162.7 ± 1.2 a	190.3 ± 19.0 a	126.2 ± 6.0 a	135.9 ± 18.8 a
		113	100.4 ± 13.8 a	82.3 ± 2.2 a	75.2 ± 5.2 a	138.1 ± 16.7 a
		154[c]	67.7 ± 6.9 a	56.5 ± 8.9 a	58.5 ± 3.5 a	58.6 ± 3.7 a
	2009	67[b]	219.8 ± 13.2 a	410.7 ± 54.1 a	371.2 ± 38.7 a	282.7 ± 43.0 a
		98	80.2 ± 4.6 ab	99.3 ± 9.6 b	112.4 ± 1.0 b	63.9 ± 5.3 a
		121[c]	75.0 ± 9.1 a	113.8 ± 5.1 a	100.1 ± 10.1 a	87.3 ± 3.0 a
tetramer	2008	85[b]	64.9 ± 3.6 b	29.4 ± 1.8 a	71.1 ± 2.6 b	79.9 ± 10.7 b
		113	25.1 ± 0.2 a	27.1 ± 0.5 a	69.0 ± 6.6 b	34.2 ± 2.1 a
		154[c]	27.2 ± 1.8 a	24.0 ± 0.8 a	45.2 ± 1.2 b	31.7 ± 3.1 a
	2009	67[b]	48.1 ± 2.6 a	85.7 ± 3.1 b	76.5 ± 7.9 ab	62.4 ± 5.4 ab
		98	41.2 ± 5.4 a	57.8 ± 3.2 a	41.3 ± 1.1 a	44.2 ± 7.9 a
		121[c]	28.0 ± 1.7 a	32.1 ± 0.2 a	40.4 ± 5.1 a	23.1 ± 3.5 a
procyanidin dimer gallate 1	2008	85[b]	35.5 ± 3.5 a	60.1 ± 6.5 ab	35.7 ± 3.5 a	84.9 ± 5.7 b
		113	59.4 ± 8.0 ab	80.6 ± 12.6 b	33.0 ± 1.0 a	32.0 ± 5.1 a
		154[c]	33.4 ± 0.5 a	50.6 ± 3.6 b	32.2 ± 3.7 a	31.9 ± 2.3 a
	2009	67[b]	87.5 ± 2.5 a	122.4 ± 13.3 a	115.3 ± 17.3 a	91.6 ± 8.6 a
		98	68.9 ± 6.4 a	67.0 ± 3.7 a	68.5 ± 4.0 a	69.7 ± 3.8 a
		121[c]	55.7 ± 8.0 a	53.8 ± 7.6 a	62.8 ± 3.3 a	36.2 ± 3.7 a
procyanidin dimer gallate 2	2008	85[b]	200.6 ± 10.1 ab	181.7 ± 7.1 a	248.5 ± 9.4 b	196.6 ± 6.3 a
		113	154.9 ± 19.4 ab	100.3 ± 6.1 a	128.3 ± 7.5 ab	164.6 ± 15.6 b
		154[c]	105.0 ± 13.2 a	107.1 ± 15.9 a	121.9 ± 9.5 a	92.5 ± 4.5 a
	2009	67[b]	407.5 ± 42.5 a	737.2 ± 27.0 b	620.0 ± 61.4 ab	493.5 ± 30.0 ab
		98	106.6 ± 13.8 a	133.7 ± 20.1 a	131.8 ± 3.6 a	76.4 ± 9.9 a
		121[c]	70.3 ± 3.0 a	78.6 ± 4.7 a	83.4 ± 5.2 a	77.0 ± 5.2 a
procyanidin trimer gallate 1	2008	85[b]	47.0 ± 3.8 a	104.6 ± 5.7 b	120.0 ± 11.6 b	224.0 ± 9.3 c
		113	93.2 ± 14.9 ab	127.0 ± 8.8 b	69.6 ± 3.1 a	74.1 ± 15.0 ab
		154[c]	111.9 ± 16.9 a	102.0 ± 9.2 a	125.5 ± 10.4 a	103.8 ± 6.7 a
	2009	67[b]	140.7 ± 13.8 a	267.2 ± 1.5 b	224.4 ± 24.1 b	127.0 ± 4.7 a
		98	74.7 ± 4.6 a	71.2 ± 6.0 a	84.6 ± 2.6 a	59.6 ± 6.9 a
		121[c]	61.9 ± 4.1 a	67.3 ± 4.3 a	68.4 ± 3.7 a	63.7 ± 5.4 a
procyanidin trimer gallate 2	2008	85[b]	290.6 ± 54.7 a	275.4 ± 4.4 a	361.0 ± 53.5 a	308.0 ± 53.3 a
		113	229.8 ± 7.6 ab	294.5 ± 24.7 b	214.1 ± 14.0 a	206.9 ± 3.1 a
		154[c]	35.1 ± 4.9 a	130.3 ± 1.7 c	134.2 ± 9.0 c	86.8 ± 6.3 b
	2009	67[b]	1009.6 ± 46.0 a	2070.1 ± 114.1 b	1930.6 ± 76.0 b	976.9 ± 131.3 a
		98	244.5 ± 34.2 a	201.0 ± 4.1 a	401.0 ± 21.8 b	156.4 ± 7.7 a
		121[c]	161.1 ± 2.0 a	167.2 ± 4.5 a	200.9 ± 10.3 b	162.2 ± 2.3 a
total phenolic compounds	2008	85[b]	6047.5 ± 168.4 a	6768.3 ± 302.8 a	6763.5 ± 181.9 a	7016.6 ± 489.7 a
		113	5240.1 ± 299.6 a	4958.4 ± 175.9 a	5490.4 ± 135.0 a	5491.3 ± 246.1 a
		154[c]	4256.4 ± 306.4 a	3773.5 ± 130.5 a	4884.3 ± 345.1 a	4260.9 ± 320.6 a
	2009	67[b]	7289.8 ± 415.8 a	14149.2 ± 536.0 b	11546.2 ± 1240.7 ab	8619.1 ± 437.9 a
		98	4660.4 ± 314.2 a	5341.1 ± 408.6 a	5838.2 ± 482.0 a	3903.9 ± 237.5 a
		121[c]	3814.0 ± 154.5 a	4342.4 ± 167.1 a	3801.5 ± 9.2 a	3563.1 ± 202.6 a

[a] Average of three replicates followed by different letters in the same row indicate significant differences between treatments for each compound and date (Tukey, $p < 0,05$). SE, standard error. DAF, days after flowering. Cluster thinning treatments: C, control; T1, early thinning; T2, veraison thinning; T3, late thinning. [b] Veraison. [c] Harvest time.

whereas in 2009, T1 positively affected the contents of dihydrokaempferol-3-glucoside and the unknown dihydroflavonol without changing the total content. Dihydroflavonols are direct precursors of flavonols; therefore, the significant differences observed in both seasons (Table 8) could be explained by the differences in flavonol content, suggesting a possibly lower activity of flavonol synthase (FLS) in 2009 samples compared to 2008. Dihydroquercetin-3-glucoside was the major compound among all nonanthocyanin phenolics detected and represented 25.7% (2008) and 39.9% (2009) of the total content at harvest. To our knowledge, this finding is reported for the first time in Malbec grapes, and it was also obtained in another experiment with Malbec wines,[11] which could represent a distinctive feature of this variety.

Phenolic Composition of Seeds during Ripening. *Total Phenolic Composition.* The seeds play a very important role during red winemaking, the flavanols being the major sensory components responsible for the bitterness and astringency of red wine, as well as also facilitate the stabilization of the anthocyanins. Table 5 shows the results of total phenolic parameters for Malbec grape seeds during ripening. During the periods evaluated, independent of treatments, total phenols and flavanols DMACH decreased 31.5 and 36.5% in 2008 and 41.9 and 15.8% in 2009, respectively, between the first and last samplings. With regard to proanthocyanidins, in 2009 the same trend was observed as in the above parameters, whereas in 2008 these compounds showed an irregular pattern of evolution. At harvest time in 2008 season, there was a significant effect of T1 on flavanols DMACH content, whereas in 2009 the T2 treatment increased the proanthocyanidin and total phenol concentrations. The findings obtained in this research were concordant with those presented in previous studies with other varieties.[14,40]

HPLC Nonanthocyanin Profile of Grape Seeds. Table 11 shows the identified and quantified low molecular weight phenolic compounds in Malbec seeds during ripening (2008−2009). Among them, three flavanol monomers were found [(+)-catechin (C), (−)-epicatechin (EC), and epicatechin-3-gallate (ECG)], five procyanidin dimers [catechin-(4α→8)-catechin (B3), epicatechin-(4β→8)-catechin (B1), catechin-(4 α→8)-epicatechin (B4), epicatechin-(4β→8)-epicatechin (B2), and catechin-(4α→6)-catechin (B6)], one procyanidin trimer [epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (C1)], five undetermined procyanidins [two dimers esterified with gallic acid (DG1, DG2), two trimers esterified with gallic acid (TG1, TG2), and one tetramer], and only one nonflavonoid compound (gallic acid).

Both flavanol monomers and seed procyanidin contents experienced a continuous fall until harvest, indicating that the extraction of these compounds declines with maturity. Kennedy et al.[41] suggest that this decline is consistent with an oxidative process. As expected, the seeds had a high concentration of flavanols in comparison with the skins. At harvest, the total flavanol contents in seeds were 4- and 9-fold higher than that of the skins in 2008 and 2009, respectively. These results coincide with the findings described previously by other authors in other varieties.[16] Also according to these studies, we observed that in Malbec seeds the major compound was (+)-catechin, followed by (−)-epicatechin. We found no significant differences of these monomers in both seasons; however, the remaining monomer (epicatequina-3-gallate) had a greater amount in 2009 compared with 2008. The relative abundance of these flavanol monomers changed during both periods evaluated. In 2008, independent of treatment, the C:EC:ECG ratio changed from 44:25:2 on first sampling to 52:25:1 on last sampling, whereas in 2009 we observed a 37:22:2 ratio at veraison and a 46:28:2 ratio at harvest. According to Kennedy et al.[41] these rate differences are consistent with expected differences when C, EC, and ECG are exposed to radical-induced oxidation under aqueous conditions. With regard to procyanidins, there were some differences in their contents at harvest. In 2008, the relative abundances of procyanidins and compounds esterified with gallic acid were 8.2 and 11.3%, and those in 2009 were 9.5 and 12.6%, respectively. Finally, the flavanol composition of seeds was differentially affected by CT treatments. At the last sampling date in 2008, the thinning treatments increased the contents of C, ECG, B4, tretamer, DG1, and TG2, whereas in 2009, the only compounds affected by CT were C, B4, B6, and TG2. The analysis of pooled data for the total content of phenolic compounds revealed no significant differences between seasons (Table 8).

The results presented in this paper show variations in some total phenolic variables analyzed and in the individual nonflavonoid and flavonoid contents of Malbec grapes from different thinning treatments, during the ripening period. In summary, during 2008, with more restrictive conditions for grapevines mainly due to climatic conditions, the early thinning had a differential effect on the various parameters evaluated on berry skins and seeds. This practice encouraged the biosynthesis of some global parameters of the grapes (soluble solids) and seeds (flavanols DMACH) and some individual compounds in grape skins (anthocyanins) and seeds (flavanols). Conversely, in 2009 the thinning treatments produced a different effect compared to 2008; there was greater biosynthesis of nonanthocyanin flavonoids (flavanols and flavonols) in skins and some global parameters and individual flavanols in seeds. On the other hand, in 2008 there was a higher concentration of most phenolic compounds, indicating greater potential to obtain high-quality wines, with polymeric pigments and color stability suitable for long aging. Finally, it is important to note that benefits from thinning should be carefully weighed with the cost involved in this viticultural practice (about $250 U.S. per hectare) and the losses of fruit, particularly if gain in grape and wine quality is not evident.

As a conclusion, our work presents for the first time the phenolic profile of Malbec berry skins and seeds from Mendoza. The phenolic composition and the range of the data obtained in the analyzed Malbec samples are in good agreement with the available international literature for other red varieties. Furthermore, our analysis of the individual phenolic composition by HPLC-DAD/ESI-MS shows that the compound family corresponding to dihydroflavonols seems to be a distinctive feature of Malbec, differing from the phenolic profile reported for other red varieties (e.g., Cabernet Sauvignon, Merlot, Carménère, and Syrah).

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