Characterisation and evolution of grape polyphenol profiles of *Vitis vinifera* L. cv. Tannat during ripening and vinification

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Abstract

Background and Aims: Changes in different polyphenol families during grape ripening and vinification were determined in Tannat grapes. This was done to establish a polyphenol profile for Tannat grapes during ripening and for young wines.

Methods and Results: We demonstrated, by high-performance liquid chromatography with diode array detection and mass spectrometry (HPLC-DAD-MS) analysis, that the content of flavan-3-ols in Tannat seeds was higher than that reported for a large number of other grape varieties analysed. Forty per cent of the total flavan-3-ols in seeds were galloylated compounds, whereas the flavan-3-ol profile in skins was characterised by the absence of galloylated forms. Prodelphinidins in skins ranged between 30 and 35% with very low values for epigallocatechin. Epicatechin gallate was not detected in Tannat wine and galloylated forms represented a low percentage of total flavan-3-ols. Tannat grapes had very high concentrations of anthocyanins in skins with levels increasing during ripening. Eleven phenolic acids were identified in Tannat grape skins and wines, but only gallic and protocatechuic acids were found in the seeds.

Conclusions: Tannat wines were shown to have one of the highest levels of phenolic compounds reported for vinifera grape varieties. The profiles for different polyphenol families present in Tannat grapes (skins and seeds) and wines were determined.

Significance of the Study: The results presented will help to better understand the phenolic winemaking potential of this variety and its functional properties within food chemistry.

Keywords: *grape ripening, polyphenol, Tannat, wine*

Introduction

Vitis vinifera L. cv. Tannat, a red grape variety with origins in the southwest of France, is widely cultivated in Uruguay and represented more than 25% of total grape production in 2009 (Carrau et al. 2011). Tannat wine production is of increasing interest to the world wine market because of its export potential. Tannat wines are considered to have a unique character with a rich structure that increases with ageing. One of the most striking features of Tannat wines is the relatively intense colour compared with red wines of other varieties; consistent with Tannat having the highest pigment content (Alcalde-Eon et al. 2006a, González-Neves et al. 2007). Tannat wines are also described as having a high phenolic content, which lends structure and colour to the wine. The phenolic fraction of this variety has thus become a focus of study from different perspectives including studies on changes in grape anthocyanins during vintage and vineyard treatments (González-Neves et al. 2004).

The colour of a wine is important for consumers and is due, in part, to anthocyanins that provide the characteristic red-purple hue to young red wines. Anthocyanins are extracted from the grapes to the must during winemaking, Once extracted, and at the end of the fermentation, the anthocyanin concentration may decline because of reactions with other molecules, or to yeast adsorption in the first steps of winemaking, as shown previously for Tannat wines (Medina et al. 2005). Finally, the qualitative and quantitative changes occurring during ageing in the main pigment families present in young Tannat wines (anthocyanins, pyranoanthocyanins and the generation of direct and acetaldehyde-mediated flavanol-anthocyanin condensation products) were also investigated (Boido et al. 2006).

Grape tannins are present in skins and seeds, but there are higher levels in seeds (Ricardo da Silva et al. 1991). Grape seed proanthocyanidins include only the procyanidins (Prieur et al. 1994, Labarbe et al. 1999), whereas grape skin tannins include both prodelphinidins and procyanidins (Souquet et al. 1996, Labarbe et al. 1999). In addition, the rich phenolic composition of Tannat was attributed to the high procyanidin content and was also correlated with vascular health and human longevity in a recent report (Corder et al. 2006).

The aim of this study was to determine the different polyphenol families (anthocyanins, phenolic acids, flavonols, and flavanols monomers and oligomers) present in Tannat grapes skins and seeds, and to establish qualitative and quantitative changes in these compounds during grape ripening and vinification. To our knowledge this is the first characterisation of

doi: 10.1111/j.1755-0238.2011.00164.x

polyphenolic families present in Tannat grapes and wines. The characterisation will enable establishment of a characteristic polyphenol profile for young Tannat wines.

Materials and methods

Grape samples

Fresh *Vitis vinifera* L. cv. Tannat grapes were obtained during a harvest in 2009 from two vineyards located in the southern region of Uruguay (34°47′ 25″ S, 56°16′ 40′ W and 34°33′ 58′ S, 56°17′ 10′ W), at three stages of ripening (m1 = 20 days after veraison; m2 = 10 days before harvest; m3 = harvest). Representative samples of grapes (~0.5 kg) were collected from several randomly selected vines and from different parts of various clusters. A portion of each sample was analysed immediately for oenological parameters, while the remainder was frozen and stored at −20°C for3 months prior to polyphenol analysis.

Wine samples

Wine samples were taken from wine produced from batches of 5 tonnes of fresh grapes from the two vineyards studied. The grapes were destemmed and crushed. SO_2 was added to the must (50 mg/L), which was then inoculated with reactivated dry yeast (*Saccharomyces cerevisiae*, strain D 254; Lallemand, Rexdale, Ontario, Canada). Fermentation was carried out at 24°C in stainless steel tanks, and when the sugar content was below 3 g/L after 15 days of maceration, the wine was separated from the skins. The skins were then pressed (maximum pressure, 1.2 bar). The free run wine and press wine were combined and transferred to new 225 L French oak barrels. Upon completion of alcoholic fermentation, malolactic fermentation (MLF) was activated by inoculation with *Oenoccocus oeni*, strain VP41 (Lallemand S.A., St. Simon, France). MLF was monitored by determining the concentrations of malic and lactic acid by thin-layer chromatography (Boido et al. 1999). Once MLF was completed, the wines were treated with 50 mg/L SO_2. Barrels were maintained at 16°C and free SO_2 was corrected to 30 mg/L periodically. Samples were taken for analysis after 4 months of ageing in the barrel.

Oenological parameters

Sugar content, pH and titratable acidity in grape samples were determined using the International Organisation of Vine and Wine (OIV) methods (OIV 2009). Total anthocyanins, easily extractable anthocyanins and total polyphenol index were analysed according to Glories (2001). Alcohol, titrable acidity, volatile acidity, pH and sugar in wine samples were determined using the OIV methods (OIV 2009). Anthocyanins were determined by the method of Ribereau-Gayon and Stonestreet (1965), and total polyphenolic index was determined by UV absorption at 280 nm (Glories 1984).

Extraction and analysis of grape polyphenols

The seeds and skins were separated manually. The seeds were lyophilised and their moisture calculated by weight difference before and after lyophilisation. Seed extraction was done by the method of García-Marino et al. (2006) using MeOH-H_2O (75:25 v/v). Skins (10 g) were macerated overnight in the dark at 4.0 ± 1°C in 100 mL 0.1% HCl in methanol. The crude extract obtained was filtered and the skins washed and filtered again with 100 mL of the same extraction solvent, and the filtrates combined. Vacuum filtration was done with Whatman (no. 2) filter paper using a Büchner funnel, the extract concentrated under reduced pressure to evaporate methanol and the aqueous residue suspended to 100 mL with ultrapure water (MilliQ, Billerica, MA, USA). This extraction process was repeated four times for each sample. For pigment and flavonol analysis, 1 mL of the aqueous extract was diluted (1:1) with 0.1 N HCl and then injected into the chromatographic system after filtration through a 0.45 µm Millex® syringe-driven filter unit (Millipore Corporation, Temecula, CA, USA). Analysis of flavanols and phenolic acids in skin extracts was done with a preliminary purification involving elution with methanol through Oasis® MCX 3 cc (60 mg) cartridges in which pigments and flavonols were retained (González-Manzano et al. 2006). Qualitative and quantitative analysis of flavan-3-ol and phenolic acid composition was studied in detail in seeds and skins, while pigments and flavonols were analysed only in the skins.

Wine polyphenol analysis

Wine samples of 1 mL were diluted (1:1) with 0.1N HCl, filtered through 0.45 µm Millex® syringe driven filter units and injected into the chromatographic system for anthocyanin and flavonol analysis. Two millilitres of each wine sample were eluted through Oasis® MCX cartridges previously conditioned with 2 mL methanol and 2 mL water, with the objective of eliminating the red pigments (García-Marino et al. (2009). After washing with 4 mL of ultrapure water, flavan-3-ols and the phenolic acids were eluted with 8 mL methanol, whereas anthocyanins and the flavonols were retained in the cartridges. A small volume of water was added to the eluate and concentrated under vacuum at lower than 30°C until complete elimination of methanol. The volume of the aqueous residue was adjusted to 0.5 mL with ultrapure water (MilliQ), filtered (0.45 µm) and analysed by HPLC–DAD–MS.

HPLC-DAD-MS analysis

HPLC-DAD-MS analysis was performed in a Hewlett-Packard 1100 series liquid chromatograph. The LC system was connected to the probe of the mass spectrometer via the UV cell. The mass analyses were done using a Finnigan TM LCQ ion trap detector (Thermoquest, San Jose, CA, USA) equipped with an atmospheric pressure ionization source, using an electrospray ionisation (ESI) interface. The HPLC–DAD–MS analysis conditions for red pigments and flavonols were done by the method of García-Marino et al. (2010), but also using an additional wavelength at 360 nm for analysis of flavonols. Analyses of flavan-3-ols was done as described by García-Marino et al. (2006). The phenolic acids were quantified using the areas of the peaks at 330 nm. The MS conditions for analysis were: sheath gas flow 1.2 L/min and the auxiliary gas flow, 6 L/min. The source voltage and the capillary voltage used were 2.50 kV and −10 V, respectively, and the capillary temperature 175°C. The mass spectra were recorded in positive and negative ion mode between m/z 120 and 2000. The mass spectrometer was programmed to do a series of three consecutive scans: a full mass, an MS^2 scan of the most abundant ion in the full mass, and an MS^3 of the most abundant ion in the MS^2, using a normalised energy of collision of 45%.

Quantification

Quantification was performed by HPLC-DAD using calibration curves of standards: delphinidin, cyanidin, petunidin, peonidin and malvidin-3-O-glucosides for each of the anthocyanidin-3-O-glucosides, acetylated forms and their derivates, respectively; myrcetin for its glycosidic derivates and quercetin for other flavonols identified; (+)-catechin for flavanol monomers;

Table 1. General characteristics of Tannat grapes during ripening and wines. Mean values and standard deviations (n = 4, samples from two vineyards or two wines analysed in duplicate).

Parameter	Grape			Wine
	m1	m2	m3	
Berry weight (g)	1.7 ± 0.4	1.5 ± 0.4	1.3 ± 0.3	–
Fresh seed weight (g/kg)	212 ± 64	192 ± 55	184 ± 41	–
Dry seed weight (g/kg)	124 ± 39	115 ± 33	114 ± 30	–
Seed humidity (%)	42 ± 3	40 ± 2	39 ± 3	–
Skin weight (g/kg)	148 ± 4	191 ± 1	191 ± 17	–
Sugar content (g/L)	197 ± 6	244 ± 18	254 ± 13	2.6 ± 0.2
Titratable acidity (g/L sulfuric acid)	5.2 ± 0.9	4.0 ± 0.4	3.6 ± 0.3	4.4 ± 0.1
pH	3.15 ± 0.01	3.36 ± 0.02	3.45 ± 0.10	3.7 ± 0.2
Total anthocyanins†	1695 ± 14	2528 ± 183	2227 ± 249	1283 ± 267
Extractable anthocyanins (mg/kg)	761 ± 178	1072 ± 134	1105 ± 196	–
Total polyphenol index	77 ± 10	96 ± 16	94 ± 30	86 ± 12
Alcohol (% vol)	–	–	–	14.8 ± 0.6
Volatile acidity (g/L sulphuric acid)	–	–	–	0.35 ± 0.05

†mg/kg in grapes samples or mg/L in wine. m1, 20 days after veraison; m2, 10 days before harvest; m3, harvest. nc, not corresponding.

(+)-gallocatechin for prodelphinidins; (−)-epicatechin gallate for galloylated flavanols; dimer B2 and epicatechin-4,8-epicatechin-4,8-catechin for dimeric and oligomeric procyanidin forms, respectively; 3,4-dyhidroxybenzoic and 4-hydroxycinnamic acids for hydroxybenzoic and hydroxycinnamic acids, respectively. Anthocyanidins were purchased from Polyphenols Labs. (Sandnes, Norway). Myricetin (+)-gallocatechin and (−)-epicatechin gallate were purchased from Extrasynthèse (Genay, France). Quercetin (+)-catechin, 3,4-dihydroxybenzoic acid and 4-hydroxycinnamic acid were purchased from Sigma (Steinheim, Germany). Dimer B2 and epicatechin-4,8-epicatechin-4,8-catechin were obtained in our laboratory following the method of Escribano-Bailón et al. (1992).

The concentration of individual phenolic compounds was expressed in mg/kg of grapes or mg/L of wine. The total content of the different groups of phenolic compounds analysed was calculated as the sum of the concentrations obtained for each individual compound, expressed in mg/kg of grapes or mg/L of wine.

Statistical analysis

Polyphenol data in grape are presented for each date of ripening as mean of 8 values (samples of two vineyards, extracted in duplicated and each extract analysis was performed in duplicate). Variance analyses with the model: replication, vineyard, stage of ripening, with interaction of the last two factors and least-significant differences test were performed. All the statistical analyses were calculated using Statistica 7.1 software (StatSoft, Tulsa, OK, USA).

Results

The general characteristics of Tannat grapes during maturation are shown in Table 1. The grape sugar content increased at sampling times up to harvest, while the titrable acidity decreased during this time with pH values increasing. The berry and the fresh seed weight decreased during grape maturation, with a simultaneous decrease in the seed humidity (Table 1), in agreement with previous reports (Kennedy et al. 2000a,b). There was a moderate increase in skin weight between the first two time points that did not increase further by harvest. A similar evolution was reported for Carménère, while a decrease in the weight of the skin was reported for Cabernet Sauvignon (Obreque-Slier et al. 2010). The total anthocyanin content increased between the first and second sampling times, but by the final stage showed a decrease (Table 1). In addition, easily extractable anthocyanins increased during ripening, with a better correlation with the content of anthocyanins determined by HPLC-DAD (see Table 4). Chemical characterisation of the wines analysed is shown in Table 1. The data indicated the anthocyanin content value was close to that obtained by HPLC-DAD quantification.

A total of 60 flavan-3-ols were identified by HPLC-DAD-MS during characterisation of the procyanidins profile in seeds and skins of Tannat, and 31 in samples of wines. Mean values summarizing the content of the different groups of flavan-3-ols in Tannat grapes skins and seeds during ripening are shown in Table 2.

Eleven flavonols were identified and quantified during ripening (Table 3) in Tannat grapes skins and wines, and thirty nine anthocyanins and anthocyanin-derived pigments were identified in the grape and wine samples analysed. Anthocyanin-derived pigments were identified only in the wine samples. Grape skin anthocyanins were quantified during ripening and the values are shown in Table 4, along with the results from the analysis of variance (ANOVA; vineyard and sampling date effects).

The analysis of phenolic acids in Tannat grapes and wines allowed for identification of eleven compounds, and variations in concentration of these compounds during maturity are indicated in Table 5. The results of the quantification of the different polyphenolic groups present in the Tannat wine samples studied are shown in Table 6.

Discussion

Flavan-3-ol characterisation in Tannat grapes and wines

Seed extracts, with 55 flavan-3-ols identified, had higher levels and greater diversity of procyanidins than skins, even when the total number of compounds can be larger considering the com-

Table 2. Mean values and standard deviations ($n = 8$, samples from two vineyards, extracted in duplicate and each extract analysis performed in duplicate), and significance level in the analysis of variance for the content sum of the different groups of flavan-3-ols in Tannat grape skins and seeds during ripening.

	m1	m2	m3	ANOVA		
				Vineyard	Date	Vineyard × Date
Seed (mg/kg)						
(+)-catechin	146 ± 13^{a} *(9%)*	160 ± 23^{b} *(9%)*	157 ± 24^{b} *(8%)*	***	*	ns
(−)-epicatechin	169 ± 11^{a} *(11%)*	213 ± 14^{b} *(11%)*	203 ± 19^{b} *(10%)*	**	***	ns
Σ monomer non-galloylated	315 ± 22^{a} *(20%)*	373 ± 35^{b} *(19%)*	360 ± 42^{b} *(18%)*	***	***	ns
Σ dimer non-galloylated	260 ± 14^{a} *(17%)*	361 ± 32^{b} *(17%)*	367 ± 32^{b} *(19%)*	***	***	*
Σ trimer non-galloylated	166 ± 14^{a} *(11%)*	218 ± 27^{b} *(11%)*	227 ± 25^{b} *(12%)*	**	***	ns
Σ tetramer non-galloylated	115 ± 6^{a} *(7%)*	148 ± 18^{b} *(7%)*	150 ± 14^{b} *(8%)*	***	***	*
Σ pentamer non-galloylated	15 ± 1^{a} *(1%)*	21 ± 3^{b} *(1%)*	22 ± 2^{b} *(1%)*	ns	***	**
Σ hexamer non-galloylated	51 ± 8^{a} *(3%)*	65 ± 11^{b} *(3%)*	69 ± 13^{b} *(4%)*	ns	*	ns
(−)-epicatechin gallate	110 ± 9^{c} *(7%)*	79 ± 14^{b} *(6%)*	70 ± 11^{a} *(4%)*	***	***	ns
Σ dimer galloylated	188 ± 7^{a} *(12%)*	221 ± 32^{b} *(12%)*	225 ± 28^{b} *(12%)*	***	***	**
Σ trimer galloylated	132 ± 7^{a} *(8%)*	168 ± 19^{b} *(9%)*	175 ± 14^{b} *(9%)*	**	***	ns
Σ tetramer galloylated	106 ± 10^{a} *(7%)*	129 ± 19^{b} *(7%)*	128 ± 19^{b} *(7%)*	***	***	ns
Σ pentamer galloylated	44 ± 4^{a} *(3%)*	56 ± 5^{b} *(3%)*	57 ± 3^{b} *(3%)*	ns	***	*
Σ hexamer galloylated	12 ± 4 *(1%)*	18 ± 9 *(1%)*	16 ± 5 *(1%)*	ns	ns	ns
Σ dimer digalloylated	19 ± 3^{a} *(1%)*	22 ± 5^{b} *(1%)*	24 ± 5^{b} *(1%)*	***	***	*
Σ trimer digalloylated	32 ± 3^{a} *(2%)*	39 ± 4^{b} *(2%)*	42 ± 5^{b} *(2%)*	**	***	ns
Σ tetramer digalloylated	12 ± 3^{a} *(1%)*	15 ± 3^{b} *(1%)*	16 ± 2^{b} *(1%)*	ns	**	*
Σ non-galloylated	922 ± 49^{a} *(58%)*	1187 ± 119^{b} *(60%)*	1194 ± 120^{b} *(61%)*	***	***	*
Σ galloylated	654 ± 35^{a} *(42%)*	746 ± 97^{b} *(40%)*	752 ± 78^{b} *(39%)*	***	***	**
Σ monomer	425 ± 26 *(27%)*	451 ± 49 *(25%)*	430 ± 52 *(22%)*	***	ns	ns
Σ dimer	467 ± 21^{a} *(30%)*	604 ± 68^{b} *(31%)*	615 ± 63^{b} *(32%)*	***	***	ns
Σ oligomer	685 ± 40^{a} *(43%)*	877 ± 101^{b} *(44%)*	901 ± 85^{b} *(46%)*	***	***	*
Σ flavan-3-oles	**1576 ± 81^{a}**	**1932 ± 216^{b}**	**1946 ± 195^{b}**	***	***	*
Skin (mg/kg)						
(+)-gallocatechin	4.1 ± 0.4 *(13%)*	4.6 ± 0.6 *(13%)*	4.5 ± 1.0 *(12%)*	ns	ns	*
(−)-epigallocatechin	0.9 ± 0.1^{a} *(3%)*	1.0 ± 0.1^{ab} *(3%)*	1.1 ± 0.1^{b} *(3%)*	***	*	ns
(+)-catechin	5.3 ± 2.5 *(17%)*	5.5 ± 1.2 *(15%)*	4.4 ± 0.5 *(12%)*	ns	ns	*
(−)-epicatechin	4.8 ± 0.5 *(16%)*	5.2 ± 2.0 *(13%)*	6.0 ± 1.4 *(16%)*	ns	ns	ns
Σ procyanidin oligomer	9.7 ± 0.8 *(33%)*	12.9 ± 4.8 *(33%)*	12.2 ± 4.2 *(32%)*	**	ns	ns
Σ prodelphinidin oligomer	5.5 ± 1.4^{a} *(18%)*	8.7 ± 2.6^{b} *(23%)*	9.4 ± 1.0^{b} *(25%)*	ns	**	**
Σ monomer	14.2 ± 3.4 *(49%)*	15.3 ± 3.8 *(44%)*	15.0 ± 2.0 *(43%)*	ns	ns	*
Σ dimer	8.2 ± 1.1^{a} *(27%)*	12.1 ± 3.8^{b} *(32%)*	12.1 ± 2.1^{b} *(32%)*	ns	*	*
Σ oligomer	6.9 ± 0.4 *(23%)*	9.6 ± 3.6 *(25%)*	9.4 ± 3.2 *(25%)*	**	ns	ns
Σ procyanidin	19.9 ± 2.6 *(66%)*	23.7 ± 8.0 *(62%)*	22.6 ± 5.3 *(60%)*	*	ns	*
Σ prodelphinidin	10.4 ± 2.0^{a} *(34%)*	14.4 ± 3.1^{b} *(38%)*	15.0 ± 1.8^{b} *(40%)*	ns	**	*
Σ flavan-3-ol	**30 ± 4**	**38 ± 11**	**38 ± 6**	**ns**	**ns**	*

Values with different letters in single rows are significantly different ($P < 0.05$). *, **, *** indicate significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively; ns, not significant. m1, 20 days after veraison; m2, 10 days before harvest; m3, harvest.

plexity of the flavonol family. Tannat seed flavanols consisted of three monomers (catechin, epicatechin and epicatechin gallate), procyanidins and galloylated procyanidins; while four monomers (catechin, epicatechin, gallocatechin and epigallocatechin), procyanidins and prodelphinidins were found in skins. The same flavanols were reported in other grape varieties (Mattivi et al. 2009).

Seeds Except for the galloylated hexamers, the different groups of flavan-3-ol identified in seeds showed significant differences for the sampling date effect in the ANOVA (Table 2). The same situation was found with the vineyard effect, except for the most polymerised procyanidins, the galloylated and non-galloylated pentamers and hexamers, and for digalloylated tetramers. Increases in concentration for most groups of

Table 3. Mean values and standard deviations ($n = 8$, samples from two vineyards, extracted in duplicate and each extract analysis performed in duplicate) and significance level in the analysis of variance for the flavonol content in Tannat grape skins during ripening.

	m1	m2	m3	ANOVA		
				Vineyard	Date	Vineyard × Date
Skin (mg/Kg)						
Myricetin-3-*O*-galactoside	17 ± 2^a *(9%)*	22 ± 2^b *(8%)*	24 ± 2^c *(8%)*	***	***	ns
Myricetin-3-*O*-glucoside	24 ± 6^a *(12%)*	49 ± 10^b *(18%)*	65 ± 16^c *(21%)*	***	***	***
Quercetin-3-*O*-galactoside	18 ± 3^a *(9%)*	24 ± 3^b *(9%)*	27 ± 2^c *(9%)*	***	***	ns
Quercetin-3-*O*-glucoside	39 ± 13^a *(20%)*	59 ± 8^b *(21%)*	65 ± 4^b *(20%)*	ns	***	ns
Laricetrin-3-*O*-glucoside	18 ± 3^a *(9%)*	26 ± 2^b *(9%)*	30 ± 2^c *(10%)*	***	***	ns
Quercetin-7-*O*-neohesperidoside	18 ± 3^a *(9%)*	26 ± 2^b *(9%)*	29 ± 2^c *(9%)*	***	***	ns
Isorhamnetin-3-*O*-glucoside	18 ± 3^a *(9%)*	23 ± 3^b *(8%)*	24 ± 3^c *(8%)*	***	***	ns
Syringetin-3-*O*-glucoside	21 ± 7^a *(11%)*	24 ± 3^b *(9%)*	26 ± 3^b *(8%)*	***	**	ns
Quercetin aglycone	22 ± 7 *(11%)*	25 ± 6 *(9%)*	24 ± 6 *(8%)*	***	ns	ns
Σ flavonol	**196 ± 39^a**	**277 ± 14^b**	**315 ± 16^c**	**	***	ns

Values with different letters in single rows are significantly different ($P < 0.05$). *, **, *** indicate significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively; ns, not significant. m1, 20 days after veraison; m2, 10 days before harvest; m3, harvest.

compounds were similar during ripening for the two vineyards. This is shown by the non-significant interaction in the ANOVA for the vineyard and sample date factors (Table 2).

Table 2 shows the total flavan-3-ol content for Tannat seeds with the highest levels reaching 1946 mg/kg of grape. This amount of flavan-3-ol is the highest reported for a large number of varieties (Núñez et al. 2006, Mattivi et al. 2009). An increase in concentration at maturity was also noted (Table 2); however, this was not significant in the samples from the last time point. Increases in concentration during ripening were found for all groups of flavan-3-ols, except for the epicatechin gallate, which showed a significant decrease over the time course of sampling.

The concentration increase can be explained mainly by the decrease in berry weight (approximately 18%) during the sampling period (Table 1). Moreover, the flavan-3-ol content expressed as mg/g of dry seed declined during maturation (data not shown), in agreement with data obtained during maturation of other red grape varieties (Downey et al. 2003, Obreque-Slier et al. 2010).

The monomer concentration did not vary significantly during ripening and therefore, as dimer and oligomer concentrations increased, the percentage of monomers decreased, indicating an increase in the mean degree of polymerisation (Table 2). Previous studies reported for Cabernet Sauvignon grapes, showed that the mean degree of polymerisation of procyanidins appeared unchanged during fruit ripening by HPLC analysis, but appeared to decrease when analysed by thiolytic degradation (Kennedy et al. 2000a).

The galloylated compound content represented about 40% of total flavan-3-ols in seeds (Table 2), with a decrease in the galloylated over non-galloylated ratio during ripening, mostly caused by the decrease in epicatechin gallate. The galloylated compound content found in this study was higher than that reported after depolymerisation by Souquet et al. (2000) in Tannat grapes as well as in other studies with other red grape varieties (Mattivi et al. 2009, Obreque-Slier et al. 2010),

Skins There were no significant differences in total flavan-3-ol amount in skins from the two vineyards, with mean values of 35 mg/kg of grapes, nor as consequence of sampling date (Table 2). The values were similar to those determined in other studies for other red grape varieties (De Freitas et al. 2000, Peña-Neira et al. 2004, Rodríguez-Montealegre et al. 2006). Moreover, the ANOVA for flavan-3-ols in skins did not indicate significant differences in material obtained on different sampling dates for most of the compounds, except for epigallocatechin and prodelphinidins (Table 2).

Galloylated forms were absent in skins of Tannat grapes, although these compounds were found in skins of Syrah grapes at low concentration (Downey et al. 2003). The prodelphinidin content represented 30–35% of the total flavan-3-ols (Table 2), which is consistent with previous reports for this variety regarding prodelphinidin content analysed after depolymerisation (Souquet et al. 2000). The relationship between procyanidin and prodelphinidin contents decreased during maturation (from 66 to 60% at harvest). Finally, very low values of epigallocatechins (3%) were detected in the flavan-3-ol profile when compared with those reported in other varieties (Mattivi et al. 2009).

Contrary to what has been reported for a large number of varieties (Downey et al. 2003, Mattivi et al. 2009, Obreque-Slier et al. 2010), flavan-3-ol monomers were the most important group quantified in Tannat skins (Table 2). At maturity, the percentage of monomers in the skin samples decreased, which corresponded to the incremental increase in dimers and oligomers, and therefore being an indicator of the increase in degree of polymerisation. These results were consistent with those of Kennedy et al. (2001), but contrast with data reported in the literature for other varieties (Downey et al. 2003, Obreque-Slier et al. 2010).

Wine Thirty-one flavan-3-ols were identified and quantified in samples of Tannat wines, but seven of them (one procyanidin trimer, one tetramer and one pentamer, as well as three prodelphynidin dimer and one trimer) were absent in grape samples (seeds and skins) of this variety. These latter seven compounds were present in all wine samples analysed suggesting their presence could be associated with hydrolysis of more highly polymerised forms.

Table 4. Mean values and standard deviations ($n = 8$, samples from two vineyards, extracted in duplicate and each extract analysis performed in duplicate), and significance level in the analysis of variance for the pigment compound content in Tannat grape skins during ripening.

	m1	m2	m3	ANOVA		
				Vineyard	Date	Vineyard × Date
Skin (mg/kg)						
Delphinidin-3,7-diglucoside	12 ± 5	15 ± 4	14 ± 5	***	ns	ns
Delphinidin-3-*O*-glucoside	318 ± 153^{a}	543 ± 89^{b}	572 ± 105^{b}	ns	***	ns
Petunidin-3,7-diglucoside	9 ± 3	12 ± 4	11 ± 2	**	ns	ns
Cyanidin-3-*O*-glucoside	50 ± 23^{a}	79 ± 11^{b}	84 ± 13^{b}	ns	***	ns
Petunidin-3-*O*-glucoside	230 ± 97^{a}	393 ± 43^{b}	430 ± 49^{b}	ns	***	ns
Malvidin-3,7-diglucoside	18 ± 8	22 ± 11	16 ± 10	*	ns	ns
Peonidin-3-*O*-glucoside	61 ± 26^{a}	116 ± 12^{b}	140 ± 19^{c}	ns	***	ns
Malvidin-3-*O*-glucoside	649 ± 215^{a}	1246 ± 55^{b}	1455 ± 108^{c}	ns	***	ns
Delphinidin-3-*O*-(6′-acetyl)-glucoside	45 ± 26^{a}	75 ± 17^{b}	91 ± 21^{b}	ns	***	ns
Cyanidin-3-*O*-(6′-acetyl)glucoside	14 ± 6^{a}	22 ± 3^{b}	27 ± 4^{c}	ns	***	ns
Petunidin-3-O-(6′-acetyl)glucoside	37 ± 20^{a}	62 ± 10^{b}	76 ± 14^{b}	ns	***	ns
Peonidin-3-O-(6′-acetyl)glucoside	11 ± 5^{a}	21 ± 3^{b}	29 ± 5^{c}	ns	***	ns
Malvidin-3-*O*-(6′-acetyl)glucoside	108 ± 52^{a}	210 ± 20^{b}	271 ± 44^{c}	ns	***	ns
Delphinidin-3-*O*-(6′-*p*-coumaroyl)glucoside	33 ± 15^{a}	51 ± 9^{b}	64 ± 14^{c}	ns	***	ns
Petunidin-3-*O*-(6′-*p*-coumaroyl)glucoside (*cis*)	8 ± 2^{a}	12 ± 1^{b}	15 ± 1^{c}	ns	***	ns
Malvidin-3-O-(6′-caffeoyl)glucoside	8 ± 1^{a}	15 ± 4^{b}	22 ± 8^{c}	***	***	***
Cyanidin-3-O-(6′-*p*-coumaroyl)glucoside	17 ± 7^{a}	26 ± 3^{b}	31 ± 5^{c}	ns	***	ns
Petunidin-3-O-(6′-*p*-coumaroyl)glucoside (*trans*)	44 ± 18^{a}	73 ± 9^{a}	90 ± 14^{b}	ns	***	ns
Malvidin-3-O-(6′-*p*-coumaroyl)glucoside (*cis*)	8 ± 3^{a}	17 ± 4^{b}	26 ± 4^{c}	ns	***	ns
Peonidin-3-*O*-(6′-*p*-coumaroyl)glucoside (*trans*)	13 ± 6^{a}	24 ± 4^{b}	33 ± 6^{c}	ns	***	ns
Malvidin-3-*O*-(6′-*p*-coumaroyl)glucoside (*trans*)	113 ± 49^{a}	234 ± 29^{b}	315 ± 50^{c}	ns	***	ns
Po/Cy	1.05 ± 0.03^{a}	1.27 ± 0.04^{b}	1.42 ± 0.02^{c}	ns	***	***
Mv/Dp	2.38 ± 0.33^{a}	2.64 ± 0.46^{ab}	2.92 ± 0.29^{b}	ns	**	*
Pt/Dp	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	ns	ns	ns
Mv/Pt	2.9 ± 0.2^{a}	3.2 ± 0.3^{b}	3.4 ± 0.1^{c}	ns	***	**
(Dp + Pt + Mv)/(Cy + Po)	9.8 ± 0.5	10.3 ± 0.8	10.0 ± 0.4	ns	ns	ns
Σ diglucoside	39 ± 17 *(2.1%)*	49 ± 18 *(1.4%)*	41 ± 15 *(1.0%)*	**	ns	ns
Σ glucoside	1307 ± 513^{a} *(72%)*	2377 ± 170^{b} *(73%)*	2680 ± 284^{b} *(70%)*	ns	***	ns
Σ acetylglucoside	215 ± 108^{a} *(12%)*	390 ± 51^{b} *(12%)*	494 ± 86^{c} *(13%)*	ns	***	ns
Σ coumaroylglucoside	237 ± 96^{a} *(13%)*	437 ± 46^{b} *(13%)*	573 ± 90^{c} *(15%)*	ns	***	ns
Σ caffeoylglucoside	8 ± 1^{a} *(0.5%)*	15 ± 4^{b} *(0.4%)*	22 ± 8^{c} *(0.5%)*	***	***	***
Σ anthocyanin	**1807 ± 718^{a}**	**3268 ± 206^{b}**	**3810 ± 443^{b}**	**ns**	*******	**ns**

Values with different letters in single rows are significantly different ($P < 0.05$). *, **, *** indicate significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively; ns, not significant. Po/Cy, peonidin and cyanidin ratio; Mv/Dp, malvidin and delphinidin ratio; Pt/Dp, petunidin and delphinidin ratio; Mv/Pt, malvidin and petunidin ratio; (Dp + Pt + Mv)/(Cy + Po), ring-B trisubstituted and disubstituted anthocyanin ratio; m1, 20 days after veraison; m2, 10 days before harvest; m3, harvest.

Epicatechin and epigallocatechin levels were at higher values than the corresponding isomers, catechin and gallocatechin, respectively (Table 6). Previous studies reported higher values for epicatechin in Merlot and Nero d'Avola wines (La Torre et al. 2006), but a higher concentration of catechin was found in wines made from Tempranillo, Graciano and Cabernet Sauvignon varieties (Monagas et al. 2003). In contrast with the concentrations found in Tannat wines, in grape skins of this variety, levels of gallocatechin were greater than the epigallocatechin isomer. Changes in the levels of these compounds during fermentation might have resulted from differences in ease of extraction during maceration or by hydrolysis of polymerised forms.

Epicatechin gallate was not detected in any of the wine samples analysed, while galloylated forms represented only 7.1% of flavan-3-ols, a very low percentage compared with that found in seeds (39% at harvest). Under the most favorable conditions for extraction of phenol compounds used in this study involving 15 days of maceration (González-Manzano et al. 2004), results indicated that these compounds are not easily extracted from Tannat seeds during winemaking.

The values obtained for prodelphinidin percentage, 18% of the total flavan-3-ol, were similar to those measured after depolymerisation in wines from Syrah and its blends (Maury et al. 2001), and in monovarietal red wines from Lisbon (Cosme et al. 2009).

Characterisation of flavonols in Tannat grapes and wines

Quercetin and myricetin, the most important aglycones in a large number of red grape varieties (Mattivi et al. 2006), were 38 and 30%, respectively, of the total flavonol content in Tannat

Table 5. Mean values and standard deviations ($n = 8$, samples from two vineyards, extracted in duplicate and each extract analysis performed in duplicate), and significance level in the analysis of variance for the phenolic acid content in Tannat grapes, skins and seeds during ripening.

	m1	m2	m3	ANOVA		
				Vineyard	Date	Vineyard × Date
Seed (mg/kg)						
Gallic acid	9.5 ± 0.9^{c}	18.5 ± 1.7^{b}	24.3 ± 5.5^{a}	***	***	*
Protocatechuic acid	1.3 ± 0.1^{c}	3.0 ± 0.3^{b}	3.2 ± 0.2^{a}	ns	***	ns
Σ phenolic acid	**10.8 ± 1.0^{a}**	**21.4 ± 1.8^{b}**	**27.5 ± 5.5^{c}**	**	***	ns
Skin (mg/kg)						
Gallic acid	1.1 ± 0.2^{a}	1.0 ± 0.7^{a}	1.7 ± 0.3^{b}	**	**	*
Protocatechuic acid	0.9 ± 0.6	1.7 ± 1.4	1.9 ± 0.5	ns	ns	ns
Methyl gallate	4.0 ± 1.5	4.0 ± 1.7	4.4 ± 0.7	ns	ns	**
trans-caftaric acid	7.4 ± 0.8^{b}	6.2 ± 0.4^{a}	6.9 ± 1.7^{b}	***	*	**
trans-fertaric acid	8.9 ± 1.1^{b}	7.5 ± 0.4^{a}	8.9 ± 2.5^{b}	**	*	*
trans-coutaric acid	3.8 ± 0.1^{c}	3.0 ± 0.2^{a}	3.4 ± 1.0^{b}	**	**	***
p-coumaroyl hexose (1)	1.8 ± 0.4^{a}	2.4 ± 0.8^{a}	2.6 ± 0.3^{b}	**	*	*
p-coumaroyl hexose (2)	1.2 ± 0.1^{a}	1.4 ± 0.4^{ab}	1.7 ± 0.2^{b}	ns	*	ns
Σ hydroxybenzoic acid	6.0 ± 1.3^{a} *(21%)*	6.6 ± 3.8^{ab} *(24%)*	8.0 ± 0.8^{b} *(25%)*	*	*	**
Σ hydroxycinnamic acid	23.1 ± 2.2^{a} *(79%)*	20.5 ± 1.7^{ab} *(76%)*	23.5 ± 5.3^{b} *(75%)*	**	*	*
Σ phenolic acid	**29.1 ± 1.2^{ab}**	**27.1 ± 5.1^{a}**	**31.5 ± 5.2^{b}**	**	*	ns

Values with different letters in single rows are significantly different ($P < 0.05$). *, **, *** indicate significance at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively; ns, not significant. m1, 20 days after veraison; m2, 10 days before harvest; m3, harvest.

grapes (Table 3). Total favonol content was increased at maturity because of a simultaneous decrease in berry weight and an increase in the content of these compounds per berry (data not shown).

Quercetin was the only free aglycone identified in grapes, accounting for about 10% of the total content of flavonols. The compound was 30% of flavonols in wine (Table 6), possibly caused by hydrolysis of glycosylated forms during winemaking (Ribereau-Gayon et al. 2006). Indeed, other free aglycones not detected in grapes, such as myricetin and laricitrin, were identified and quantified in Tannat wine samples.

ANOVA showed significant differences in the concentration of all flavonols during ripening, and significant differences in the samples depending on the vineyard origin of the grapes, except for quercetin-3-O-glucoside. Besides, only for myricetin-3-O-glucoside was the evolution during ripening different for the two vineyard samples with significant interaction in the ANOVA for the vineyard and sample date effects (Table 3).

When compared with other grape varieties investigated, the total flavonol content determined for Tannat grapes was higher than that reported for Italian grapes varieties (Tamborra and Esti 2010), Jaen Tinto and Palomino Negro (Guerrero et al. 2009), and lower than that reported for Tintilla Rota, Cabernet Sauvignon and Tempranillo (Guerrero et al. 2009). The flavonol content in the Tannat wines analysed were higher than that reported for Merlot and Nero d'Avola (La Torre et al. 2006), Cabernet Sauvignon (Puértolas et al. 2010) and wines made from a native Turkish grape variety (Kelebek et al. 2010).

Characterisation of anthocyanins in Tannat grapes and wines

The total anthocyanin content at harvest was 3810 mg/kg of grape (Table 4), a higher value than that reported by Mattivi et al. (2006) for 56 of the 64 varieties investigated in their study. In Tannat wine samples, the anthocyanin content was 1299 mg/L (Table 6). Considering a wine yield of 750 mL per kilogram of grapes during Tannat vinification, the total anthocyanins content in wine was near 45% of the value at harvest. This percentage indicates the degree of extraction during maceration as well as losses caused by 4 months of ageing in barrels.

The ANOVA for content of individual compounds in grapes showed a significant increase during maturation (Table 4). The 3,7-diglucosides found in small quantities also described in a previous report (Castillo-Muñoz et al. 2010), were the only compounds that showed no significant differences in levels or modification during maturation. However, these compounds and malvidin-3-O-caffeoylglucoside were the only ones exhibiting significant differences depending on the vineyard used. The evolution of the concentration of most anthocyanin compounds during ripening was similar for the two vineyards. This was illustrated by the non-significant interaction in the ANOVA for the vineyard and sample date factors (Table 4).

Malvidin, delphinidin and petunidin-3-O-glucosides were the main pigments within this family in the samples. The same hierarchy, in quantitative terms, was observed for the acetyl and coumaroyl glucosides. In the same way, the nonacylated monoglucosides of the anthocyanins represented the most abundant group of pigments in Tannat grapes, while acetylglucosides and coumaroylglucosides represented 13.0 and 15.2%, respectively. These results were in agreement with previous studies by our group (Boido et al. 2006).

In contrast, the acetylglucoside content was higher than that of coumaroylglucoside in wines analysed (Table 6). This was in agreement with previous reports (García-Beneytez et al. 2002, Alcalde-Eon et al. 2006a,b, Boido et al. 2006, Ristic et al. 2010), and was attributed to hydrolysis during fermentation and wine ageing, and lower extractability and solubility of

Table 6. Mean values and standard deviations (n = 4, two wines analysed in duplicate) of phenolic components of Tannat wines.

Flavan-3-ol (mg/L)	
(+)-gallocatechin	14 ± 1
(−)-epigallocatechin	60 ± 6
(+)-catechin	43 ± 6
(−)-epicatechin	65 ± 7
Dimer non-galloylated	205 ± 19
Trimer non-galloylated	194 ± 9
Tetramer non-galloylated	73 ± 12
Pentamer non-galloylated	27.1 ± 0.7
Dimer galloylated	48 ± 8
Trimer galloylated	3.7 ± 0.2
Σ non-galloylated	681 ± 32 *(92.9%)*
Σ galloylated	52 ± 8 *(7.1%)*
Σ procyanidin	601 ± 33 *(82.0%)*
Σ prodelphinidin	132 ± 9 *(18.0%)*
Σ monomer	182 ± 18 *(24.7%)*
Σ dimer	253 ± 27 *(34.5%)*
Σ oligomer	298 ± 12 *(40.8%)*
Σ flavan-3-ol	**733 ± 40**
Phenolic acid (mg/L)	
Gallic acid	86 ± 11
Protocatechuic acid	13 ± 1
Methyl gallate	38 ± 1
cis-caftaric acid	48 ± 39
trans-caftaric acid	41 ± 25
p-coumaroyl hexose (1)	25 ± 2
trans-caffeic acid	86 ± 48
p-coumaroyl hexose (2)	24 ± 1
p-coumaric acid	30 ± 22
Σ hydroxybenzoic acid	137 ± 13 *(34.9%)*
Σ hydroxycinnamic acid	255 ± 8 *(65.1%)*
Σ phenolic acid	**391 ± 19**
Flavonols (mg/L)	
Myricetin-3-*O*-galactoside	12.6 ± 0.2
Myricetin-3-*O*-glucoside	30 ± 2
Quercetin-3-*O*-galactoside	14 ± 1
Quercetin-3-*O*-glucoside	17.8 ± 0.9
Laricetrin-3-*O*-glucoside	17.1 ± 0.4
Syringetin-3-*O*-glucoside	27 ± 2
Myricetin aglycone	17.4 ± 0.8
Quercetin aglycone	22 ± 4
Laricetrin aglycone	12.9 ± 0.8
Σ flavonol	**170 ± 4**
Pigment compounds (mg/L)	
DCP between malvidin-3-*O*-glucoside and gallocatechin	8.4 ± 0.6
DCP between petunidin-3-*O*-glucoside and catechin	8 ± 1
DCP between malvidin-3-*O*-glucoside and catechin	14 ± 1

Pigment compounds (mg/L) (continuation)	
Vitisin A of delphinidin-3-*O*-glucoside	10 ± 3
Delphinidin-3-*O*-glucoside	98 ± 8
Cyanidin-3-*O*-glucoside	14 ± 1
Petunidin-3-*O*-glucoside	106 ± 13
Vitisin A of Petunidin-3-*O*-glucoside	10 ± 1
Malvidin-3,7-diglucoside	21 ± 2
Peonidin-3-*O*-glucoside	27 ± 3
Malvidin-3-*O*-glucoside	470 ± 36
Vitisin A of Malvidin-3-*O*-glucoside	20 ± 2
Delphinidin-3-*O*-(6′-acetyl)-glucoside	48 ± 8
Vitisin A of malvidin-3-*O*-(6′-acetyl)glucoside	13 ± 2
Cyanidin-3-*O*-(6′-acetyl)glucoside	16 ± 2
Petunidin-3-O-(6′-acetyl)glucoside	40 ± 7
Malvidin-3-*O*-glucoside-8-ethyl-catechin	11 ± 1
DCP between malvidin-3-*O*-(6′-*p*-coumaroyl)glucoside and catechin	11 ± 2
Malvidin-3-*O*-glucoside-8-ethyl-epicatechin	7.9 ± 0.9
Peonidin-3-O-(6′-acetyl)glucoside	16 ± 3
Malvidin-3-*O*-(6′-acetyl)glucoside	115 ± 8
Delphinidin-3-*O*-(6′-*p*-coumaroyl)glucoside	11.7 ± 0.5
Malvidin-3-O-(6′-caffeoyl)glucoside	10 ± 2
Cyanidin-3-O-(6′-*p*-coumaroyl)glucoside	16 ± 2
Petunidin-3-O-(6′-*p*-coumaroyl)glucoside	27 ± 3
Malvidin-3-O-(6′-*p*-coumaroyl)glucoside (*cis*)	18 ± 2
Malvidin-3-*O*-(6′*p*-coumaroil)glucoside-8-ethylcatechin	8 ± 1
Peonidin-3-*O*-(6′-*p*-coumaroyl)glucoside	9.6 ± 0.6
Malvidin-3-*O*-(6′-*p*-coumaroyl)glucoside (*trans*)	63 ± 7
Malvidin 3-*O*-glucoside-4-vinylcatechol adduct	11 ± 2
Malvidin 3-*O*-glucoside-4-vinylphenol adduct	11 ± 2
Malvidin 3-*O*-glucoside-4-vinylguaiacol adduct	7 ± 1
Malvidin-3-*O*-(6′-acetyl)glucoside-4-vinylphenol adduct	7 ± 1
Malvidin-3-*O*-(6′-acetyl)glucoside-4-vinylguaiacol adduct	4.5 ± 0.6
Malvidin-3-*O*-(6′-*p*-coumaroyl)glucoside-4-vinylphenol adduct	4.9 ± 0.7
Malvidin-3-*O*-(6′-*p*-coumaroyl)glucoside-4-vinylguaiacol adduct	3.5 ± 0.4
Σ diglucoside	21 ± 2 *(1.6%)*
Σ glucoside	716 ± 62 *(55.1%)*
Σ acetylglucoside	236 ± 26 *(18.2%)*
Σ coumaroylglucoside	146 ± 12 *(11.2%)*
Σ caffeoylglucoside	10 ± 2 *(0.8%)*
Σ direct condensation dimer	41 ± 2 *(3.2%)*
Σ acetaldehyde mediated dimer	27 ± 3 *(2.1%)*
Σ pyranoanthocyanin	102 ± 13 *(7.8%)*
Σ pigment compounds	**1299 ± 119**

coumaroylglucoside compounds. In wines analysed, anthocyanin-derived pigments represented 13% of total pigments. Quantitatively, the most abundant group were the pyranoanthocyanins (Table 6), consistent with previous reports on the evolution of pigments in Tannat wines (Boido et al. 2006). We also studied the relationship between different anthocyanins in grapes and their evolution during maturation (Table 4). During biosynthesis, two primary anthocyanins (cyanidin and delphinidin) are synthesised in the cytosol of berry epidermal cells (Holton and Cornish 1995). The B-ring of cyanidin is di-hydroxylated at the 3′ and 4′ positions, whereas delphinidin has a tri-hydroxylated B-ring with a hydroxyl group also at the 5′ position (Holton and Cornish 1995). These anthocyanins are derived from parallel pathways in which flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′,5′-hydroxylase (F3′5′H) generate cyanidin and delphinidin, respectively (Bogs et al. 2006). The 3′ position of cyanidin and delphinidin and subsequently the 5′ position of delphinidin can be methoxylated by O-methyl transferase (OMT) to generate peonidin, petunidin and malvidin, respectively (Castellarin et al. 2007).

In Tannat grapes, peonidin over cyanidin (Po/Cy) and malvidin over delphinidin (Mv/Dp) ratios increased significantly during ripening (Table 4), peonidin was formed by methoxylation of cyanidin at the 3′ position of the B-ring, and malvidin of delphinidin at the corresponding 3′ and 5′ positions (Holton and Cornish 1995, Castellarin et al. 2007). This result suggested a difference in the rate of biosynthesis of these compounds, and may reflect changes in OMT enzyme activity during maturation (Holton and Cornish 1995, Lücker et al. 2010). However, during ripening no significant differences were observed in the levels of delphinidin and petunidin, derived from methoxylation of B-ring 3′ position of delphinidin. This suggests that petunidin might undergo additional methoxylation to produce malvidin, which is consistent with an increase in the malvidin over petunidin (Mv/Pt) ratio (Table 4). The evolution of the relative levels of these compounds differed between vineyards, shown by the significant values of the interaction in the ANOVA. We suggest that this may indicate differences in the biosynthesis of these compounds that resulted from the different culture conditions. Variations in OMT activity have been reported for different conditions in a water stressed vineyard (Castellarin et al. 2007).

It is important to note that the changes in the relative levels of methoxylated anthocyanins was not observed for the methoxylated flavonols (data not shown), although the biosynthesis of flavonols was closely related to that of anthocyanins (Bogs et al. 2006). There is no evidence, however, that the enzymes involved in the O-methylation of the flavonols and the anthocyanins are related. Furthermore, the flavonols are bonded to different sugars and O-methylation occurs after the glycosylation step (Lücker et al. 2010).

The ratio of trisubstituted B-ring anthocyanins (delphinidin, petunidin and malvidin) to those disubstituted (cyanidin and peonidin) did not change during maturation (Table 4), suggesting similar rates for biosynthesis for the two groups of compounds and similar activities for the F3′H and F3′5′H enzymes (Holton and Cornish 1995). In ripe berries, there was a predominance of blue tri-hydroxylated anthocyanins (90%). This percentage was similar to that obtained in Tempranillo (89.7%) and Aglianico (93.2%) varieties, and far from the 70.0% and 14.5% found in Pinot Noir and Grignolino varieties, respectively (Castellarin and Di Gaspero 2007).

Characterisation of phenolic acids in Tannat grapes and wines

Eleven phenolic acids were identified and quantified. Only gallic and protocatechuic acid were identified in the seeds, in agreement with the data reported for other red grape varieties (Rodríguez-Montealegre et al. 2006), and only the free forms of caffeic and *p*-coumaric acids were found in wine samples. Syringic acid was not identified in either Tannat grape skins or wine samples even though it is found in grape skin (Obreque-Slier et al. 2010) and wines (La Torre et al. 2006) of other varieties.

The two acids quantified in the seeds, gallic and protocatechuic acid, showed a significant increase in concentration during maturation (Table 5). However, in skins, total phenolic acids did not present a clear increase during ripening, while the concentration of methyl gallate and protocatechuic acid showed no significant differences at different samples times.

The hydroxycinnamic acid content was higher than that of hydroxybenzoic acid in Tannat skins, representing 75% of the total phenolic acids at harvest. In addition, the concentration of *trans*-caftaric and *trans*-coutaric acids reported in Cabernet Sauvignon and Tempranillo and in native varieties of Andalucia, was lower than in the Tannat variety (Guerrero et al. 2009).

In wines, the phenolic acid content was higher than that found in grapes (Table 6), possibly caused by a contribution of phenolic acids presented in the pulp (Tian et al. 2009). Moreover, the relationship between hydroxybenzoic and hydroxycinnamic acids was different from that found in skins, in which the percentage of the latter decreased to 65%. This modification can be explained by the contribution of hydroxybenzoic acid from seeds or by the hydrolysis of galloylated flavan-3-ols during the vinification (Monagas et al. 2005).

Caffeic and *p*-coumaric acids, products of hydrolysis of coutaric and caftaric acids, respectively (Puértolas et al. 2010), were quantified only in the wine samples. The caftaric acid *cis*/*trans* ratio was near one (Table 6), this ratio was 4.8 in Malbec wines (Fanzone et al. 2010), and less than one in Öküzgözü wines, a native grape variety of Turkey (Kelebek et al. 2010). In Tannat wines, unlike in grape, fertaric acid was not detected, nor its hydrolysis product, ferulic acid.

The gallic, protocatechuic and hydroxycinnamic acid content found in Malbec wines from different regions of Argentina (Fanzone et al. 2010) and in Öküzgözü wines (Kelebek et al. 2010), was smaller than that found in the Tannat wines analysed. The protocatechuic and hydroxycinnamic acid content found in Nero d'Avola wines and in wines from other varieties grown in the Sicilian region (Merlot, Syrah, Cabernet Sauvignon) were also reported as having lower values compared with those found in Tannat. The gallic acid content was similar in wine from these five varieties (La Torre et al. 2006).

Conclusions

In this study we showed that Tannat seeds had a higher content of flavan-3-ols than that reported for a large number of other grape varieties. The variations in the monomer concentration were not significant during ripening, but an increase in the dimer and oligomer forms was observed. Galloylated compounds represented around 40% of the total flavan-3-ols, which decreased during ripening most likely because of a corresponding decrease of epicatechin gallate.

The total flavan-3-ols in skins showed no significant differences during ripening, with values similar to those found in other red grape varieties.

The profile of flavan-3-ol in skins was characterised by the absence of galloylated forms, the presence of monomeric forms as the most abundant, a 30–35% prodelphinidin content, and very low values for epigallocatechin.

Epicatechin gallate was not detected in Tannat wine and galloylated forms represented a low percentage of the total

flavan-3-ols, indicative of ineffective extraction or hydrolysis of these compounds during vinification.

Tannat grapes were characterised as having very high concentrations of anthocyanin in skins, with an increase in anthocyanin and flavonol concentrations during ripening. The highest levels of anthocyanins found were malvidin, delphinidin and petunidin monoglucosides, and flavonols, quercetin and myricetin.

In Tannat grapes, Po/Cy and Mv/Dp ratios increased significantly during ripening, indicating differences in the biosynthetic rate for these compounds that may be caused by the differences in activity or expression of OMTs. In ripe berries, there was a predominance of blue tri-hydroxylated anthocyanins (90%), with no change during maturation.

Eleven phenolic acids were identified in Tannat grapes and wines, but only gallic and protocatechuic acid were found in seeds. These acids increased in concentration in seeds during ripening; however, a corresponding increase in these phenolic acids in the skins was not evident.

Acknowledgements

The authors thank the Instituto Nacional de Investigación Agropecuaria for the financial support (INIA grant FPTA-240). Boido thanks the Comisión Sectorial de Investigación Científica (CSIC) for providing support for studies at Salamanca University. The authors also thank the Spanish MICINN (Project ref. AGL2008-05569-C02-01) and the Junta de Castilla y León (group GR133) for financial support.

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Manuscript received: 31 January 2011
Revised manuscript received: 3 July 2011
Accepted: 10 July 2011