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Tannin profiles of *Vitis vinifera* L. cv. red grapes growing in Lisbon and from their monovarietal wines

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ABSTRACT

The tannin profiles of five grape (*Vitis vinifera* L.) varieties 'Touriga Nacional', 'Trincadeira', 'Castelão', 'Syrah' and 'Cabernet Sauvignon' and the profiles of their red monovarietal wines from their 2004 and 2005 vintages were studied. Depending on the variety, the polymeric fractions represented 77–85% in seeds and 91–99% in skins. The distribution of the mean degree of polymerisation (mDP) of the proanthocyanidins ranged from 2.8 to 12.8 for seeds and from 3.8 to 81.0 for skins. In the monovarietal wines, the distribution of the mDP of the proanthocyanidins ranged from 2.1 to 9.6. Of the total proanthocyanidins the polymeric fraction represented 77–91% in vintage 2004 and 82–95% in vintage 2005. The wine proanthocyanidins of Trincadeira and Cabernet Sauvignon showed similar tannin profiles in each vintage. After six months of storage, noticeable decreases in total proanthocyanidins concentration were measured. These were accompanied by slight decreases in prodelphinidin percentages but the percentage of galloylation and mDP remained similar.

1. Introduction

Proanthocyanidins are found in all the parts of a grape cluster but skins contain lower amounts of proanthocyanidins than do seeds, and their structural characteristics also differ (Bourzeix, Weyland, & Heredia, 1986; Ricardo-da-Silva, Rigaud, Cheynier, Cheminat, & Moutounet, 1991b). Grape seed tannins comprise only the procyanidins (Labarbe, Cheynier, Brossaud, Souquet, & Moutounet, 1999; Prieur, Rigaud, Cheynier, & Moutounet, 1994), whereas grape skin tannins comprise both prodelphinidins and procyanidins (Labarbe et al., 1999; Souquet, Cheynier, Brossaud, & Moutounet, 1996). Skin proanthocyanidins have a higher mDP and a lower percentage of galloylated subunits than those from seeds (Cheynier, Prieur, Guyot, Rigaud, & Moutounet, 1997; Moutounet, Rigaud, Souquet, & Cheynier, 1996).

According to Prieur et al. (1994) the grape seed proanthocyanidins (*Vitis vinifera*, var. Alicante Bouchet) showed mDP values ranging from 2.3 (fraction 1) to 15.1 (fraction 5), and the percentage of galloylation (% gal) increased with mDP from 13.2% to 30.2%. Therefore, Sun, Leandro, Ricardo-da-Silva, and Spranger (1998) determined a mDP of 9.8 and 31.5 and a % gal of 23.0% and 26.2%, in oligomeric and polymeric proanthocyanidins of seed extracts (*V. vinifera*, var. Tinta Miúda). The mDP of the seed proanthocyanidins (*V. vinifera*, var. Cabernet Franc) characterised by Labarbe et al. (1999) ranged from 4.7 (fraction 1) to 15.7 (fraction 8). However, these authors showed that the % gal remained constant (20%) in each fraction, which seems to indicate that the extension of galloylation is independent of mDP. Vidal et al. (2003) also studied the structural characteristics of seed proanthocyanidins from *V. vinifera*, var. Syrah separated into two fractions and verified mDP values of 2.8 and 8.9 with % gal of 16.2 and 22.5 in these two fractions, respectively. Perret, Pezet, and Tabacchi (2003) separated seed proanthocyanidins from *V. vinifera*, var. Gamay into ten fractions and observed that their mDP varied from 1.8 to 19.3. Kennedy and Taylor (2003) separated seed proanthocyanidins from *V. vinifera*, var. Pinot noir into five fractions with mDP of 2.0 to 24.1. The mDP and the % gal of the seed polymeric proanthocyanidins from Tempranillo (mDP = 7.1, % gal = 14.3), Graciano (mDP = 7.3, % gal = 10.9) and Cabernet Sauvignon (mDP = 6.4, % gal = 12.9) were determined by Monagas, Gómez-Cordovés, Bartolomé, Laureano, and Ricardo-da-Silva (2003). The mDP determined in seeds from *V. vinifera* cv. Cabernet Sauvignon at harvest was 5.6 (Kennedy, Matthews, & Waterhouse, 2000) and in seed from Syrah around 5.0 (Downey, Harvey, & Robinson, 2003; Kennedy, Troup, et al., 2000).

The mDP of skin proanthocyanidins (*V. vinifera* var. Merlot) determined by Souquet et al. (1996) ranged from 3 (fraction 1) to 80 (fraction 6), and the percentage of prodelphinidins (% prodelph) ranged from 17% to 31%. Nevertheless, these authors showed that the % gal was low (3–6%), and seems to be independent of mDP.

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Likewise, skin proanthocyanidins (*V. vinifera*, var. Cabernet Franc) analysed by Labarbe et al. (1999), represented mDP values that rose from 9.3 (fraction 1) to 73.8 (fraction 11). These authors also showed that the % gal (2.7%) was low and independent of mDP, and that the % prodelph increased slightly with increasing mDP. Vidal et al. (2003) in skin proanthocyanidins from *V. vinifera*, var. Syrah separated into three fractions found that the mDP ranged from 3.0 to 19.8 and the % prodelph ranged from 9.0 to 16.3; however, the % gal was around 4%. Kennedy and Taylor (2003) separated skin proanthocyanidins from *V. vinifera*, var. Pinot noir into seven fractions and observed that the mDP varied from 3.8 to 39.0. Monagas et al. (2003) also determinate the mDP, the % gal and the % prodelph of the skin polymeric proanthocyanidin fraction from Tempranillo (mDP = 72.3, % gal = 2.9, % prodelph = 13.3), Graciano (mDP = 33.8, % gal = 6.5, % prodelph = 10.7) and Cabernet Sauvignon (mDP = 85.7, % gal = 3.8, % prodelph = 31.2) varieties. The mDP determined in skins from *V. vinifera* cv. Syrah at commercial harvest was about 27.0 (Kennedy, Hayasaka, Vidal, Waters, & Jones, 2001) and 28.5 (Downey et al., 2003).

Wine proanthocyanidins are extracted during wine making from the more solid parts of clusters, mainly from the skins and seeds but also from stems if they are present (Bourzeix et al., 1986; Sun, Pinto, Leandro, Ricardo da Silva, & Spranger, 1999). Consequently wine proanthocyanidins include both procyanidins and prodelphinidins (González-Manzano, Rivas-Gonzalo, & Santos-Buelga, 2004). Working with red wine of Tinta Miúda, Sun et al. (1998) determined that the mDP and % gal of oligomeric proanthocyanidins were 4.8 and 3.0, respectively, and of polymeric proanthocyanidins were 22.1 and 7.3, respectively. Working with red wines, obtained by various winemaking technologies, Sun, Spranger, Roque-do-Vale, Leandro, and Belchior (2001) later found that the mDP of oligomeric proanthocyanidins ranged from 3.7 to 5.0 and % gal from 1.8 to 3.3, and that the mDP of polymeric proanthocyanidins ranged from 11.1 to 15.6 and % gal from 5.9 to 8.3. More recently, Monagas et al. (2003) measured mDP, % gal and the % prodelph of the polymeric proanthocyanidins from Tempranillo wines finding mDP to be 13.0; % gal, 2.8 and % prodelph, 11.3. In Graciano wines were found mDP = 6.9, % gal = 2.8 and % prodelph = 8.2, and in Cabernet Sauvignon wines mDP was 9.0; % gal, 3.4 and % prodelph, 10.6. Sarni-Manchado, Deleris, Avallone, Cheynier, and Moutounet (1999) estimated mDP of 6.2, % gal of 3.9 and % prodelp of 19.2 for the proanthocyanidins of a wine made from *V. vinifera*, var. Merlot (50%) and var. Carignan (50%). Maury, Sarni-Manchado, Lefebvre, Cheynier, and Moutounet (2001) determined wine proanthocyanidins from Syrah finding mDP to be 9.5, % gal, 5.0; and % prodelph, 19.2 and from Merlot mDP to be 5.8; % gal, 8.3; and % prodelph, 17.7. These compare with Maury, Sarni-Manchado, Lefebvre, Cheynier, and Moutounet (2003) who report for Syrah mDP = 10.3, % gal = 5.1, % prodelph = 19.5 and for Merlot mDP = 5.8, % gal = 8.3, % prodelph = 12.8. In a wine made from 75% Syrah and 25% Grenache, Maury et al. (2001) report mDP to be 10.4; % gal, 5.0; and % prodelph, 19.9 whilst in a wine made from 25% Syrah and 75% Grenache they found mDP to be 12.3; % gal, 4.8; and % prodelph, 22.6.

Cheynier et al. (1997) observed that after four months maturation a red wine showed a decrease in total proanthocyanidins, particularly of the prodelphinidins, but to a lesser extent of the galloylated compounds. These authors also confirmed that mDP may be related to easier degradation of higher molecular weight proanthocyanidins. Vidal, Cartalade, Souquet, Fulcrand, and Cheynier (2002) also attributed the decrease in mDP to a cleavage reaction that occurs in acidic media like wine. In this case these probably dominate in relation to the polymerisation reaction of proanthocyanidins that can also occur (Haslam, 1974).

According to several studies, proanthocyanidins are important in relation to the sensory characteristics of red wines, such as colour, bitterness and astringency. It has been shown that astringency depends on the structural characteristics of proanthocyanidin such as mDP and the% gal (Peleg, Gacon, Schlich, & Noble, 1999; Vidal et al., 2003). Therefore, knowledge of wine structural composition of proanthocyanidins could well be essential to defining the sensorial characteristics of wine. It has also been shown by some authors that the mDP and % gal of wine proanthocyanidins are essential structural characteristics affecting the action of wine fining agents (Cosme, Ricardo-da-Silva, & Laureano, 2008; Maury et al., 2001; Maury et al., 2003; Ricardo-da-Silva et al., 1991a; Sarni-Manchado et al., 1999).

Because there is a dearth of information on the subject, with respect to *V. vinifera* L. cv. Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah grown in Portugal, this work intended to study the tannin profile from the seeds and skins. The monovarietal wines produced from these varieties were also studied. We also compared the tannin profiles from the monovarietal wines from two vintages (2004 and 2005) and changes in the 2004 wine after six months of storage.

2. Materials and methods

2.1. Reagents

All solvents and acids were of HPLC grade. Toluene-α-thiol (phenylmethanethiol) was purchased from Fluka (Buchs, Switzerland).

2.2. Grapes

V. vinifera L. cv. Touriga Nacional, Trincadeira, Castelão, Syrah and Cabernet Sauvignon berries grown during the 2005 harvest season on the vineyards of the Tapada da Ajuda at the Instituto Superior de Agronomia located in Lisbon were used in this study. Approximately 250 berries at their technological maturity were randomly selected. The more solid parts of the grapes, skins and seeds were manually separated for subsequent analysis.

2.3. Preparation of phenolic extracts from grape seeds and skins

Grape seeds were ground to a fine powder using a coffee-bean mill. The phenolic compounds were extracted from samples of grape seeds (≈9 g) and skins (≈50 g) following the method described by Bourzeix et al. (1986).

2.4. Monovarietal wines

Monovarietal red wines were made from grapes from *V. vinifera* L. cv. Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah grown in the same geographical area (Instituto Superior de Agronomia vineyard, Lisbon) and harvested at their technological maturity (vintages 2004 and 2005) to produce the wines for the study. The wines were made at the Instituto Superior de Agronomia experimental cellar located in Lisbon, by classic vinification with maceration processes over approximately 12 days. The 2004 and 2005 wines were analysed after about five months (malolactic fermentation was already completed). The 2004 wine was also analysed second time, six months later. The chemical characteristics of wines from vintage 2004 and 2005 are Touriga Nacional 2004: alcohol content 11.8% v/v, 7.1 g/L tartaric acid, pH 3.51; Touriga Nacional 2005: alcohol content 11.6% v/v, 5.1 g/L tartaric acid, pH 3.84; Trincadeira 2004: alcohol content 11.0% v/v, 7.5 g/L tartaric acid, pH 3.43; Trincadeira 2005: alcohol content 12.4% v/v, 6.8 g/L tartaric acid, pH 3.62; Cabernet Sauvignon 2004: alcohol content 13.0% v/v, 7.5 g/L tartaric acid, pH 3.39; Cabernet Sauvignon 2005: alcohol content 13.0% v/v, 7.1 g/L tartaric acid, pH

3.62; Castelão 2004: alcohol content 11.8% v/v, 8.0 g/L tartaric acid, pH 3.20; Castelão 2005: alcohol content 11.9% v/v, 6.9 g/L tartaric acid, pH 3.50; Syrah 2004: alcohol content 14.7% v/v, 6.8 g/L tartaric acid, pH 3.53; and Syrah 2005: alcohol content 14.4% v/v, 6.6 g/L tartaric acid, pH 3.75.

2.5. Separation of proanthocyanidins by C_{18} Sep-Pak cartridges and determination of the flavan-3-ol content by the vanillin assay

The separation of flavanols was performed using a C_{18} Sep-Pak cartridge (Waters, Milford, Ireland) according to their degree of polymerisation into three fractions, monomeric, oligomeric and polymeric, following the method described by Sun et al. (1998). The total flavan-3-ol content of each fraction was determined using the vanillin assay according to the method described by Sun et al. (1998). Quantification was carried out by means of standard curves prepared from monomers, oligomers, and polymers of flavan-3-ol isolated from grape seeds and as described elsewhere (Sun et al., 1998; Sun et al., 2001).

2.6. Fractionation of proanthocyanidins (wine, seed and skin) according to their degree of polymerisation using a sequential dissolving procedure on an inert glass powder column

Proanthocyanidins (oligomeric and polymeric) extracted from seeds, skins and wines were separated from phenolic monomers, by fractionation in a C_{18} Sep-Pak cartridge (Waters, Milford, Ireland), and in line with the method described by Sun et al. (2001). The proanthocyanidin extract from seeds (Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah), skins (Touriga Nacional, Cabernet Sauvignon, and Castelão) or wines (Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah) were separated according to their degree of polymerisation following the method described by Labarbe et al. (1999). The elution gradient (methanol/chloroform) used for wines and seeds was FI-25:75 (v/v); FII-30:70 (v/v); FIII-35:65 (v/v); FIV-40:60 (v/v); FV-45:55 (v/v); FVI-50:50 (v/v); FVII-55:45 (v/v); FVIII 100:0 (v/v). For skins a slightly different gradient was used this being: FI-25:75 (v/v); FII-30:70 (v/v); FIII-35:65 (v/v); FIV-40:60 (v/v); FV-45:55 (v/v); FVI-50:50 (v/v); FVII-55:45 (v/v); FVIII 60:40 (v/v); FIX 65:35 (v/v); FX 70:30 (v/v); FXI 100:0 (v/v).

The tannin fractions were analysed by HPLC after thiolysis (Monagas et al., 2003), to estimate their structural characteristics (mean degree of polymerisation, percentage of galloylation and percentage of prodelphinidins) and to determine their concentrations.

2.7. Characterisation of wine, seed and skin proanthocyanidins by acid-catalysed depolymerisation in the presence of toluene-α-thiol followed by reversed-phase HPLC analysis

The acid-catalysed degradation was carried out according to the method of Monagas et al. (2003), and the thiolysed samples were then analysed by reversed-phase HPLC. The equipment and elution conditions employed for analytical HPLC were the same as used by Cosme et al. (2008). The amounts of monomers (terminal units) and of toluene-α-thiol adducts (extension units) released from the depolymerisation reaction in the presence of toluene-α-thiol were calculated from the areas of the chromatographic peaks at 280 nm by comparison with calibration curves (Kennedy et al., 2000; Prieur et al., 1994; Rigaud, Perez-Ilzarbe, Ricardo-da-Silva, & Cheynier, 1991).

Table 1
Concentration of seed, skin and wine monomeric flavanols; oligomeric and polymeric proanthocyanidins of *Vitis vinifera* L. seeds, skins and monovarietal wines of cvs

	Monomeric flavanols		Oligomeric proanthocyanidins		Polymeric proanthocyanidins		Total proanthocyanidins[a]	
	Concentration	mDP	Concentration	mDP	Concentration	mDP	Concentration	mDP
Touriga Nacional								
Seed (mg/g)	0.3 ± 0.0	–	7.7 ± 0.1	3.8 ± 0.2	27.1 ± 3.6	6.2±0.5	34.8 ± 0.1	–
Skin (mg/g)	0.02 ± 0.00	–	0.01 ± 0.00	7.5±0.7	2.36 ± 0.39	26.4±2.9	2.36 ± 0.56	–
2004 wine (mg/L)	14.9 ± 1.2	–	152.5 ± 2.2	–	507.0 ± 6.0	–	659.5 ± 22.1	4.5 ± 0.6
2004 (S) wine (mg/L)	7.7 ± 0.3	–	62.3 ± 1.0	–	288.3 ± 8.2	–	350.6 ± 10.7	4.6 ± 0.2
2005 wine (mg/L)	11.5 ± 1.0	–	73.9 ± 6.9	–	670.0 ± 8.0	–	743.6 ± 16.1	5.0 ± 0.3
Trincadeira								
Seed (mg/g)	1.1 ± 0.3	–	18.0 ± 0.6	4.3 ± 0.4	64.1 ± 0.9	6.6 ± 0.7	82.1 ± 2.2	–
Skin (mg/g)	0.03 ± 0.01	–	0.23 ± 0.11	6.1 ± 0.5	2.95 ± 0.35	33.4±3.7	3.17 ± 0.65	–
2004 wine (mg/L)	3.5 ± 1.2	–	22.1 ± 0.5	–	171.2 ± 3.9	–	193.3 ± 21.3	4.8 ± 0.9
2004 (S) wine (mg/L)	2.7 ± 0.2	–	21.2 ± 5.9	–	72.1 ± 9.6	–	93.3 ± 7.9	5.1 ± 0.6
2005 wine (mg/L)	16.3 ± 1.6	–	61.4 ± 2.3	–	816.4 ± 9.8	–	877.8 ± 11.1	4.6 ± 0.8
Cabernet Sauvignon								
Seed (mg/g)	1.8 ± 0.2	–	17.7 ± 2.5	2.3 ± 0.1	74.3 ± 0.6	5.1 ± 0.7	91.9 ± 2.7	–
Skin (mg/g)	0.02 ± 0.00	–	0.04 ± 0.01	9.0 ± 0.8	1.05 ± 0.03	43.9 ± 3.9	1.09 ± 0.04	–
2004 wine (mg/L)	5.5 ± 0.1	–	27.8 ± 1.5	–	261.3 ± 9.1	–	289.1 ± 4.7	4.3 ± 0.5
2004 (S) wine (mg/L)	2.2 ± 0.7	–	12.1 ± 0.3	–	103.6 ± 12.7	–	115.7 ± 13.5	4.7 ± 0.7
2005 wine (mg/L)	30.4 ± 3.7	–	87.5 ± 3.0	–	689.2 ± 9.3	–	776.7 ± 17.1	4.4 ± 0.3
Castelão								
Seed (mg/g)	0.3 ± 0.0	–	7.8 ± 0.8	5.2 ± 0.6	49.7 ± 1.6	8.8 ± 0.4	57.5 ± 1.1	–
Skin (mg/g)	0.01 ± 0.00	–	0.08 ± 0.02	9.0 ± 0.5	5.76 ± 0.54	22.5 ± 2.7	5.84 ± 0.74	–
2004 wine (mg/L)	5.6 ± 0.6	–	40.6 ± 1.8	–	405.4 ± 8.2	–	446.0 ± 16.4	5.9 ± 0.2
2004 (S) wine (mg/L)	4.3 ± 1.0	–	12.9 ± 3.3	–	253.4 ± 5.7	–	266.3 ± 8.1	5.5 ± 0.3
2005 wine (mg/L)	8.5 ± 0.7	–	42.4 ± 0.5	–	792.8 ± 12.7	–	835.2 ± 19.2	6.2 ± 0.5
Syrah								
Seed (mg/g)	2.0 ± 0.4	–	15.1 ± 0.4	3.3 ± 0.2	57.9 ± 1.6	7.8 ± 0.4	72.9 ± 1.7	–
Skin (mg/g)	0.01 ± 0.00	–	0.09 ± 0.02	7.6 ± 1.4	2.43 ± 1.33	45.1 ± 2.6	2.52 ± 1.91	–
2004 wine (mg/L)	12.7 ± 0.8	–	65.9 ± 8.7	–	427.9 ± 7.8	–	493.8 ± 18.5	5.2 ± 0.4
2004 (S) wine (mg/L)	6.0 ± 0.1	–	10.9 ± 5.6	–	244.4 ± 6.1	–	255.3 ± 10.5	4.5 ± 0.6
2005 wine (mg/L)	28.8 ± 1.1	–	228.3 ± 5.1	–	1002.3 ± 15.2	–	1230.6 ± 25.1	5.3 ± 0.5

Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah and the mean degree of polymerisation (mDP) of the oligomeric and polymeric seed and skin proanthocyanidins and of the wine total proanthocyanidins (mean ± SD).
2004 (S) – analysis performed second time on the 2004 wine, six months later.
[a] Sum of oligomeric and polymeric proanthocyanidins.

3. Results and discussion

3.1. Grape tannin profile

The concentration and structural composition of the proanthocyanidins from seeds and skins differed greatly amongst the *V. vinifera* L. cv grape varieties studied, which agrees with previous studies (Labarbe et al., 1999; Monagas et al., 2003; Souquet, Cheynier, & Moutounet, 2000; Sun et al., 2001). On a mg/g basis, the seed proanthocyanidin concentration was always higher than in the skins (Table 1).

3.1.1. Structural characterisation and quantification of grape seed proanthocyanidin fractions

The flavan-3-ols, of the seed fractions (monomeric, oligomeric and polymeric) determined by the vanillin reaction, are shown in Table 1. The seeds of Cabernet Sauvignon contained higher levels of oligomeric and polymeric flavan-3-ols when compared with the other *V. vinifera* L. cv grape seed proanthocyanidins analysed (Table 1). The lowest values of monomeric, oligomeric and polymeric flavan-3-ols were measured for Touriga Nacional seed. The highest mDP for the polymeric seed fraction was found for Castelão followed by Syrah. The mDP values for Syrah grown in Portugal are in the same range as those published for Syrah seed proanthocyanidins (Vidal et al., 2002; Vidal et al., 2003).

Proanthocyanidins extracted from seeds were also fractionated according to their mean degree of polymerisation, on an inert glass powder column eluted with a gradient of methanol/chloroform. The data on structural characteristics of all fractions of seed proanthocyanidins after toluene-α-thiol derivatisation are summarised in Table 2. The percentage of galloylation ranged from 9.4% to 32.2%. It is noted that the degree of galloylation of the proanthocyanidins increased with increasing mDP as had previously been observed for seeds from Alicante Bouchet (Prieur et al., 1994) but not in seeds from Cabernet Franc (Labarbe et al., 1999).

The proanthocyanidins of seeds showed a mDP ranging from 2.8 to 12.8 (Table 2). Different tannin profiles were observed amongst the varieties analysed (Fig. 1). Touriga Nacional recorded the lowest concentration of total proanthocyanidins and showed a distribution of tannin fractions as follows: 36% of 2–4 mDP, 44% of 5–8 mDP and 17% of 12–13 mDP. Trincadeira and Syrah had the largest quantities of proanthocyanidins (85% and 76%, respectively) for mDP 4–7 and for mDP 3–6, respectively, and a lower amount at a higher mDP (13% at 10–11 mDP and 23% at 12–13 mDP, respectively). As already noted Cabernet Sauvignon had the highest concentration of total proanthocyanidins. This variety showed a major quantity of tannins for mDP 3–5 (59%) and for mDP 6–7 (30%), and a smaller quantity of proanthocyanidins for the higher mDP 11–12 (10%). For Castelão proanthocyanidins were for mDP 3–6, 40%; for mDP 8–9, 24% and for mDP 11–12, 33%.

3.1.2. Structural characterisation and quantification of skin proanthocyanidin fractions

For skins, quantification by the vanillin assay revealed that the concentrations of monomeric and oligomeric flavan-3-ol were similar for all five varieties studied. The exception was for the skin content of the oligomeric fraction in Trincadeira which was higher than the other skin oligomeric proanthocyanidins (Table 1). The polymeric proanthocyanidin fraction represented the highest proportion of total flavan-3-ols content in each of the grape varieties studied, of which the highest belonged to Castelão. The highest mDP for the polymeric skin fraction was found for Syrah followed by Cabernet Sauvignon, and the lowest value was for Castelão skins

Table 2
Structural characteristics (mDP – mean degree of polymerisation, % gal – percentage of galloylation) and concentration (mg/g) of the proanthocyanidin fractions of seeds of cvs

Proanthocyanidin fractions	Touriga Nacional			Trincadeira			Cabernet Sauvignon			Castelão			Syrah		
	mg/g	mDP	% gal	mg/g	mDP	% gal	mg/g	mDP	% gal	mg/g	mDP	% gal	mg/g	mDP	% gal
FI	6.5 ± 0.2	2.8 ± 0.1	10.9 ± 0.6	12.6 ± 0.3	4.1 ± 0.9	9.4 ± 0.1	12.6 ± 0.2	2.9 ± 0.2	10.4 ± 0.5	8.3 ± 0.2	3.3 ± 0.5	11.6 ± 0.6	9.2 ± 0.2	3.1 ± 0.4	11.5 ± 0.3
FII	2.6 ± 0.1	2.9 ± 0.6	11.8 ± 0.2	12.4 ± 0.5	4.2 ± 0.7	13.0 ± 0.9	9.4 ± 0.2	3.0 ± 0.1	12.3 ± 0.8	2.9 ± 0.2	4.0 ± 0.3	12.2 ± 0.7	6.3 ± 0.3	3.5 ± 0.4	13.5 ± 0.5
FIII	1.9 ± 0.1	3.1 ± 0.6	12.7 ± 0.4	3.5 ± 0.2	4.9 ± 0.3	14.4 ± 0.3	3.5 ± 0.2	3.1 ± 0.3	14.6 ± 0.5	2.7 ± 0.7	4.2 ± 0.2	12.3 ± 0.4	1.5 ± 0.4	3.9 ± 0.6	14.2 ± 0.3
FIV	1.6 ± 0.3	3.4 ± 0.3	13.6 ± 0.2	4.5 ± 0.3	5.0 ± 0.9	15.2 ± 0.7	4.5 ± 0.6	3.3 ± 0.2	15.4 ± 0.8	3.4 ± 0.3	4.5 ± 0.7	12.6 ± 0.4	3.1 ± 0.6	4.3 ± 0.6	16.7 ± 0.4
FV	2.1 ± 0.2	5.1 ± 0.5	14.2 ± 0.9	4.9 ± 0.6	5.5 ± 0.9	16.1 ± 0.1	4.9 ± 0.4	3.4 ± 0.2	16.6 ± 0.5	2.9 ± 0.4	5.2 ± 0.6	14.6 ± 0.2	2.1 ± 0.8	4.9 ± 0.9	19.1 ± 0.2
FVI	5.5 ± 0.3	6.6 ± 0.7	15.3 ± 0.4	9.2 ± 0.6	5.9 ± 0.7	20.7 ± 0.6	19.2 ± 0.8	4.9 ± 0.4	23.6 ± 0.5	2.9 ± 0.5	5.7 ± 0.9	15.0 ± 0.7	15.0 ± 0.5	5.3 ± 0.8	20.3 ± 0.3
FVII	7.7 ± 0.4	7.0 ± 0.8	17.5 ± 0.3	23.4 ± 2.0	6.5 ± 0.3	21.5 ± 0.7	27.4 ± 0.6	6.3 ± 0.9	24.1 ± 0.6	13.6 ± 0.6	8.9 ± 0.8	20.7 ± 0.6	18.6 ± 0.2	5.9 ± 0.9	20.5 ± 0.2
FVIII	6.1 ± 0.5	12.8 ± 0.4	28.7 ± 0.2	10.8 ± 0.8	11.7 ± 0.4	32.2 ± 0.5	8.8 ± 0.2	11.1 ± 0.7	28.9 ± 0.9	18.8 ± 0.9	11.6 ± 0.6	26.1 ± 1.7	16.9 ± 0.6	12.4 ± 0.7	22.5 ± 0.3
Total seed extract	34.0	6.2	16.4	81.3	6.1	18.5	90.3	5.1	19.7	55.6	7.6	18.6	72.6	6.5	18.7

Touriga Nacional, Trincadeira, Castelão, Syrah and Cabernet Sauvignon (mean ± SD).

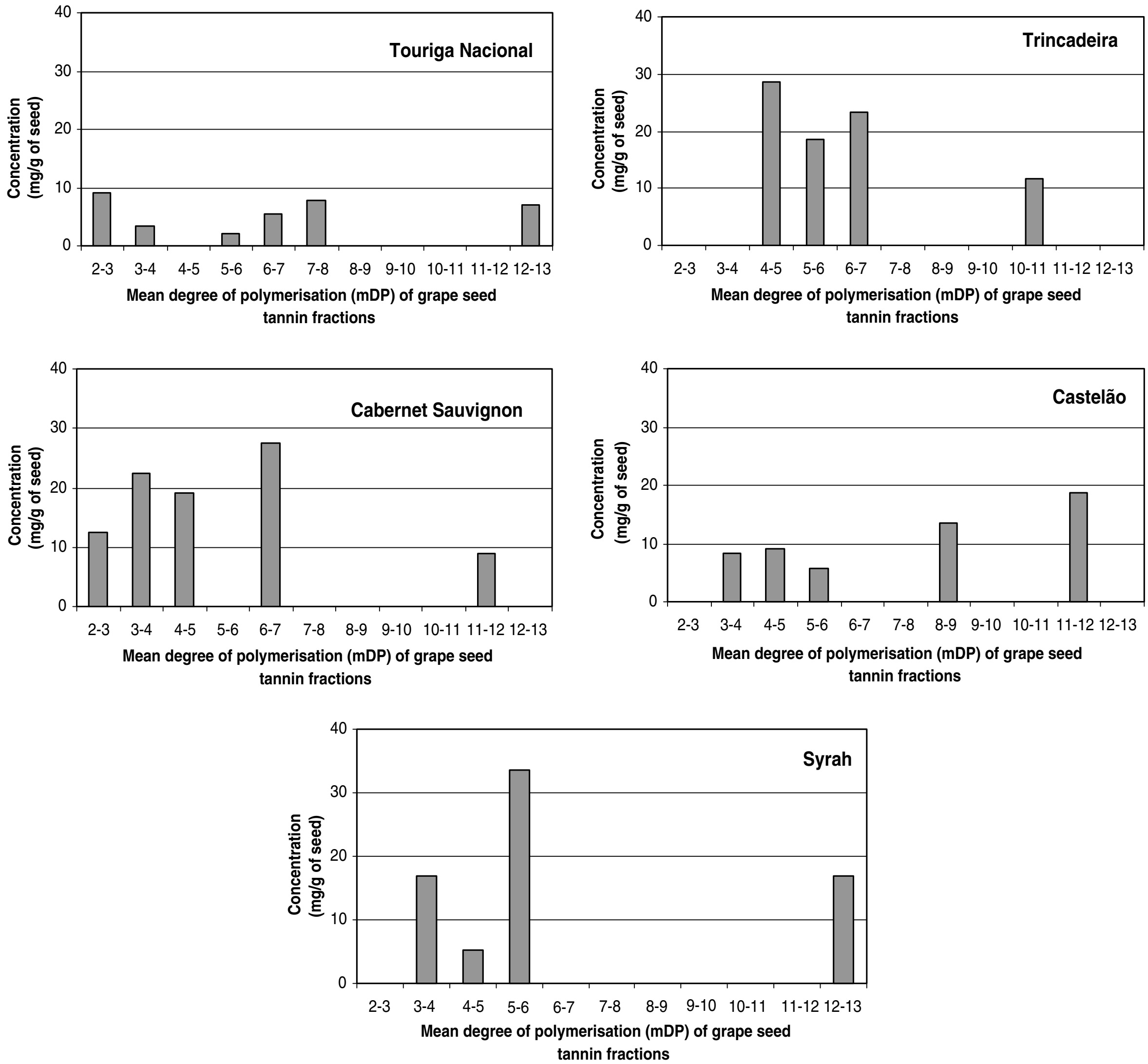

Fig. 1. Tannin profile of *Vitis vinifera* L. seed for cvs. Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah.

(Table 1). The mDP values for the polymeric fraction of Syrah skin proanthocyanidins are in agreement with the previous results (Moutounet et al., 1996; Vidal et al., 2002).

The tannin profiles for grape skins and their structural characteristics were found for three varieties, Touriga Nacional, Cabernet Sauvignon and Castelão (Table 3). As expected from other studies

Table 3
Structural characteristics (mDP – mean degree of polymerisation, % gal – percentage of galloylation, % prodelph – percentage of prodelphinidins) and concentration (mg/g) of the proanthocyanidin fractions of skins of cvs

Proanthocyanidin fractions	Touriga Nacional				Cabernet Sauvignon				Castelão			
	mg/g	mDP	% gal	% prodelph	mg/g	mDP	% gal	% prodelph	mg/g	mDP	% gal	% prodelph
FI	0.08 ± 0.02	4.6 ± 0.2	4.9 ± 0.6	17.9 ± 0.6	0.02 ± 0.01	6.0 ± 0.9	2.9 ± 0.2	22.6 ± 0.7	0.09 ± 0.03	3.8 ± 0.6	4.5 ± 0.8	12.9 ± 0.7
FII	0.03 ± 0.01	5.4 ± 0.6	5.8 ± 0.8	19.8 ± 0.7	0.01 ± 0.00	6.2 ± 0.3	3.8 ± 0.4	24.4 ± 0.9	0.05 ± 0.01	4.1 ± 0.6	4.8 ± 1.0	15.8 ± 0.5
FIII	0.04 ± 0.01	7.7 ± 0.4	5.7 ± 0.4	22.7 ± 0.4	0.01 ± 0.00	7.1 ± 0.8	4.1 ± 0.6	26.2 ± 1.1	0.06 ± 0.01	4.6 ± 0.4	5.2 ± 0.4	18.7 ± 0.9
FIV	0.09 ± 0.03	8.7 ± 0.6	2.6 ± 0.2	23.6 ± 0.6	0.01 ± 0.00	7.3 ± 0.8	5.6 ± 0.6	25.9 ± 0.6	0.07 ± 0.01	5.8 ± 0.8	3.6 ± 0.2	22.6 ± 0.7
FV	0.05 ± 0.02	10.5 ± 0.7	3.2 ± 0.9	24.2 ± 0.9	0.02 ± 0.01	7.7 ± 0.9	6.2 ± 1.2	27.5 ± 1.2	0.06 ± 0.01	5.9 ± 0.9	4.2 ± 0.9	26.2 ± 1.1
FVI	0.16 ± 0.03	11.7 ± 0.9	3.3 ± 0.4	25.3 ± 0.4	0.04 ± 0.01	7.9 ± 0.8	7.3 ± 0.7	29.7 ± 0.9	0.19 ± 0.01	6.1 ± 0.3	2.3 ± 0.4	25.9 ± 0.6
FVII	0.19 ± 0.02	12.9 ± 0.8	3.5 ± 0.5	27.5 ± 0.3	0.03 ± 0.01	9.1 ± 1.0	4.5 ± 0.7	34.5 ± 0.4	0.22 ± 0.03	6.9 ± 0.9	3.5 ± 0.5	29.5 ± 1.2
FVIII	0.25 ± 0.02	13.6 ± 0.9	3.7 ± 0.2	28.7 ± 0.9	0.09 ± 0.02	11.7 ± 1.1	3.7 ± 0.3	42.1 ± 1.3	0.39 ± 0.09	8.3 ± 0.7	5.7 ± 0.3	27.7 ± 1.1
FIX	0.28 ± 0.08	15.2 ± 0.9	3.3 ± 0.4	32.3 ± 0.8	0.33 ± 0.01	26.9 ± 1.2	3.3 ± 0.5	40.3 ± 0.7	1.78 ± 0.02	12.9 ± 1.1	4.3 ± 0.5	32.9 ± 0.7
FX	0.47 ± 0.04	23.8 ± 1.9	3.5 ± 0.3	27.5 ± 0.7	0.27 ± 0.09	38.9 ± 1.1	3.5 ± 0.6	41.1 ± 1.1	1.47 ± 0.09	16.6 ± 2.0	4.5 ± 0.5	29.1 ± 2.0
FXI	0.62 ± 0.07	64.5 ± 2.8	3.7 ± 0.3	33.7 ± 1.1	0.26 ± 0.08	81.0 ± 4.4	4.7 ± 0.6	40.1 ± 1.3	1.18 ± 0.08	49.3 ± 3.2	4.7 ± 0.4	37.1 ± 1.1
Total skin extract	2.3	28.4	3.6	28.5	1.1	41.7	4.3	41.8	5.6	19.8	4.3	30.5

Touriga Nacional, Castelão and Cabernet Sauvignon (mean±SD).

(Labarbe et al., 1999; Souquet et al., 1996) proanthocyanidins also contained both (−) epigallocatechin units (prodelphinidin) and procyanidin (Fulcrand, Remy, Souquet, Cheynier, & Moutounet, 1999; Kennedy et al., 2000; Labarbe et al., 1999; Souquet et al., 1996; Souquet et al., 2000). In addition, skin proanthocyanidins differed from seed proanthocyanidins by their lower percentage of galloylation and higher mDP in agreement with findings of Labarbe et al. (1999), Monagas et al. (2003), Souquet et al. (2000) and Sun et al. (2001). The percentage of galloylation in the skin proanthocyanidins ranged from 2.3% to 7.3%. No relationship was apparent between mDP and the percentage of galloylation as previously observed for skins of Merlot (Souquet et al., 1996), Cabernet Franc (Labarbe et al., 1999) and Syrah (Vidal et al., 2003). The percentage of prodelphinidins in the skins ranged from 12.9% to 42.1%. A tendency for proanthocyanidins with higher mDP to also show a higher percentage of epigallocatechins units was also observed. This tendency had previously been noted in the varieties Merlot, Cabernet Franc and Syrah (Labarbe et al., 1999; Souquet et al., 1996; Vidal et al., 2003).

The proanthocyanidins of skins of the three varieties studied showed mDP ranging from 3.8 to 81.0 (Table 3). For skin proanthocyanidins it was also observed that the tannin profile differed amongst the varieties analysed. Castelão showed the lowest mDP and Cabernet Sauvignon, the highest mDP. Cabernet Sauvignon measured the lowest concentration of total proanthocyanidins in the skins and the proanthocyanidins distribution was mainly

Table 4
Structural characteristics (mDP – mean degree of polymerisation, % gal – percentage of galloylation, % prodelph – percentage of prodelphinidins) and concentration (mg/L) of the proanthocyanidin fractions of monovarietal wines from cvs

Proanthocyanidin fractions	2004				2004 (S)				2005			
	mg/L	mDP	% gal	% prodeph	mg/L	mDP	% gal	% prodeph	mg/L	mDP	% gal	% prodeph
Touriga Nacional												
FI	218.3	2.4	3.9	20.8	64.5	2.8	3.3	16.2	184.1	2.9	4.2	24.8
FII	134.4	3.8	9.9	20.4	53.9	4.9	9.4	18.4	124.7	4.2	10.9	23.3
FIII	52.9	4.5	3.6	16.2	53.6	5.0	3.4	14.1	39.0	4.5	4.6	18.1
FIV	107.4	5.4	5.3	18.2	35.8	5.4	4.9	16.1	87.7	4.9	5.9	18.9
FV	45.0	5.7	6.3	8.2	33.0	5.5	5.8	6.2	42.1	5.2	6.8	9.5
FVI	67.8	6.4	1.8	8.5	33.8	5.5	1.5	7.9	65.9	5.8	2.8	8.7
FVII	48.1	6.9	2.2	22.3	33.0	5.6	2.1	19.7	90.8	7.6	2.7	23.1
FVIII	45.9	8.1	2.1	13.7	24.6	5.9	2.0	12.1	83.7	8.9	2.9	12
Total	719.8	4.5	4.9	17.5	332.2	4.6	4.1	13.7	718.0	5.0	5.2	19.0
Trincadeira												
FI	67.6	2.1	5.4	18.4	26.7	3.3	4.7	17.1	194.1	2.9	5.6	20.1
FII	44.9	3.3	7.3	33.2	12.6	5.0	6.8	29.3	123.3	3.4	7.5	33.4
FIII	24.2	4.3	7	29.4	13.0	5.4	6.5	23.2	80.1	4.1	7.3	29.6
FIV	25.0	4.6	7.2	16.8	10.0	5.9	6.4	13.9	78.4	4.6	7.5	15.9
FV	26.4	4.8	6.4	16.9	12.5	5.9	6	12.9	94.7	4.7	6.5	16.7
FVI	21.6	5.2	2.9	16.6	5.4	6.5	2.3	12.7	60.9	5.2	2.8	16.8
FVII	21.4	5.4	5.4	15.9	5.5	6.9	5	12.7	67.7	5.4	5.5	16.1
FVIII	57.0	9.6	4.3	19.1	6.8	7.0	3.5	16.3	156.5	8.1	4.4	19.6
Total	288.1	4.8	5.5	20.6	92.5	5.1	5.4	18.0	855.6	4.5	5.7	21.0
Cabernet Sauvignon												
FI	87.7	2.7	7.1	27.6	35.83	3.7	6.6	24.6	216.6	2.4	7.6	29.1
FII	44.9	3.4	6.5	29.5	12.4	4.4	5.6	25.2	115.4	3.9	6.7	29.2
FIII	22.0	4.9	8.7	19.9	10.9	4.6	8.2	17.4	45.5	4.4	8.9	20.1
FIV	24.5	5.1	8.7	20.8	9.8	4.8	7.7	17.4	55.6	4.7	8.8	20.9
FV	29.6	5.3	5.6	15.7	11.3	5.1	7.0	13.3	61.1	5.2	7.9	15.9
FVI	18.9	5.5	4.6	20.2	8.9	5.5	4.6	16.5	49.3	5.3	5.1	20.7
FVII	22.3	5.9	6.2	17.2	9.1	5.7	6.0	15.9	54.8	5.7	6.7	17.8
FVIII	49.3	6.6	4.3	18.2	16.8	6.2	4.1	14.9	186.1	6.6	4.5	18.9
Total	299.2	4.3	6.2	22.1	114.9	4.7	6.2	19.4	784.4	4.4	6.5	22.6
Castelão												
FI	104.3	3.2	9.7	32.9	18.5	5.0	9.6	30.9	159.1	4.5	9.9	32.9
FII	54.1	5.1	4.5	15.9	17.9	5.2	4.4	13.8	91.5	5.2	5.2	17.9
FIII	43.4	5.7	4.8	19.1	15.9	5.9	4.6	18.1	78.7	5.8	5.3	21.8
FIV	38.7	5.9	4.9	17.2	19.9	6.1	4.6	16.6	69.7	6.2	5.3	19.6
FV	39.9	5.98	6.1	17.8	21.5	6.2	5.9	17.0	59.5	6.4	6.7	19.4
FVI	38.0	6.1	6.4	15.0	21.2	6.4	6.1	14.9	58.1	6.6	6.9	18.1
FVII	52.3	7.1	2.4	18.5	71.9	7.3	2.3	16.9	95.3	8.1	2.8	19.2
FVIII	94.3	9.3	3.2	19.2	32.9	8.1	3.1	18.3	186.0	8.5	3.9	20
Total	465.0	5.9	5.4	20.8	219.8	5.5	3.6	14.8	797.9	6.2	5.5	21.0
Syrah												
FI	125.3	2.2	8.7	19.8	47.3	3.2	8.5	16.8	267.8	2.9	9.5	22.6
FII	56.0	4.9	6.4	18.6	19.5	5.1	6.3	17.2	121.9	4.1	7.1	21.4
FIII	41.7	5.4	8.1	18.4	42.4	5.2	7.8	18.2	91.5	5.1	8.7	19.8
FIV	40.9	5.8	3.0	17.5	19.0	5.2	2.8	16.3	112.3	5.3	3.3	18.2
FV	44.6	6.0	4.7	19.1	20.7	5.6	4.5	18.7	103.8	5.8	4.9	21.7
FVI	48.4	6.2	1.4	17.6	19.5	5.7	1.3	17.0	131.3	6.3	1.9	19.2
FVII	59.5	6.6	4.7	16.4	21.1	5.8	4.4	15.9	156.5	6.7	5.2	18.9
FVIII	75.6	7.8	3.3	18.7	32.8	7.2	2.9	17.7	223.3	8.1	3.9	20.9
Total	492.0	5.2	5.5	18.4	222.3	4.5	4.76	15.1	1208.4	5.3	5.6	19.8

Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah (mean ± SD).
2004 (S) – analysis performed second time on the 2004 wine, six month later.

(84%) at the higher mDP (mDP > 30), with only 23% of the proanthocyanidins associated with mDP 6–12. The tannin profile of Touriga Nacional was proanthocyanidins with mDP 3–18 (51%), with mDP 24 (20%) and with mDP 65 (27%). The tannin profile of Castelão skins was proanthocyanidins with mDP 3–6 (19%), with mDP 12–18 (57%) and with mDP 44 (20%).

3.2. Wine tannin profile

Table 1 shows the flavan-3-ols of wine fractions (monomeric, oligomeric and polymeric) measured by the vanillin reaction. The data showed that the concentration of the total proanthocyanidins of all the five monovarietal wines made from grapes cultivated in the same geographical area and under the same winemaking conditions was lower in the 2004 vintage than in 2005. However, the highest concentration of oligomeric plus polymeric proanthocyanidins in vintage 2004 was measured in wines from Touriga Nacional and in vintage 2005 in wines from Syrah. The polymeric fraction of the total proanthocyanidins from the five monovarietal wines ranged from 77% to 91% in vintage 2004 and 82% to 95% in 2005 (Table 1). The data from Table 4 show that the higher concentrations of proanthocyanidins measured in vintage 2005 seem not to be associated with a higher mDP of the total proanthocyanidins. The distribution of the proanthocyanidin fractions with different mDP values in the wines from Trincadeira and Cabernet Sauvignon was similar in vintages 2004 and 2005 as shown in Fig. 2. The two vintages wines from Castelão did not show proanthocyanidin fractions with mDP amongst 2 and 3 and the wines from Cabernet Sauvignon did not show proanthocyanidins fraction with mDP above 7. The structural characteristics presented in Table 4 show that the percentage of galloylation and the percentage of prodelphinidins were very close in the two vintages. The values measured for the percentage of galloylation are in agreement with other wine studies with Tinta Miúda (Sun et al., 1998), Syrah and blends from

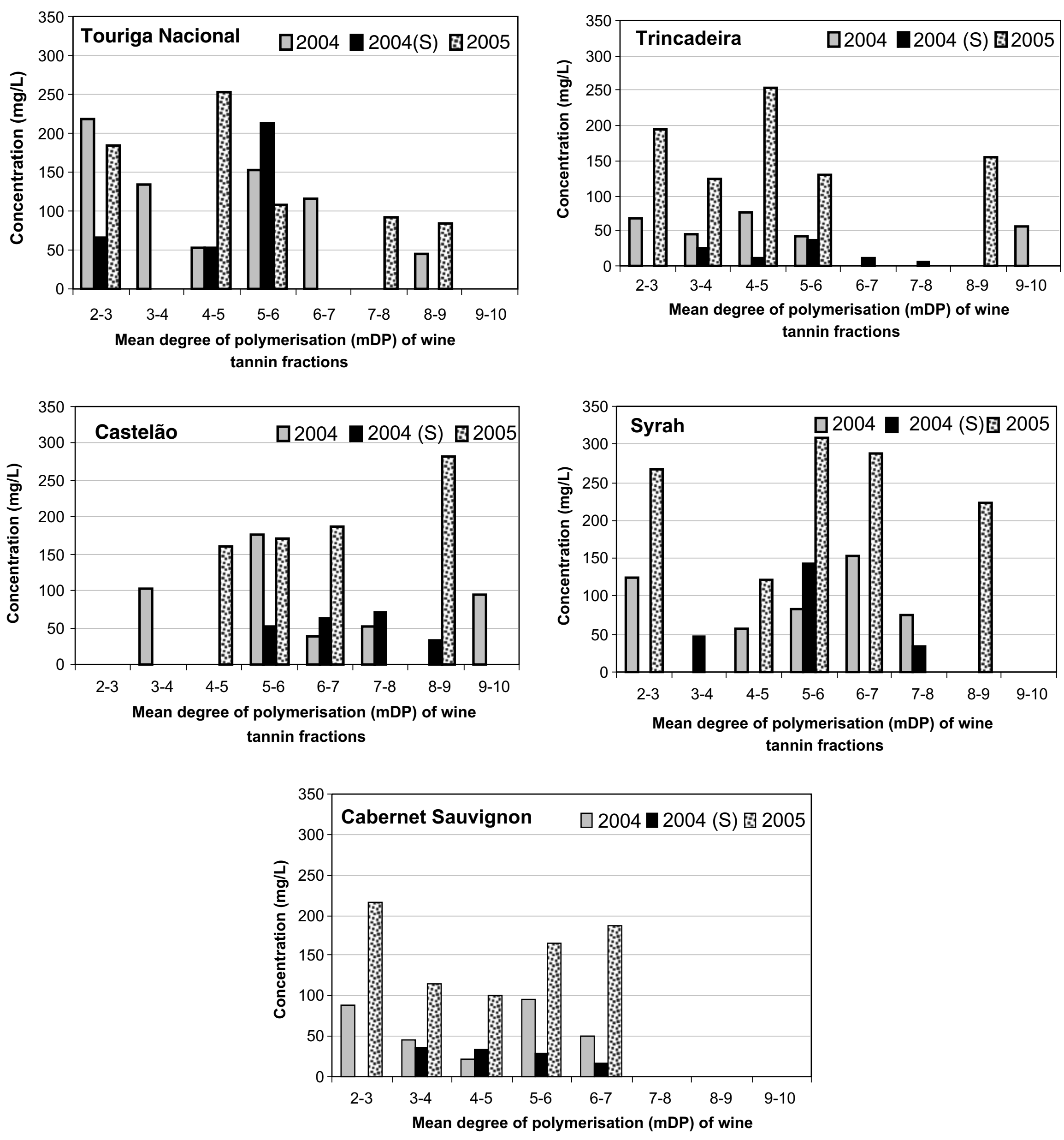

Fig. 2. Tannin profile of *Vitis vinifera* L. monovarietal wines for cvs. Touriga Nacional, Trincadeira, Cabernet Sauvignon, Castelão and Syrah. 2004(S) analysis performed second time on the 2004 wine, six months later.

Syrah (Maury et al., 2001; Maury et al., 2003). Also, the values obtained for the percentage of prodelphinidins were similar to those measured in wines from Syrah and blends from Syrah (Maury et al., 2001; Maury et al., 2003).

Data on proanthocyanidin content of the five monovarietal wines of vintage 2004 showed that the concentration of proanthocyanidins in wines decreased by 39–59% over six months. In Fig. 2 we see that the changes were not only in the proanthocyanidin concentration but also in the distribution of the different proanthocyanidin fractions. It seems that a polymerisation of the lower mDP fraction and a loss of the higher mDP fraction occur simultaneously. However, no changes in the mDP and percentage of galloylation (except in Castelão) of the total proanthocyanidins were observed (the small differences found are within experimental error). The percentage of prodelphinidins decreased slightly during storage. Changes during ageing lead to structural changes but not to proanthocyanidins with higher mean degree of polymerisation as shown in Table 4 and in Fig. 2. Similar results were observed for the percentage of galloylation and of prodelphinidins in a blended wine of Merlot and Carignan, cellared for three months (Sarni-Manchado et al., 1999).

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