Relationship Between Antioxidant Capacity, Proanthocyanidin and Anthocyanin Content During Grape Maturation of Touriga Nacional and Tinta Roriz Grape Varieties

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To investigate antioxidant capacity in seeds and skins during grape maturation and its relationship with anthocyanin and proanthocyanidin content, two Portuguese red grape varieties, Touriga Nacional and Tinta Roriz (*Vitis vinifera* L.) were studied. Two analytical methods were used for antioxidant capacity analysis: the DPPH and ABTS methods. Proanthocyanidins from seeds and skins were separated into monomers, oligomers and polymers, while 13 individual anthocyanins from the skins were also evaluated by HPLC. For both grape varieties studied, antioxidant capacity from the skins and seeds was characterised during grape maturation by a general decrease, mainly in the first weeks after véraison, followed by stabilisation and a slight increase in the values in the last three weeks of ripening. A similar tendency was observed for the amount of all the different proanthocyanidin fractions quantified. Our results also showed that seeds are an important source of proanthocyanidins with respect to the grape berry skins. Seeds were also the grape berry fraction with the highest antioxidant capacity when compared to the antioxidant capacity content of the skins. For the 13 individual monomeric anthocyanins quantified during grape maturation, evolution was generally characterised by a continuous increase in the values. However, for some of the individual anthocyanins, the continuous increase was followed by stabilisation or a decrease in the values in the last weeks of ripening. Finally, there was a positive relationship between the different proanthocyanidin fractions and antioxidant capacity of both grape varieties studied; while a negative relationship during grape maturation was obtained for individual anthocyanins.

INTRODUCTION

Grapes have long been appreciated for their rich content of phenolic compounds such as gallic acid, catechin, anthocyanins and resveratrol, and a wide variety of procyanidins (Cheynier & Rigaud, 1986; Ricardo-da-Silva *et al*., 1992a; Jordão *et al*., 1998a, 1998b; Kennedy *et al*., 2000a, 2001; Jordão *et al*., 2001a,b; Sun *et al*., 2001). Phenolic compounds can be classified into two groups: flavonoids and nonflavonoids. The main C_6-C_3-C_6 flavonoids in wine include conjugates of the flavonols, quercetin and myricetin; flavin-3-ols (+)-catechin and (-)-epicatechin; and anthocyanins. The nonflavonoids incorporate the C_6-C_1 hydroxy-benzoic acids, and gallic and ellagic acids; the C_6-C_3 hydroxycinnamates caffeic, caftaric and *p*-coumaric acids, and the C_6-C_2-C_6 stilbenes trans-resveratrol, *cis*-resveratrol, and *trans*-resveratrol glucoside.

In the grape berry, flavonoids such as anthocyanins are located mainly in the skins (Jordão *et al*., 1998a), while the flavan-3-ols (catechins and proanthocyanidins) are present in the skins, seeds and stems (Ribéreau-Gayon, 1972; Ricardo-da-Silva *et al*., 1992b; Fuleki & Ricardo-da-Silva, 1997; Kennedy *et al*., 2000a, 2000b; Jordão *et al*., 2001a,b; Sun *et al*., 2001).

Anthocyanin accumulation in the vacuoles of the skin begins in véraison and reaches maximum concentration around harvest time (Kennedy *et al*., 2002; Canals *et al*., 2005). A decrease in the total amount of anthocyanins just before harvest and during over-ripening has been recorded in some works (Ryan & Revilla, 2003; Fournand *et al*., 2006). The highest levels of proanthocyanidins in grapes were usually found at the onset of ripening, after which they decreased (Jordão *et al*., 1998b; Kennedy *et al*., 2000a, 2000b; Hanlin & Downey, 2009). However, for some grape varieties and for some procyanidins, a maximum is sometimes observed at véraison (Jordão *et al*., 2001b).

According to several studies, flavan-3-ols, flavonols and anthocyanins are the most important compounds contributing to the antioxidant proprieties of red grapes and

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wine (Wang *et al*., 1997; Simonetti *et al*., 1997; Ghiselli *et al*., 1998; Beecher, 2003). In recent years, several authors have reported on the phenolic composition and antioxidant capacity of several red and white grapes from different varieties, grape berry fractions and countries (Orak, 2007; Bozan *et al*., 2008; Poudel *et al*., 2008; Yang *et al*., 2009; Xu *et al*., 2010; Breksa *et al*., 2010). However, in these studies the grapes were only generally harvested at optimum maturity or technological maturation. Thus the antioxidant capacity of different fractions of the grape berry during grape maturation and its relationship with proanthocyanidin and individual anthocyanin evolution had not been investigated.

The main object of this study therefore was to investigate the evolution of the antioxidant capacity of the different fractions of grape berry (seeds and skins) and its relationship with the different proanthocyanidin fractions (monomeric, oligomeric and polymeric fractions) and the individual anthocyanin content of two important Portuguese red grape varieties during ripening (Touriga Nacional and Tinta Roriz).

MATERIALS AND METHODS

Samples

Touriga Nacional and Tinta Roriz (*Vitis vinifera* L.) grapes were harvested at grape maturation in 2009 from a vineyard located in Viseu (northern Portugal). Two hundred berries per variety were selected from two clusters per plant among a total of 100 plants. Samples were harvested weekly (except in the last three weeks of maturation), starting at véraison and continuing until technological maturity (56 days after véraison). The berries were frozen at -18ºC until processing.

General physicochemical parameters

From the initial 200 berries, a subsample of 100 berries was taken from the Touriga Nacional and Tinta Roriz grapes and analysed at technical maturity for estimated alcohol degree, pH, titratable acidity and total phenolic index using the analytical methods recommended by the OIV (1990). Total anthocyanins were measured as recommended by Glories (1999). All determinations were carried out on the must (except total phenols and total anthocyanins). Total phenols and total anthocyanins were determined from an extract obtained by macerating the crushed grapes at 25ºC for 24 h in a pH 3.7 buffer (Carbonneau & Champgnol, 1993). All laboratory analyses were done in duplicate.

The general physicochemical composition of the Touriga Nacional and Tinta Roriz grape cultivars at harvest are presented in Table 1. The total phenol index in the Tinta Roriz grapes was much higher than that in Touriga Nacional, while Touriga Nacional showed the highest values for total anthocyanins.

TABLE 1
General physicochemical parameters of Touriga Nacional and Tinta Roriz grapes at technological maturity.

Parameters	Grape varieties	
	Touriga Nacional	Tinta Roriz
Estimated alcohol degree (%, V/V)	11.6	12.6
Titratable acidity (g/L tart. acid)	7.8	5.2
pH	3.0	3.2
Total phenols index (o.d. x 100)	45.4	60.7
Total anthocyanins (mg/100 g berry)	112.5	87.8

Sample preparation for antioxidant capacity, proanthocyanidins and individual anthocyanin analyses

Skins and seeds (from the other subsample of 100 berries) were separated by hand from the clusters (approximately 60 and 20 g respectively) of each grape cultivar. After this separation, each fraction was washed separately several times with distilled water to remove foreign particles, and moisture was absorbed with blotting paper. The seeds were crushed manually before the extraction process.

According to the procedure described by Sun *et al*. (1996), grape skins and seeds were submitted separately to an extraction process using 50 mL of 80% methanol first, followed by 50 mL of 75% acetone (each for 3 h under agitation). After centrifugation (10 min at 3 500 rpm), an aliquot of each extract was filtered (Whatman 0.45 µm) and frozen at -18 ºC until processing.

Antioxidant capacity

There are various methods to evaluate the antioxidant capacity of different foods, such as wine, which involve different mechanisms. The values for antioxidant capacity change according to the method used. The lack of a strong correlation between different methods, e.g. DPPH and ABTS, can probably be attributed to the fact that every individual phenol compound contained in wine causes a different response to the specific radical used in the assay (Lachman *et al*., 2007). Thus the use of a single method cannot provide a comprehensive prediction of the antioxidant efficacy of the different compounds (Arts *et al*., 2003).

The total antioxidant capacity was performed separately from the skin and seed extracts produced according to the methodology described previously. For the analysis of antioxidant capacity, two analytical methods were used: ABTS (2.2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2.2-diphenyl-1-picrylhydrazyl).

The ABTS method is based on discoloration, which occurs when the radical cation $ABTS^{\cdot+}$ is reduced to ABTS (Re *et al*., 1999). The radical was generated by reacting a 7 mM solution of ABTS in water with 2.45 mM of potassium persulphate (1:1). The assay was made with 980 µl of $ABTS^{\cdot+}$ solutions and 20 µL of the diluted sample (1:50 in water). The reaction takes place in darkness at room temperature. Absorbance measurements at 734 nm were taken after 15 min of reaction time.

The procedure used to determine antioxidant capacity using the DPPH method is described by Brand-Williams *et al*. (1995). This spectrophotometric technique employs the 2.2-diphenyl-1-picrylhydrazyl free radical ($DPPH^{\bullet}$), which shows a characteristic UV-vis spectrum with a maximum absorbance close to 515 nm in methanol. Briefly, 0.1 mL of different sample concentrations was added to 3.9 mL of a DPPH methanolic solution (25 mg/L). The DPPH solution was prepared daily and protected from light. Absorbance at 515 nm was measured after 30 min of reaction at 20ºC. The reaction was carried out under shaking in closed Eppendorf

tubes at 20ºC. Methanol was used as a blank reference.

The antioxidant capacity results were expressed as Trolox® equivalents (TEAC mM) by a calibration curve obtained with a standard Trolox®. All laboratory measurements were performed in duplicate.

Proanthocyanidins according to their degree of polymerisation

Seed and skin extracts were separated into three fractions containing flavan-3-ol monomers, oligomeric (degree of polymerisation ranging from 2 to 12–15) and polymeric (degree of polymerisation > 12–15) fraction, using the C_{18} Sep-Pack column as described by Sun *et al*. (1998a). Thus, each sample was passed through the two preconditioned neutral C_{18} Sep-Pack cartridges connected in series. To eliminate phenolic acids, a 4 mL de-alcoholised medium was adjusted to pH 7.0 and then passed through the two connected C_{18} Sep-Pack cartridges preconditioned with 10 mL of water adjusted to pH 7.0.

After drying the column with nitrogen, elutions were carried out, first with 25 mL of ethyl acetate to elute catechins and oligomeric proanthocyanidins, and then with 10 mL of methanol to elute polymeric fraction. The ethyl acetate eluate was taken to dryness under vacuum, re-dissolved in 3 mL of 67 mM phosphate buffer at pH 7.0, and reloaded onto the same series of cartridges that had been conditioned again as described above. The cartridges were dried with nitrogen and eluted sequentially with 25 mL of diethyl ether (fraction containing flavan-3-ol monomers) and 10 mL of methanol (fraction containing oligomers). The three fractions obtained were evaporated to dryness under vacuum and re-dissolved in 3 mL of methanol.

For each fraction obtained previously, the quantification of flavanols was performed by the modified vanillin assay as described by Sun *et al*. (1998b). Thus, the vanillin reaction with the catechin fraction was carried out in a 30ºC water bath for 15 min, and a measurement of 500 nm was also taken at 30ºC. For oligomeric and polymeric fractions, both the vanillin reaction and the measurement of 500 nm were performed at room temperature, and the maximum of 500 nm was taken as the measured value. Oligomeric and polymeric proanthocyanidins isolated from grape seeds previously isolated by column chromatography on Lichroprep RP-18, in accordance with what was described earlier by Sun *et al*. (1998a), were dissolved in methanol to prepare the standard solutions used to build the standards curves. All laboratory analyses were done in duplicate.

Chromatographic analysis of individual anthocyanins

For the analysis of individual skin anthocyanins, an HPLC Dionex Ultimate 3000 Chromatographic System (Sunnyvale, California, USA) equipped with a quaternary pump Model LPG-3400 A, an auto sampler Model ACC-3000, a thermostated column compartment (adjusted to 25ºC) and a multiple Wavelength Detector MWD-300 was used. The column (250 x 4.6 mm, particle size 5 µm) was a C_{18} Acclaim® 120 (Dionex, Sunnyvale, California, USA) protected by a guard column of the same material. The solvents were (A) 40% formic acid, (B) pure acetonitrile and (C) bi-distilled water. The individual anthocyanins were analysed by HPLC using the method described by Dallas and Laureano (1994). Thus, initial conditions were 25% A, 10% B and 65% C, followed by a linear gradient from 10 to 30% B, and 65 to 45% C for 40 min, with a flow rate of 0.7 mL/min. The injection volume was 40 µl. Detection was done at 520 nm and Chromeleon software program version 6.8 (Sunnyvale, California, USA) was used.

The individual anthocyanins were quantified by a calibration curve obtained with standard solutions of malvidin-3-glucoside chloride (> 95% purity) from Extrasynthese (Genay, France). The chromatographic peaks of anthocyanins were identified according to reference data previously described by Dallas and Laureano (1994). All HPLC analyses were done in duplicate.

Statistical analysis

Analysis of variance (ANOVA, one-way) and comparison of treatment means by Duncan's test ($\alpha = 0.05$) were carried out using the SPSS software program version 11.0 (SPSS Inc. Headquarters, Chicago, IL, USA). In addition, the correlation coefficient between antioxidant capacity values and the content of the different phenolic compounds was determined using the Microsoft Office Excel 2003 software program.

RESULTS AND DISCUSSION

Evolution of proanthocyanidin fraction

Proanthocyanidins from the seeds and skins of Tinta Roriz and Touriga Nacional were separated into monomers (catechins), oligomers and polymers, and then quantified during grape berry development (Figs 1 and 2). In the seeds and skins of both grape varieties, a general decrease in the values was observed for the three fractions analysed, followed by stabilisation in the last weeks of ripening.

The decrease in proanthocyanidin fractions observed for both grape fractions in both grape varieties has previously been reported for other grape varieties and in other regions by various authors (Jordão *et al*., 2001a,b; Downey *et al*., 2003; Ó-Marques *et al*., 2005). In a study on condensed tannin accumulation in the skin during berry development in Shiraz and Cabernet Sauvignon grape varieties, Hanlin and Downey (2009) reported a maximum tannin concentration just after fruit set, with lower tannin concentrations at véraison and harvest. The tendency for higher concentrations of condensed tannins in the early stages of berry development may be related to their metabolisation throughout ripening. For Kennedy *et al*. (2000a), the decrease in proanthocyanidin content in the seeds after véraison could be explain by oxidation reactions, while for Cheynier *et al*. (1997) the decrease could be attributed to reduced extractability resulting from the conjugation of proanthocyanidins with other cell components. Valero *et al*. (1989) considered that the concentration decrease during grape maturation is only a consequence of the increasing weight of the berries or seeds. However, for Bogs *et al*. (2005) the level of proanthocyanidins detected in the grapes represents a balance between the accumulation of proanthocyanidins through synthesis and decreased extractability, and may not be a true reflection of biosynthesis in the fruit. Finally, Bordiga *et al*. (2011) recently reported a progressive proanthocyanidin decrease

(with the exception of epigallocatechin) during grape ripening and, at same time, an increase in the percentage of prodelphinidins between the first and the last sampling. These authors also reported an inter-conversion between catechin and epigallocatechin during the maturation of the different red grapes analysed.

During grape berry ripening, in both grape varieties, the seeds represented the highest concentration of all proanthocyanidin fractions (from 72.0 mg/g of seeds at the beginning of véraison to 30.0 mg/g of seeds at the end of maturation for the polymeric fraction in the Tinta Roriz grape variety, and from 56.0 mg/g of seeds at the beginning of véraison to 8.0 mg/g of seeds at the end of maturation for the oligomeric fraction in the Touriga Nacional grape variety), followed by the skins (from 15.4 mg/g of skins at the beginning of véraison to 7.8 mg/g of skins at the end of maturation for the polymeric fraction, and from 17.1 mg/g of skins at the beginning of véraison to 8.2 mg/g of skins at the end of maturation for the oligomeric fraction in the Tinta Roriz grape variety). The quantification of high proanthocyanidin concentrations in the seeds in relation to the skins is in accordance with the results obtained by other authors (Ricardo-da-Silva *et al*., 1992a; Jordão *et al*., 2001a; Ó-Marques *et al*., 2005). In addition, the results show (Fig. 1 and 2) that, during the entire maturation process and for all berry fractions and for both varieties, the monomeric

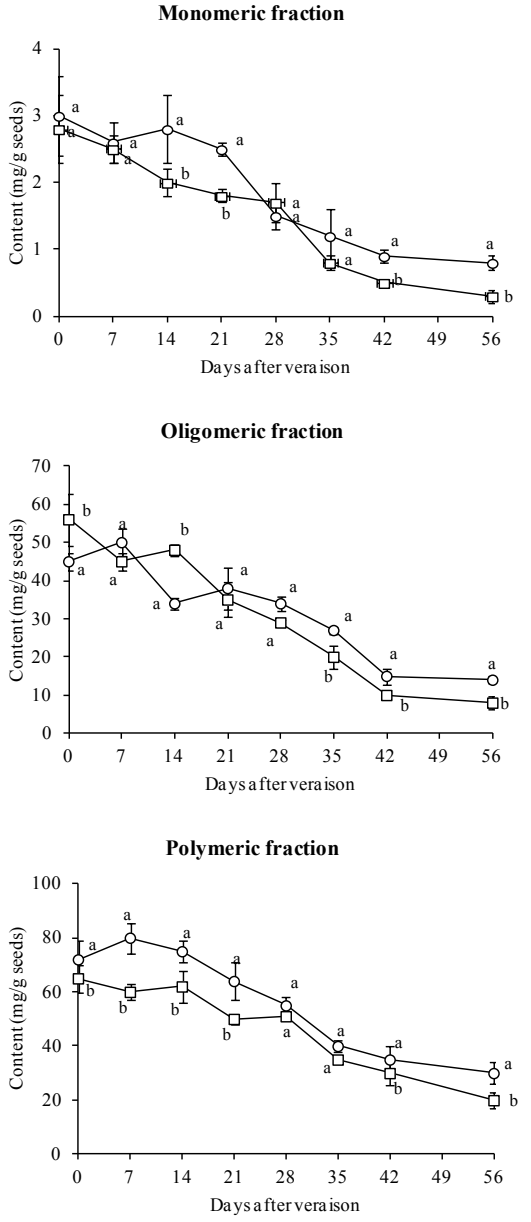

FIGURE 1

Evolution of the different seed proanthocyanidin fractions during the ripening of two red grape varieties for the same sampling day. Different letters show significant differences between means according to the Duncan test (α = 0.05); Error bars indicate standard deviation. (O) Tinta Roriz; (□) Touriga Nacional

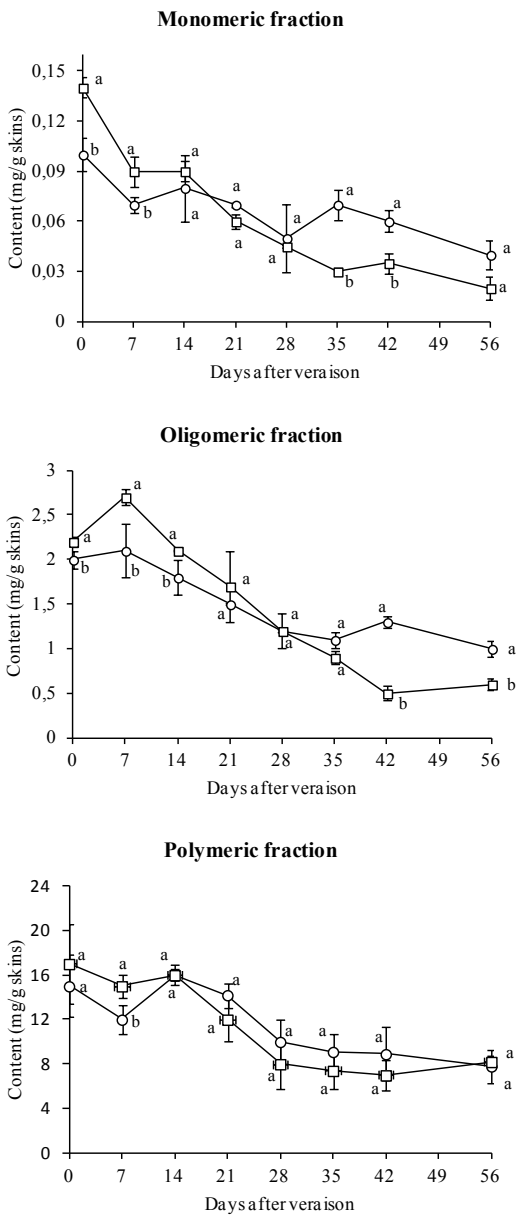

FIGURE 2

Evolution of the different skin proanthocyanidin fractions during the ripening of two red grape varieties for the same sampling day. Different letters show significant differences between means according to the Duncan test (α = 0.05); Error bars indicate standard deviation. (O) Tinta Roriz; (□) Touriga Nacional

fraction represented the lowest concentration and the polymeric fraction the highest concentration of condensed tannins. These results confirm research by Sun *et al*. (2001) and Ó-Marques *et al*. (2005) on proanthocyanidin evolution in skins and seeds during grape berry ripening in the Cabernet Sauvignon and Tinta Roriz varieties.

Finally, the results show significant differences between the two varieties, namely a significant high concentration of proanthocyanidins in the seed and skins (except for the polymeric fraction) for the Tinta Roriz grape variety in contrast to Touriga Nacional. In addition, the values obtained for Tinta Roriz are similar to those obtained by Ó-Marques *et al*. (2005) for the same grape variety, but from other growing regions with other vineyard management practices and environmental conditions. This fact may lead us to consider that it is the caste factor that most influences proanthocyanidin content in the different grape berry fractions with respect to other factors such as climatic and geographical factors, cultural practices and the plants' vegetative vigour.

Individual anthocyanin evolution

Figs. 3 and 4 show the evolution of different individual monomeric anthocyanins extracted from the skins during ripening. In both grape varieties, a general increase followed by a slight oscillation in the individual anthocyanins was observed during the ripening process. However, for the majority of individual anthocyanins in the Touriga Nacional variety (delphinidin-3-glucoside, petunidin-3-glucoside, malvidin-3-glucoside, delphinidin-3-acetylglucoside, petunidin-3-acetylglucoside, malvidin-3-*p*-coumaroyl glucoside and petunidin-3-*p*-coumaroyl glucoside), a slight decrease in the values was quantified during the last two weeks of the ripening process.

For both varieties, the results indicate that the group of anthocyanin-3-glucoside pigments were the most abundant, followed by the 3-acetyl-glucoside forms and finally the coumaroyl-glucoside pigments. Considering the individual anthocyanin content, malvidin-3-glucoside (from 0.40 to 7.79 mg/g skin at the beginning of véraison and at the end

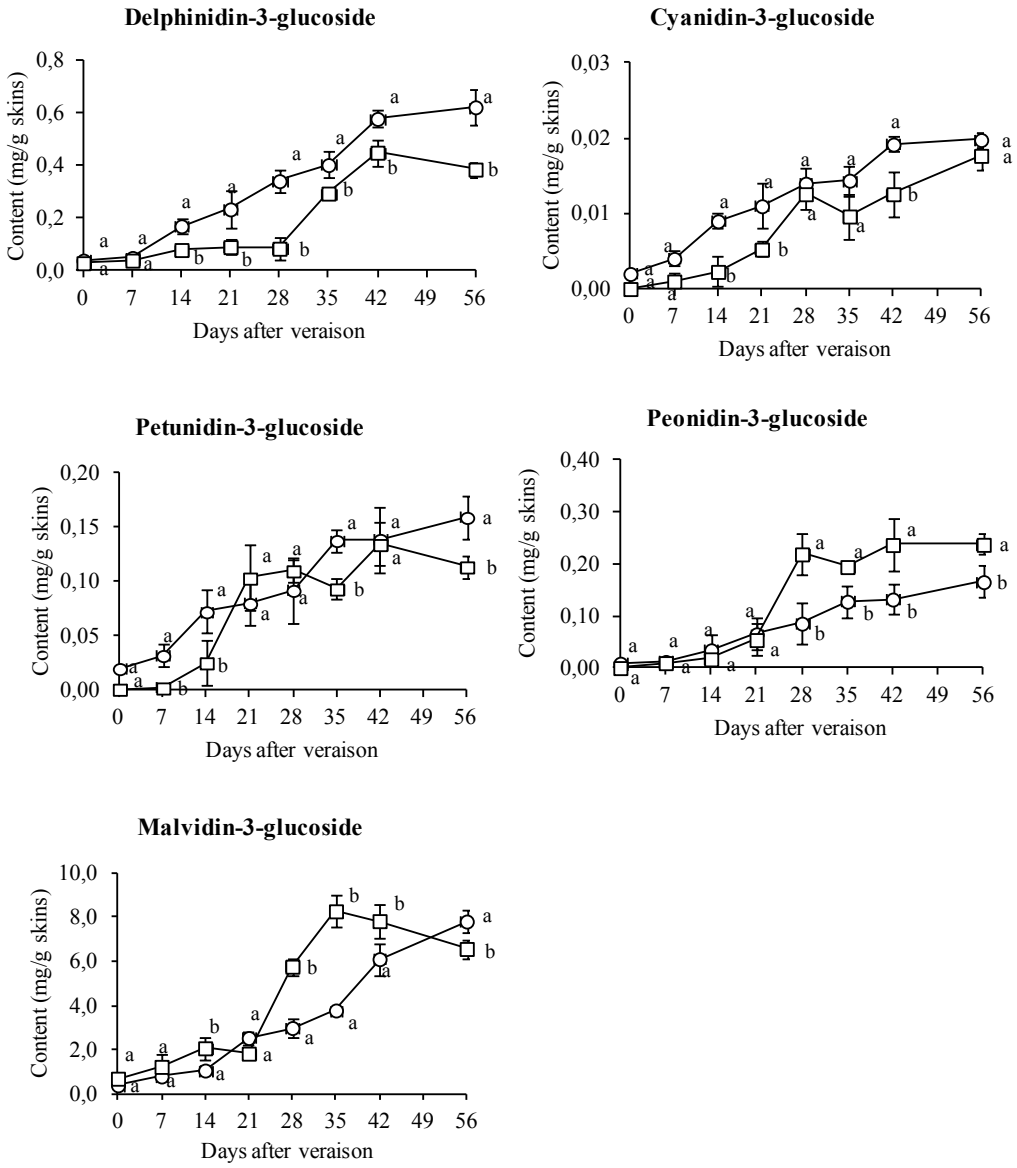

FIGURE 3

Evolution of skin anthocyanin glucoside derivatives during the ripening of two red grape varieties for the same sampling day. Different letters show significant differences between means according to the Duncan test (α = 0.05); Error bars indicate standard deviation. (O) Tinta Roriz; (□) Touriga Nacional

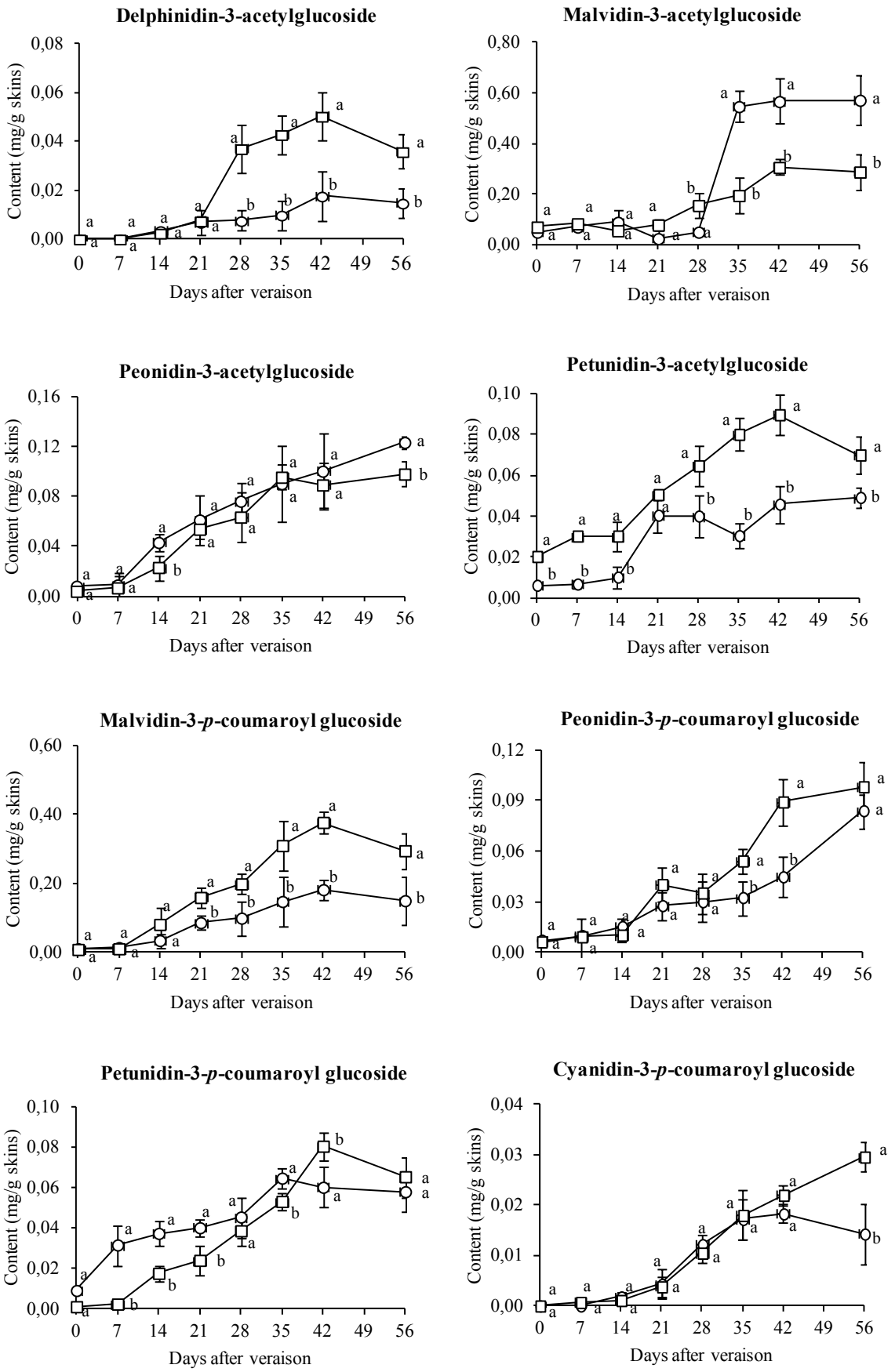

FIGURE 4
Evolution of skin anthocyanin acetyl and coumaroyl glucoside derivatives during the ripening of two red grape varieties for the same sampling day. Different letters show significant differences between means according to the Duncan test (α = 0.05); Error bars indicate standard deviation. (O) Tinta Roriz; (□) Touriga Nacional

of maturation respectively), followed by delphinidin-3-glucoside (from 0.030 to 0.62 mg/g of skins at the beginning of véraison and at the end of maturation respectively) and malvidin-3-acetylglucoside (from 0.05 to 0.57 mg/g of skins at the beginning of véraison and at the end of maturation respectively), was the most abundant in both varieties. In addition, cyanidin-3-glucoside (from 0.0 to 0.019 mg/g of skins at the beginning of véraison and at the end of maturation respectively) and cyanidin-3-*p*-coumaroyl-glucoside (from 0.0 to 0.029 mg/g of skins at the beginning of véraison and at the end of maturation respectively) were the least abundant pigments in both varieties. According to Roggero *et al*. (1986), cyanidin derivatives are one of the primary pigments in the biosynthetic pathway, constituting the smallest group during maturation, while for other authors malvidin-3-glucoside represents the ultimate form in biosynthesis transformation chains (Jordão *et al*., 1998a). Several authors (Bakker & Timberlake, 1985; Roggero *et al*., 1986; Jordão *et al*., 1998a; Romero-Cascales *et al*., 2005; Mulinacci *et al*., 2008) have reported that, during all stages of ripening, anthocyanin-3-glucosides are the most abundant pigment group, while malvidin-3-glucoside is the most abundant individual anthocyanin, regardless of the international wine grape variety. However, the main anthocyanin in a pool of red table grapes was peonidin-3-glucoside (Cantos *et al*., 2002). For some indigenous Spanish *Vitis vinifera* L. red grape varieties in danger of extinction, Gómez-Alonso *et al*. (2007) reported that delphinidin-3-glucoside (Tinto Velasco variety), peonidin-3-glucoside (Gordera Roja and Teta de Vaca Tinta varieties) and cyanidin-3-glucoside (Rojal variety) were the predominant individual anthocyanins. Furthermore, some authors believe that the ratio of total anthocyanins to acetyl and coumaroyl derivates can be usefully correlated to the specificity of each grape cultivar (Mulinacci *et al*., 2008).

It is commonly accepted that the anthocyanin composition of each cultivar is closely linked to its genetic inheritance and, from a qualitative point of view, is quite independent of seasonal conditions or production area (Férnandez-Lopez *et al*., 1998). In our study, and in general, the Touriga Nacional variety presented the highest content of acetyl and coumaroyl derivatives, but the lowest content of the 3-glucosides derivatives during grape ripening.

The evolution of antioxidant capacity and its relationship with proanthocyanidins and anthocyanins.

The evolution of antioxidant capacity in the seeds and skins measured by the ABTS and DPPH methods during grape maturation of the Touriga Nacional and Tinta Roriz grape cultivars is presented in Figs. 5 and 6 respectively. For both grape varieties and for all grape fractions, a general decrease was observed in the early stages of maturation, followed by stabilisation of the values in the last weeks of ripening. However, a tendency for a slight increase in the values in the skins of Tinta Roriz was observed in the last two weeks of ripening. These results were independent of the antioxidant capacity method used. In addition, the seeds represented the highest antioxidant capacity during grape maturation in both varieties. In general, these results are according to others that analysed the antioxidant capacity of seeds and skins in several red grape varieties (Poudel *et al*., 2008; Xu *et al*., 2010). However, these works only presented the antioxidant capacity results at technical grape maturation, and not over the grape maturation process. It is also important to consider that the majority of the cited works only report the results of the total antioxidant capacity from the grape must, without the contribution of seeds during the extraction process or without an individual analysis for each grape berry fraction. Thus, it is difficult to compare our results with the cited results obtained for other grape varieties. It is important to highlight that, in the literature, there generally are large variations in values of antioxidant capacities obtained for different grape varieties as a result of different analytical methods, different sample preparation and extraction processes, and different grape varieties and species studied (*Vitis vinifera*, *Vitis labruscana*, *Vitis amurensis* and several Euro-American and Asian hybrids). In addition, the impact of climatic and soil conditions on the phenolic composition of grapes should be considered (Yokotsuka *et al*., 1999; Mateus *et al*., 2002).

In general, the antioxidant capacity of Tinta Roriz during maturation was significantly higher than that of Touriga Nacional (Figs. 5 and 6). This was more evident for the results obtained using the ABTS method. At harvest, the content of antioxidant capacity (measured by the ABTS method) of Tinta Roriz was 445.1 and 72.0 µmol trolox/g for seeds and skins respectively, while for Touriga Nacional the values in the seeds and skins were 245.8 and 52.5 µmol trolox/g respectively. However, at technical maturity the

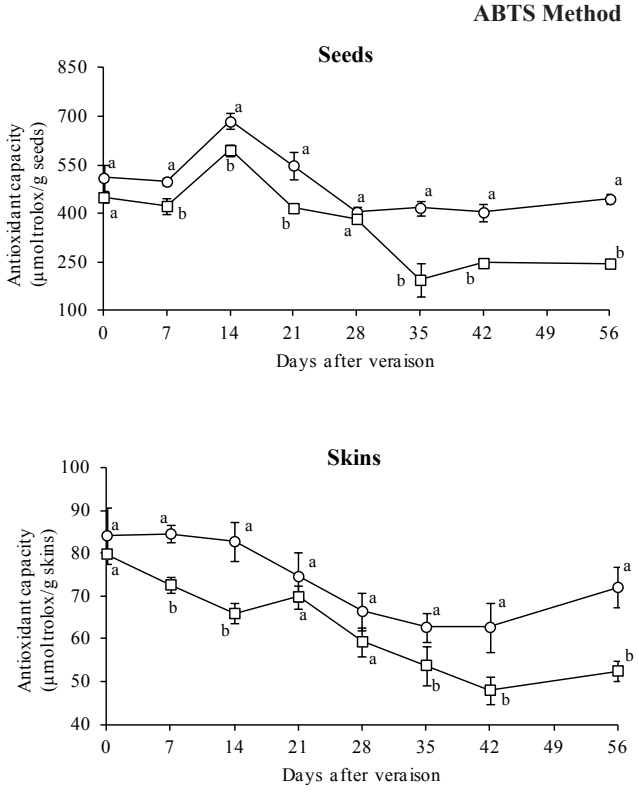

FIGURE 5

Evolution of the antioxidant capacity in seeds and skins measured by the ABTS method during the ripening of two red grape varieties for the same sampling day. Different letters show significant differences between means according to the Duncan test (α = 0.05); Error bars indicate standard deviation. (O) Tinta Roriz; (□) Touriga Nacional

quantitative values obtained for the seeds and skins of the two Portuguese varieties are lower than the values reported for the Cabernet Sauvignon grape variety by Xu *et al*. (2010), but similar to the results reported for several other grape varieties by Poudel *et al*. (2008).

The data in Table 2 show the linear correlation coefficients (R^2) between the different proanthocyanidin fractions and the antioxidant capacity quantified in the seeds and skins during grape maturation in the two varieties. The correlation coefficients generally indicated an important role of the different proanthocyanidin fractions of the skins and seeds in the antioxidant capacity during grape maturation. The correlation coefficient values for skins ranged from 0.72 to 0.82, from 0.72 to 0.86 and from 0.52 to 0.82 for the monomeric, oligomeric and polymeric fraction of proanthocyanidins respectively, while for seeds the correlation values ranged from 0.57 to 0.79, from 0.72 to 0.86 and from 0.47 to 0.79 for the monomeric, oligomeric and polymeric fraction of proanthocyanidins respectively.

Several authors have reported considerable correlations between antioxidant capacity and the total polyphenolic content of a large number of grape seed and skin extracts from different varieties (Bakkalbase *et al*., 2005; Yang *et al*., 2009; Xu *et al*., 2010). However, other authors (Bozan *et al*., 2008) reported no significant correlations between individual flavanols analysed by HPLC or total polyphenols and antioxidant capacity values in seed extracts from several grape varieties. Thus, there is conflicting evidence in the literature about the correlation between polyphenol content and the antioxidant capacity of grapes. Furthermore, it is important to consider that there is a quantitative and qualitative change in the phenolic profile of the grapes during the maturation process and this consequently will affect the evolution of antioxidant capacity values.

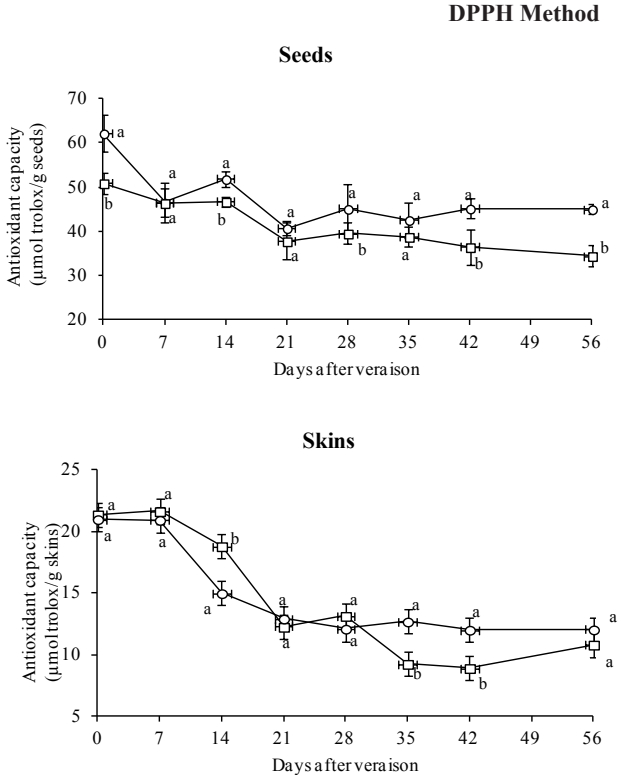

FIGURE 6
Evolution of the antioxidant capacity from seeds and skins measured by the DPPH method during the ripening of two red grape varieties for the same sampling day. Different letters show significant differences between means according to the Duncan test (α = 0.05); Error bars indicate standard deviation. (O) Tinta Roriz; (□) Touriga Nacional

TABLE 2
Correlation coefficients between the levels of different proanthocyanidin fractions and their antioxidant capacity from seeds and skins during the maturation of Touriga Nacional and Tinta Roriz grape varieties.

		Correlation coefficient (adjusted R^2) Antioxidant methods	
	Proanthocyanidin fractions	DPPH	ABTS
Tinta Roriz			
Seeds	Monomeric	0.73	0.57
	Oligomeric	0.85	0.77
	Polymeric	0.72	0.47
Skins	Monomeric	0.72	0.75
	Oligomeric	0.86	0.76
	Polymeric	0.52	0.59
Touriga Nacional			
Seeds	Monomeric	0.79	0.62
	Oligomeric	0.86	0.72
	Polymeric	0.79	0.74
Skins	Monomeric	0.76	0.82
	Oligomeric	0.72	0.83
	Polymeric	0.69	0.82

Anthocyanins are considered very good antioxidant agents, with their high activity being attributed to their peculiar structure, namely the oxonium ion in the C ring. The antioxidant functions of anthocyanins have been attributed to the aglycone moiety, and this was demonstrated for cyanidin and some of its glycosides (Wang *et al*., 1999). The number of sugar residues at the 3-position, the oxidation state of the C ring (Lapidot *et al*., 1999), the hydroxylation and methylation pattern (Wang *et al*., 1997), as well as acylation by phenolic acids (Degenhardt *et al*., 2000) are considered crucial factors for the expression of antioxidant effects.

Correlation coefficients between the levels of individual anthocyanins and the antioxidant capacity from the skins during grape maturation of the two grapes varieties studied are presented in Table 3.

There was a negative relationship between individual anthocyanins and antioxidant capacity during grape skin maturation. These results were independent of the antioxidant capacity method used and the grape varieties studied, suggesting that anthocyanins were not the most powerful radical scavengers that occur in grape berry skins during the maturation process. Thus, grape skins contain other molecules, like proanthocyanidins, but perhaps also flavonols

TABLE 3
Correlation coefficients between the concentration of individual anthocyanins and their antioxidant capacity during the skin maturation of Touriga Nacional and Tinta Roriz grape varieties.

	Correlation coefficient[a] (adjusted R^2) Antioxidant methods	
Individual anthocyanins	DPPH	ABTS
Tinta Roriz		
Delphinidin-3-glucoside	0.79	0.65
Cyanidin-3-glucoside	0.84	0.69
Petunidin-3-glucoside	0.84	0.67
Peonidin-3-glucoside	0.84	0.68
Malvidin-3-glucoside	0.67	0.48
Delphinidin-3-acetylglucoside	0.82	0.68
Petunidin-3-acetylglucoside	0.82	0.64
Peonidin-3-acetylglucoside	0.85	0.67
Malvidin-3-acetylglucoside	0.53	0.45
Peonidin-3-*p*-coumaroyl glucoside	0.50	0.29
Malvidin-3-*p*-coumaroyl glucoside	0.93	0.84
Cyanidin-3-*p*-coumaroyl glucoside	0.84	0.91
Petunidin-3-*p*-coumaroyl glucoside	0.79	0.71
Touriga Nacional		
Delphinidin-3-glucoside	0.41	0.80
Cyanidin-3-glucoside	0.66	0.77
Petunidin-3-glucoside	0.85	0.68
Peonidin-3-glucoside	0.63	0.84
Malvidin-3-glucoside	0.58	0.90
Delphinidin-3-acetylglucoside	0.58	0.87
Petunidin-3-acetylglucoside	0.68	0.88
Peonidin-3-acetylglucoside	0.77	0.84
Malvidin-3-acetylglucoside	0.39	0.80
Peonidin-3-*p*-coumaroyl glucoside	0.54	0.74
Malvidin-3-*p*-coumaroyl glucoside	0.72	0.90
Cyanidin-3-*p*-coumaroyl glucoside	0.48	0.80
Petunidin-3-*p*-coumaroyl glucoside	0.66	0.93

[a] Negative linear correlations values.

and phenolic acids, that have a more powerful effect on the antioxidant capacity of grape skins during maturation. Meyer *et al*. (1997) showed that anthocyanins in grape extracts are rather moderately related to the inhibition of low-density lipoprotein oxidation, which has been attributed mainly to total phenol content. In addition, Kallithraka *et al*. (2005) reported a low and statistically insignificant correlation for the total anthocyanin content and antioxidant capacity of skin extracts from several Greek grape cultivars at harvest. According to Orak (2007), the antioxidant capacity of red grape cultivars does not always have a relationship with the presence of their anthocyanin content.

Despite their low contribution to the antioxidant power of the grape, anthocyanins have been claimed to exhibit various activities of biological importance, such as an antioxidant and anti-inflammatory function (Wang *et al*., 1999) and peroxynitrile scavenging (Tsuda *et al*., 2000).

CONCLUSIONS

The antioxidant capacity of both Portuguese grape varieties studied showed a tendency to decrease in all the grape berry fractions studied during grape maturation. A similar tendency was observed for the different proanthocyanidin fraction contents analysed in the skins and seeds. These tendencies were particularly more highly pronounced in the first five weeks after véraison. At the same time, our results show that seeds are an important source of proanthocyanidins and consequently present a high antioxidant capacity with respect to the grape berry skin. As for the different individual monomeric anthocyanins quantified during grape maturation, a continuous increase in the values was generally observed during the ripening process. However, for some of the individual anthocyanins analysed, this continuous increase was followed by a slight stabilisation or decrease in the values in the last weeks of ripening. Finally, for both grape varieties studied, a negative relationship was obtained between individual anthocyanins and antioxidant capacity during grape skin maturation.

Meanwhile, although the data presented may contribute towards an understanding of the role of grape maturation in the antioxidant capacity of the different grape berry fractions, these aspects need to be studied in other grape varieties and taking several other factors into account, such as the influence of vintage conditions and cultural practices.

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