Vitis **51** (3), 137–139 (2012)

Evolution of antioxidant capacity in seeds and skins during grape maturation and their association with proanthocyanidin and anthocyanin content

A. M. Jordão[1), 2)], A. C. Correia[2)] and F. J. Gonçalves[2)]

[1)] Polytechnic Institute of Viseu, Centre for the Study of Education, Technologies and Health, Viseu, Portugal
[2)] Agrarian Higher School of Viseu, Department of Food Industries, Viseu, Portugal

Summary

In order to investigate the antioxidant activity in seeds and skins during grape maturation and their relation with anthocyanin and proanthocyanidin content, two Portuguese red grape varieties, 'Touriga Nacional' and 'Tinta Roriz' were studied. For antioxidant activity analysis, one analytical method was used (ABTS method). Different proanthocyanidins fractions according to their degree of polymerization were analyzed, while 13 different individual anthocyanins were evaluate by HPLC as well. During the maturation period the antioxidant activity content quantified from the different grape berry fractions was characterized by a general decrease with a slight oscillation in the early stages of maturation, followed by a stabilization and slight increase of the values in the last weeks of ripening. Similar evolution was observed for the different proanthocyanidins fractions quantified. For the individual anthocyanins, in general the evolution was characterized by a slowly increase in the first 3 weeks of ripening process, followed by a marked increase and finally a slight decrease or stabilization in the last weeks of ripening. Finally, high linear positive correlations between proanthocyanidin fractions and the antioxidant activity evolution during the grape maturation were obtained while for the individual anthocyanins a high negative correlation values was quantified.

Introduction

Flavan-3-ols, flavonols and anthocyanins are the most important compounds that contribute to the antioxidant proprieties in red grapes and wine.

In recent years several authors have reported the phenolic composition and antioxidant capacity of several red and white grapes from different varieties, fractions of the grape berry and countries (Xu *et al.* 2010, Breska *et al.* 2010). These studies looked into the grapes only at harvest (generally at optimum maturity or technological maturation). However, there is a considerable lack of information for the evolution of the antioxidant capacity from the different fractions of the grape berry during grape maturation and their relationship with the different proanthocyanidin fractions and individual anthocyanin evolution content.

Thus the main objective of this study was to investigate the antioxidant capacity evolution from the different fractions of the grape berry (seeds and skins) and their relationship with the different proanthocyanidin fractions (monomeric, oligomeric and polymeric fraction) and individual anthocyanin content of two Portuguese red grape varieties. This paper will help towards a better understanding of the antioxidant capacity that is quantified in different fractions of grape berry, especially during maturation and the role of two important flavonoid compound groups in the evolution of antioxidant capacity values.

K e y w o r d s : antioxidant capacity; anthocyanins; grapes; maturation; proanthocyanidins.

Material and Methods

S a m p l e p r e p a r a t i o n : Two Portuguese red grape varieties (*Vitis vinifera* L.), 'Touriga Nacional' and 'Tinta Roriz', were harvested in 2009 from a vineyard in the region of Viseu (northern Portugal). Grapes (200 berry samples) were kept frozen at -18 ºC until processing. For the two grape varieties used, sampling started at veraison and lasted until technological maturity. Skins and seeds, were removed manually from the berries and washed separately several times with distilled water, and moisture was absorbed on blotting paper. Before the extraction process, seeds were crushed. For the extraction process we used 50 mL of 80 % methanol followed by 50 mL of 75 % acetone, each for 3 h with agitation. After clarification of the suspension by centrifugation all extracts were assembled. Aliquots of each extract were filtered. All extractions were done in duplicate for each grape berry fraction analyzed.

A n t i o x i d a n t c a p a c i t y : The total antioxidant capacity evolution for each grape berry fraction was determined from the extracts produced according to the methodology previously described, using the ABTS method (Re *et al.* 1999). The results were expressed as Trolox equivalents (TEAC mM), using the relevant calibration curve. All measurements were performed in duplicate.

P r o a n t h o c y a n i d i n s a n d i n d i v i d u a l a n t h o c y a n i n s : Seeds and skin extracts were separated into three fractions containing catechins (monomers), oligomeric (degree of polymerisation ranging from 2 to 12-15) and polymeric (degree of polymerisation > 12-15) fraction, using C_{18} Sep-Pack column as described by Sun *et al.*

Correspondence to: Prof. A. M. Jordão, Agrarian Higher School of Viseu, Department of Food Industries, Estrada de Nelas, Quinta da Alagoa, Ranhados, 3500-606 Viseu, Portugal. E-mail: antoniojordao@esav.ipv.pt

(1998). The individual anthocyanins from skins were analyzed by HPLC using the method described by Dallas and Laureano (1994). All analyses were done in duplicate.

Results and Discussion

Antioxidant capacity evolution and its relationship with proanthocyanidins and individual anthocyanins: For the two grape varieties studied and for the two grape fractions analyzed, a general decrease in antioxidant capacity was observed in the early stages of maturation, followed by stabilization and slight increase in the values in the last weeks of ripening (Figure). Additionally, during grape maturation, seeds presented the major antioxidant capacity values. In general, these results are in accordance with other published works which analyzed antioxidant capacity in seeds and skins in several red *Vitis vinifera* grape varieties (Poudel *et al.* 2008, Xu *et al.* 2010). However, the antioxidant capacity values presented by these works are only measured at technical grape maturation and not during the grape maturation. Additionally, it is important to consider that the majority of the works only report the results of the total antioxidant capacity from the grape extract without the contribution of seeds during the extraction process or without individual analysis for each grape berry fraction. Thus, it is difficult to compare our results with the results obtained for other grape varieties. It is important to note that generally speaking, there are large variations of antioxidant capacity values quantified in the different grape varieties mentioned in the literature as a result of the different antioxidant capacity methods used, different grape varieties and species studied, vintage factors, and climatic and soil conditions.

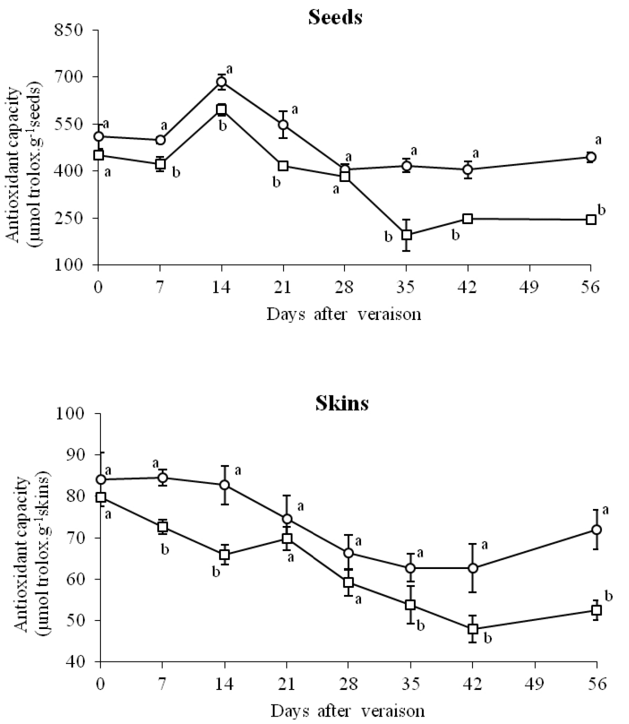

Figure: Evolution of the antioxidant capacity from seeds and skins during the maturation of two red grape varieties. Data points derived for given day showing the same letter are not significantly different according to the Duncan test ($\alpha = 0.05$); error bars indicate standard deviation. —○— = Tinta Roriz; —□— = Touriga Nacional.

Table

Linear correlations between the levels of different proanthocyanidins fractions, individual anthocyanins, and the antioxidant capacity from seeds and skins during the maturation of two red grape varieties.

Variables	Correlation coefficient (adjusted R^2)
Tinta Roriz	
Proanthocyanidins fractions	
Seeds	
Monomeric	0.57
Oligomeric	0.17
Polymeric	0.47
Skins	
Monomeric	0.35
Oligomeric	0.76
Polymeric	0.59
Individual anthocyanins[a]	
Delphinidin-3-glucoside	0.65
Cyanidin-3-glucoside	0.69
Petunidin-3-glucoside	0.67
Peonidin-3-glucoside	0.68
Malvidin-3-glucoside	0.48
Delphinidin-3-acetylglucoside	0.68
Petunidin-3-acetylglucoside	0.64
Peonidin-3-acetylglucoside	0.67
Malvidin-3-acetylglucoside	0.45
Peonidin-3-p-coumaroyl glucoside	0.29
Malvidin-3-p-coumaroyl glucoside	0.84
Cyanidin-3-p-coumaroyl glucoside	0.91
Petunidin-3-p-coumaroyl glucoside	0.71
Touriga Nacional	
Proanthocyanidins fractions	
Seeds	
Monomeric	0.62
Oligomeric	0.72
Polymeric	0.74
Skins	
Monomeric	0.82
Oligomeric	0.83
Polymeric	0.82
Individual anthocyanins[a]	
Delphinidin-3-glucoside	0.80
Cyanidin-3-glucoside	0.77
Petunidin-3-glucoside	0.68
Peonidin-3-glucoside	0.84
Malvidin-3-glucoside	0.90
Delphinidin-3-acetylglucoside	0.87
Petunidin-3-acetylglucoside	0.88
Peonidin-3-acetylglucoside	0.84
Malvidin-3-acetylglucoside	0.80
Peonidin-3-p-coumaroyl glucoside	0.74
Malvidin-3-p-coumaroyl glucoside	0.90
Cyanidin-3-p-coumaroyl glucoside	0.80
Petunidin-3-p-coumaroyl glucoside	0.93

[a] Negative linear correlations values for individual anthocyanins from skins.

In general, the antioxidant values of the 'Tinta Roriz' grape variety during maturation were significantly higher than the values quantified for the 'Touriga Nacional' grape variety (Figure). This result was more evident in the last ripening weeks. At harvest the content of antioxidant capacity of the 'Tinta Roriz' grape variety was 445.1 and 72.0 µmol trolox·g^{-1} for seeds and skins respectively, while for the 'Touriga Nacional' grape variety the values quantified in seeds and skins were 245.8 and 52.5 µmol trolox·g^{-1} respectively. However, at technical maturity the quantitative values obtained for both Portuguese grape varieties studied for seeds and skins 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 the Table show the correlations between different proanthocyanidins fractions, individual anthocyanins and the antioxidant capacity during the grape maturation for both grape varieties studied. In general the correlation coefficients calculated indicated good correlations between monomeric, oligomeric and polymeric fractions of proanthocyanidins from skins and seeds (except for the correlations obtained for 'Tinta Roriz' grape variety) and the antioxidant capacity.

Several authors have also reported considerable correlations between antioxidant capacity and the total polyphenolic content of a great number of grape seed and skin extracts from different grape varieties (Xu *et al.* 2010). However, other authors (Bozan *et al.* 2008) reported no significant correlations between individual flavanols 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.

For the linear correlations between individual anthocyanins and antioxidant capacity from skins during the grape maturation, the results show that there was a negative relationship between individual anthocyanins and antioxidant capacity. These results suggest that, in this case anthocyanins were not the most powerful radical scavengers that occur in grape berry skins during the maturation process. Meyer *et al.* (1997) showed that anthocyanins in grape extracts are rather moderately related to the inhibition of LDL oxidation, which has mainly been attributed to total phenol content. Additionally, Kallithraka *et al.* (2005) reported a low and statistically insignificant correlation between total anthocyanin content and antioxidant capacity of skin extracts from several Greek grape cultivars at harvest. This result may be clearly illustrated taking into consideration that grape skins also contain other flavonoid polyphenols, principally flavanols and flavonols, which is at same time consistent with the results that we obtained in the relationship between antioxidant capacity and the different proanthocyanidin fractions during grape maturation.

Although the data presented here contributed to understanding the role of grape maturation in antioxidant capacity values from the different grape berry fractions, it must remembered that the results need to be analyzed in other grape varieties and several other factors must be analyzed such as the influence of vintage conditions and the cultural practices.

References

Bozan, B.; Tosun, G.; Özcan, D.; 2008: Study of polyphenol content in the seeds of red grape (*Vitis vinifera* L.) varieties cultivated in Turkey and their antiradical activity. Food Chem. **109**, 426-430.

Breska, A. P.; Takeoka, G. R.; Hidalgo, M. B.; Vilches, A.; Vasse, J.; Ramming, D. W.; 2010: Antioxidant activity and phenolic content of 16 raisin grape (*Vitis vinifera* L.) cultivars and selections. Food Chem. **121**, 740-745.

Dallas, C.; Laureano, O.; 1994: Effects of pH, sulphur dioxide, alcohol content, temperature and storage time on colour composition of a young Portuguese red table wine. J. Sci. Food Agric. **65**, 477-485.

Kallithraka, S.; Mohdaly, A. A.; Makris, D. P.; Kefalas, P.; 2005: Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): association with antiradical activity. J. Food Comp. Anal. **18**, 375-386.

Meyer, A. S.; Yi, O. S.; Pearson, D. A.; Waterhouse, A. L.; Frankel, E. N.; 1997: Inhibition of human low-density lipoprotein oxidation in relation to composition of phenolic antioxidants in grapes (*Vitis vinifera*). J. Agric. Food. Chem. **45**, 1638-1643.

Poudel, P. R.; Tamura, H.; Kataoka, I.; Mochioka, R.; 2008: Phenolic compounds and antioxidant activities of skins and seeds of five wild grapes and two hybrids native to Japan. J. Food Comp. Anal. **21**, 622-625.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C.; 1999: Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Medic. **26**, 1231-1237.

Sun, B. S.; Leandro, C.; Ricardo-da-Silva, J. M.; Spranger, I.; 1998: Separation of grape and wine proanthocyanidins according to their degree of polymerization. J. Agric. Food Chem. **46**, 1390-1396.

Wang, H.; Nair, M. G.; Strasburg, G. M.; Chang, Y. C.; Booren, A. M.; Gray, J. I.; De Witt, D. L.; 1999: Antioxidant and anti-inflammatory activities of anthocyanins and their aglycone, cyaniding, from tart cherries. J. Nat. Prod. **62**, 294-296.

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

Received October 11, 2011