ENVIRONMENTAL AND VITICULTURAL PRACTICE EFFECTS ON THE PHENOLIC COMPOSITION OF GRAPES: IMPACT ON WINE SENSORY PROPERTIES

Stefanos KOUNDOURAS

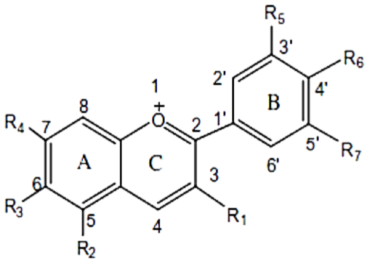
Laboratory of Viticulture, School of Agriculture, Faculty of Agriculture, Forrestry and Natural Environment, Aristotle University of Thessaloniki, University Campus 541 24 Thessaloniki, Greece
E-mail: skoundou@agro.auth.gr

Abstract
Grape phenolic compounds are located in the internal layers of grape skins and seeds. They are synthesized via the phenyl-propanoid biosynthetic pathway which is modulated by both biotic and abiotic factors. Considerable research has been conducted to clarify the evolution pattern of grape phenolic compounds and the role of environmental and viticultural factors that can manipulate their levels at harvest. The accumulation of phenolic compounds in grapes may be influenced by grape variety, environmental conditions and viticultural practices. More notably, the influence of irrigation on the accumulation of anthocyanins in grapes has been treated by several authors reporting an overall positive impact of mild water deficit, attributed to changes in berry skin-to-pulp ratio, modifications in grape microclimate or differences in the partitioning of assimilates among vine organs. Moreover, light environment of the grapes, as affected directly by leaf removal, is reported to modify skin anthocyanin content, profile and extractability. However, under hot climate conditions, increased temperatures of exposed berries may hasten phenolic ripening and decouple skin and seed sensory traits. Concerning berry tannins, reports on the effects of environmental and viticultural conditions are fewer and inconsistent. Moreover, there is limited information available concerning the effects of environmental and viticultural conditions on the structural characteristics of grape proanthocyanidins, such as polymerization, galloylation and subunit composition, which define wine sensory properties.

Keywords: grapevine, anthocyanins, tannins, flavan-3-ols, astringency, bitterness, polymerization, irrigation, microclimate.

1 PHENOLIC COMPOUNDS IN GRAPES: LOCALIZATION AND BIOSYNTHESIS

Grape-derived secondary metabolites are the principal sources of wine color, aroma and flavor. Among them, phenolic compounds are classified as non-flavonoid (benzoic and cinamic acids and stilbenes) and flavonoid (anthocyanins, flavonols and tannins).



Anthocyanidin	R1	R2	R3	R4	R5	R6	R7
cyanidin	OH	OH	H	OH	OH	OH	H
delphinidin	OH	OH	H	OH	OH	OH	OH
petunidin	OH	OH	H	OH	OMe	OH	OH
peonidin	OH	OH	H	OH	OMe	OH	H
malvidin	OH	OH	H	OH	OMe	OH	OMe

Figure 1: General structure of grape anthocyanidins

Among the latter, anthocyanidins are pigmented compounds located in the skins of grape berries in red cultivars while tannins derive from both skins and seeds of berries. Anthocyanins are glycosides of anthocyanidins. In red grape *Vitis vinifera* varieties, the most common 3-O-glucoside derivatives of anthocyanidins are delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside, the latter being the most abundant (Figure 1) (Kallithraka et al. 2005). *Vitis labrusca* and *Vitis rotundifolia* grapes contain both anthocyanidin monoglucosides and diglucosides. Although anthocyanin profile is mostly genetically driven, the relative amounts of anthocyanins were also shown to depend to a lesser extent on the degree of grape ripeness (Roggero et al. 1986) and on the growing conditions (Kotseridis et al. 2012)

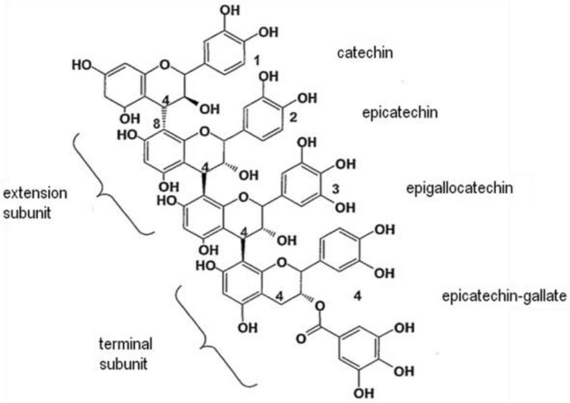


Figure 2: General structure of a proanthocyanidin polymer.

Proanthocyanidins are polymers composed of terminal and extension subunits of flavan-3-ols, mainly (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin (EGC) (Figure 2). Grape tannins derived from skins and seeds vary in their relative amount, length, subunit composition and sensory properties. Tannin content of the skins is reported to be lower than that of seeds (Prieur et al. 1994). Seed tannins are also shorter, with a lower mean degree of polymerization (mDP) while skin tannins are generally longer with a higher mDP (Chira et al. 2009). Seed proanthocyanidins are usually composed of C, EC and ECG (Hanlin and Downey 2009) whereas skin proanthocyanidins also contain EGC as extension subunit (Li et al. 2014) and have a lower proportion of ECG (Gil et al. 2012). EC is the major extension subunit in the skins (Monagas et al., 2003) while seeds were found to contain similar amounts of C and EC subunits (Downey et al. 2003).

Flavonoid compounds are synthesized via the phenyl-propanoid biosynthetic pathway which is modulated by both the biotic and abiotic factors (Cassasa et al. 2015). Biosynthesis of tannins occurs after anthesis, reaching a maximum at veraison (Ollé et al. 2011). Downey et al. (2003) observed the highest concentration of flavan-3-ols in Shiraz skins before veraison followed by a continuous decrease until complete ripeness while in other studies, skin tannins were found to change little from veraison to harvest (Harbertson et al. 2002). Concerning seed tannins, most studies report a general decline during ripening associated with seed coat browning (Kennedy et al. 2000). The degree of polymerization of skin tannins was generally reported to increase with ripening especially in the polymeric fraction (Kyraleou et al. 2015b). On the contrary, seed proanthocyanidins mDP was either found to remain approximately constant (Pastor del Rio and Kennedy 2006) or it followed a decreasing trend during ripening (Bordiga et al. 2011). Accumulation of anthocyanins commences at veraison and presents a maximum around harvest period (Castellarin et al. 2007) but some authors have observed a decline just before harvest or during over-ripening (Bucchetti et al. 2011). Kyraleou et al. (2016b) found that maximum anthocyanin accumulation of Syrah berries, grown under semiarid conditions, occured 18-24 days after veraison.

2 SENSORY PROPERTIES OF PHENOLIC COMPOUNDS

The types and amounts of various anthocyanins in grape skins determine the color and quality of the produced wines as they undergo co-pigmentation with other compounds to produce more stable pigments (Bolton 2001) but they are tasteless or indistinctly flavored (Vidal et al. 2004). On the other hand, proanthocyanidins are responsible for the bitter and astringent sensation of red wines. The intensity of astringency is positively related with proanthocyanidin concentration but it is mostly determined by polymer size, the longer molecules being more astringent than the smaller ones (Vidal et al. 2003). However, astringency of proanthocyanidins could also decrease at high mDP since large molecules become either less soluble or too bulky to bind with proteins (Sun et al. 2013). Moreover, the extraction of tannins becomes more difficult as their polymerization degree increases, thus, larger tannins may be less easily released from skins during the maceration phase (Quijada-Morin et al. 2015). Astringency also increases with the degree of galloylation (presence of ECG subunits) while it decreases in the presence of prodelphinidins (proanthocyanidins with subunits constituted of EGC) (Vidal et al. 2003). Bitterness is more associated with lower molecular weight compounds such as flavan-3-ols monomers and oligomers (Peleg et al. 1999). Seed and skin tannins were found to be equally astringent when tasted at the same concentration in a wine or buffer medium in some studies (Brossaud et al. 2001) while other works reported a higher astringency sensation of seed extracts compared to those of the skins (Kyraleou et al., 2016a). However, skin tannins commonly have a higher contribution to the polymer composition of the wine (Monagas et al. 2003) due to the increasing extractability of skin cell walls with the progress of ripening (Nunan et al. 1998) as compared to the lignified seed coat (Cadot et al. 2006). Finally, the bitter and astringent perception of tannins is also affected by the formation of complexes with soluble polysaccharides and proteins present in the grape must (Gil et al. 2012).

3 VINEYARD FACTORS AFFECTING PHENOLIC COMPOSITION OF GRAPES

Grape and wine phenolic content has been related to many agronomical factors such as soil and climate (Koundouras et al. 2006), rootstocks (Koundouras et al. 2009), irrigation (Kyraleou et al. 2016b), training systems (Kyraleou et al. 2015a) and summer pruning techniques (Kotseridis et al. 2012).

Environmental factors (topographical, agro-pedological, climatic) have been acknowledged to influence grape and wine phenolic composition, mainly by controlling vine vigour as a result of soil rooting depth, fertility and water storage (Koundouras et al. 1999). Therefore, phenolic composition of grapes usually shows a well structured and time-stable spacial pattern within the vineyard, mostly related to soil physical properties. Bramley (2003) demonstrated how variation in soil depth controlled by variation in topography affected vine growth in a Coonawarra vineyard. In a work conducted in a cv. Agiorgitiko vineyard in Central Greece situated on a steep slope (Figure 3, unpublished data), berries with the highest phenolic content were located in the upper part of the vineyard with the more shallow soil and the lowest water reserves, associated with low vine vigour (estimated as winter pruning weight).

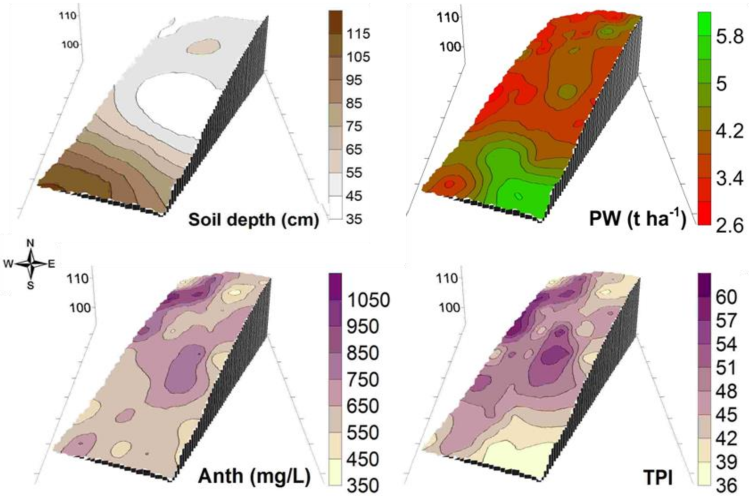


Figure 3: Spatial variability of the phenolic composition of 'Agiorgitiko' grapes as affected by topography (2010, Central Greece); PW, winter pruning weight; Anth, Total Anthocyanins in the juice; TPI, Total Grape Phenolics Index (OD280).

However, it is generally accepted that the direct manipulation of water availability through irrigation is the single most important management factor in determining berry and wine phenolic composition. More notably, many studies have reported a positive effect of a moderate water restriction imposed by deficit irrigation on the accumulation of anthocyanins in grapes in a variety of cultivars. The positive effects of water deficits on grape anthocyanin content are mostly attributed to suppressed vegetative growth, associated with improved canopy microclimate and better carbohydrate partitioning to the ripening berries (Romero et al. 2010) or to increased berry skin-to-pulp ratio (Koundouras et al. 2009). However, according to Roby et al. (2004), increases in skin polyphenol levels under limited irrigation can occur independently of any effect related to berry size modification. Recent studies have shown that the expression of genes of the flavonoid pathway is triggered earlier in grapevines submitted to water deficit thus stimulating anthocyanin biosynthesis (Castellarin et al. 2007). Anthocyanin accumulation pattern should also be considered when studying the effects of irrigation, since anthocyanin content is reported to undergo a decrease prior to harvest, especially under warm climate. In a recent study conducted in Greece (Kyraleou et al. 2016b), Syrah skin anthocyanins were higher in berries of water stressed vines 2 to 3 weeks after veraison, but no differences were observed compared to irrigated vines by harvest time.

In contrast to anthocyanins, reports on the effects of water availability on berry tannins are fewer. As regards skin tannins, most studies show an increasing trend of skin proanthocyanidins with water restriction (Roby et al. 2004, Casassa et al. 2015). Less uniform results are published concerning seed polyphenols, some studies showing increased concentration of seed flavanols with water deficiency (Chacón et al. 2009, Casassa et al. 2015) while other authors found higher levels of polyphenols in the seeds of irrigated vines (Kennedy et al. 2000, Koundouras et al. 2009). In the case of an irrigation trial under the semiarid conditions of Greece (Kyraleou et al. 2016a), irrigation was found to increase contents of total tannins and flavan-3-ol monomers and dimers in Syrah seeds, which might explain the stronger astringency of seed extracts of irrigated vines as perceived by sensory analysis (Figure 4). Regarding the effects of water conditions on the structural properties of skin and seed proanthocyanidins, such as polymerization, galloylation, and subunit composition, the amount of research is limited. In a recent study (Kyraleou et al. 2015b) water deficit increased skin tannin polymerization throughout ripening leading to increased levels of polymeric tannins at harvest as opposed to grapes of irrigated vines; in the same study, an opposite trend was observed for seed tannins, decreasing polymerization with water limitation (data not shown).

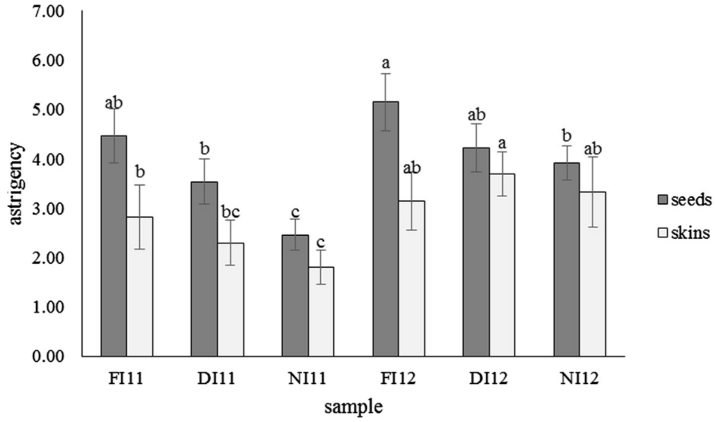


Figure 4: Mean astringency intensity of seed and skin extracts of Full Irrigated (FI), Deficit irrigated (DI) and Non Irrigated (NI) Syrah vines in 2011 (FI11, DI11, NI11) and 2012 (FI12, DI12, NI12). Grapes were collected at commercial harvest. Different letters within each year's samplings indicate statistical significant difference ($p<0.05$). Skin samples were treated separately from seed samples (from Kyraleou et al., 2016a).

Among the many seasonal practices that affect the phenolic composition of grapes, cluster exposure by selective leaf removal is accepted as a powerful technique to manipulate flavonoid content of grapes and wines. High light incidence on grapes is generally considered to promote greater anthocyanin accumulation in the skins (Jeong et al. 2004). According to Matus et al. (2009), the specific anthocyanin biosynthetic gene encoding UDP glucose: flavonoid-3-O-glucosyltransferase (UFGT) was particularly enhanced under increased exposure to solar radiation in Cabernet Sauvignon grapes. However, fruit-zone defoliation effects on grape composition are not always consistent depending on the timing (Tardaguila et al. 2010) and the severity of application (Kotseridis et al. 2012). Of particular importance in defoliation trials is the interaction between light intensity and temperature since the concomitant increase in exposed berry temperature, especially under warm climate conditions, may cause lower pigmentation in red grapes (Spayd et al. 2002). Mori et al. (2007) observed a significant reduction of anthocyanin content of Cabernet Sauvignon grapes at 35ºC as compared to 25ºC.

Contrary to skin anthocyanins, fewer data exist regarding the effect of cluster exposure to light on skin and seed proanthocyanidins. Sunlight exposure increased the accumulation of skin proanthocyanidins in Shiraz (Downey et al. 2004) and Pinot noir (Cortell et al. 2006) and decreased seed tannins (Cortell et al. 2006). Moreover, berry shading was reported to reduce the transcription of the specific proanthocyanidin biosynthesis genes in the skins during berry development while no significant effect was observed in the seeds (Fujita et al. 2007). A significant influence of vine vigor on total flavan-3-ol monomers in seeds has also been reported (Koundouras et al. 2009) with higher levels in high vigor vines possibly because more dense canopies increase shading in the fruit zone. In a post bloom defoliation trial conducted in Greece (Kotseridis et al. 2012), defoliation increased skin anthocyanins in Merlot and Cabernet Sauvignon but significantly reduced seed flavan-3-ols mainly as a result of the reduction in catechin and epicatechin amount. These effects were also largely independent of any variation in berry mass.

4 CONCLUSION

While considerable research has been conducted on the impact of environmental and cultural practice on grape anthocyanins, additional knowledge is required in order to elucidate the dependence of polymeric flavonoids, as well as of their structural properties, on site and viticultural factors. Irrigation and microclimate manipulation are highlighted as the most important tools influencing grape phenolic potential but results are often contradicting depending on variety and experimental conditions. Moreover, the influence of other vineyard factors such as rootstocks, row orientation or training systems remain relatively less studied. The building of a comprehensive database per phenolic compound and cultural practice across a wide range of cultivars would greatly assist grape growers in adjusting vineyard management to winery specifications.

5 LITERATURE CITED

Bordiga, M., F. Travaglia, M. Locatelli, J.D. Coisson, and M. Arlorio. 2011. Characterization of polymeric skin and seed proanthocyanidins during ripening in six Vitis vinifera L. cv. Food Chem. 127:180-187.

Boulton, R. 2001. The copigmentation of anthocyanins and its role in the colour a red wine: a critical review. Am. J. Enol. Vitic. 52:67-87.

Bramley, R. 2003. Smarter thinking on soil survey. Austral. New Zealand Wine Industry J. 18:88–94

Brossaud, F., V. Cheynier, and A.C. Noble. 2001. Bitterness and astringency of grape and wine polyphenols. Aust. J. Grape Wine Res. 7:33–39.

Bucchetti, B., M.A. Matthews, L. Falginella, E. Peterlunger, and S.D. Castellarin. 2011. Effect of water deficit on Merlot grape tannins and anthocyanins across four seasons. Sci. Hortic. 128:297–305.

Cadot, Y., M.T. Minana-Castello, and M. Chevalier. 2006. Anatomical, histological, and histochemical changes in grape seeds from Vitis vinifera L. cv Cabernet franc during fruit development. J. Agric. Food Chem. 29:9206−9215

Casassa, L.F., M. Keller, and J.F. Harbertson. 2015. Regulated deficit irrigation alters anthocyanins, tannins and sensory properties of Cabernet Sauvignon grapes and wines. Molelules 20:7820-7844.

Castellarin, S.D., M.A. Matthews, G. Di Gaspero, and G.A. Gambetta. 2007. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. Planta 227:101–112.

Chacón, J.L., E. Garcia, J. Martinez, R. Romero, and S. Gomez. 2009. Impact of the vine water status on the berry and seed phenolic composition of 'Merlot' (Vitis vinifera L.) cultivated in a warm climate: Consequence for the style of wine. Vitis 48:7-9.

Chira, K., G. Schmauch, C. Saucier, S. Fabre, and P.L. Teissedre. 2009. Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). J. Agric. Food Chem. 57:545–553.

Cortell, J.M., and J.A. Kennedy. 2006. Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot Noir and extraction in a model system. J. Agric. Food Chem. 54:8510–8520.

Downey, M.O., J.S., Harvey, and R. Simon. 2003. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust. J. Grape Wine Res. 9:15–27.

Downey, M.O., J.S. Harvey, and S.P. Robinson. 2004. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. Aust. J. Grape Wine Res. 10:55-73.

Fujita, A., N. Soma, N. Goto-Yamamoto, A. Mizuno, K. Kiso, and K. Hashizume. 2007. Effect of shading on proanthocyanidin biosynthesis in the grape berry. J. Japan. Soc. Hort. Sci. 76:112-119.

Gil, M., N. Kontoudakis, E. González, M. Esteruelas, F. Fort, J.M. Canals, and F. Zamora. 2012. Influence of grape maturity and maceration length on color, polyphenolic composition, and polysaccharide content of Cabernet Sauvignon and Tempranillo wines. J. Agric. Food Chem. 60:7988-8001.

Hanlin, R.L., and M.O. Downey. 2009. Condensed tannin accumulation and composition in Shiraz and Cabernet Sauvignon grapes during berry development. Am. J. Enol. Vitic. 60:13-23.

Harbertson, J.F., J.A. Kennedy, and D.O. Adams. 2002. Tannin in seeds and skins of Cabernet Sauvignon, Syrah and Pinot noir berries during ripening. Am. J. Enol. Vitic. 53:54–59.

Jeong, S.T., N. Goto-Yamamoto, S. Kobayashi, and M. Esaka. 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape skins. Plant Sci. 167:247-252.

Kallithraka, S., A.A.A. Mohdalya, D.P. Makris, and P. Kefalas. 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.

Kennedy, J.A., M.A. Matthews, and A.L. Waterhouse. 2000. Changes in grape seed polyphenols during ripening. Phytochemistry 55:77-85.

Kotseridis, Y., A. Georgiadou, P. Tikos, S. Kallithraka, and S. Koundouras. 2012. Effects of severity of post-flowering leaf removal on berry growth and composition of three red Vitis vinifera L. cultivars grown under semiarid conditions. J. Agric. Food Chem. 60:6000–6010.

Koundouras, S., C. Van Leeuwen, G. Seguin, and Y. Glories. 1999. Influence de l'alimentation en eau sur la croissance de la vigne, la maturation des raisins et les caractéristiques des vins en zone méditerranéenne (exemple de Némée, Grèce, cépage Saint-Georges, 1997). J. Int. Sci. Vinge Vin 33:149-160.

Koundouras, S., V. Marinos, A. Gkoulioti, Y. Kotseridis, and C. van Leeuwen. 2006. Influence of vineyard location and vine water status on fruit maturation of nonirrigated cv. Agiorgitiko (*Vitis vinifera* L.). Effects on wine phenolic and aroma components. J. Agric. Food Chem. 54:5077-5086.

Koundouras, S., E. Hadjidimitriou, M. Karamolegkou, E. Dimopoulou, S. Kallithraka, J.T. Tsialtas, E. Zioziou, N. Nikolaou and Y. Kotseridis. 2009. Irrigation and rootstock effects on the phenolic content and aroma potential of Vitis vinifera L. cv. Cabernet-Sauvignon grapes. J. Agric. Food Chem. 57:7805–7813.

Kyraleou, M., S. Kallithraka, S. Koundouras, K. Chira, S. Haroutounian, H. Spinthiropoulou, and Y. Kotseridis. 2015a. Effect of vine training system on the phenolic composition of red grapes (Vitis vinifera L. cv. Xinomavro). J. Int. Sci. Vinge Vin 49:71-84.

Kyraleou, M., S. Koundouras, S. Kallithraka, N. Theodorou, N. Proxenia, and Y. Kotseridis. 2015b. Effect of water availability on the phenolic content and tannin polymerization of Vitis vinifera L. cv. Syrah berry skins under semiarid climate. *In* Proceedings of the [2015] 19th International GiESCO Symposium, pp. 185-189, Pech Rouge, Gruissan France.

Kyraleou, M., Y. Kotseridis, S. Koundouras, K. Chira, P.L. Teissedre, and Kallithraka S. 2016a. Effect of irrigation regime on perceived astringency and proanthocyanidin composition of skins and seeds of Vitis vinifera L. cv. Syrah grapes under semiarid conditions. Food Chem. 203:293-300.

Kyraleou, M., S. Koundouras, S. Kallithraka, N. Theodorou, N. Proxenia, and Y. Kotseridis. 2016b. Effect of irrigation regime on anthocyanin content and antioxidant activity of Vitis vinifera L. cv. Syrah grapes under semiarid conditions. J. Sci. Food Agric. 96:988-996.

Li, Q., Y.X. Liu, Q.H. Pan, C.Q. Duan, and Y. Shi. 2014. Comparison of Proanthocyanidins with Different Polymerisation Degrees among Berry Skins of ‘Shiraz’, ‘Cabernet Sauvignon’, and ‘Marselan’. S. Afr. J. Enol. Vitic. 35:51-58.

Matus, J.T., R. Loyola. A. Vega, A. Peña-Neira, E. Bordeu, P. Arce-Johnson, and J. Antonio Alcalde. 2009. Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin and flavonol synthesis in berry skins of Vitis vinifera. J. Exp. Bot. 60:853–867.

Monagas, M., C. Gomez-Cordoves, B. Bartolome, O. Laureano, and J.M. Riccardo Da Silva. 2003. Monomeric, Oligomeric, and Polymeric Flavan-3-ol Composition of Wines and Grapes from Vitis vinifera L. cv. Graciano, Tempranillo, and Cabernet Sauvignon J. Agric. Food Chem. 51:6475-6481.

Mori, K., N. Goto-Yamamoto, M. Kitayama, and K. Hashizume. 2007. Loss of anthocyanins in red-wine grape under high temperature. J. Exp. Bot. 58:1935-1945.

Nunan, K.J., I.M. Sims, A. Bacic, S.P. Robinson, and G.B. Fincher. 1998. Changes in cell wall composition during ripening of grape berries. Plant Physiol. 118:783−792.

Ollé, D., J.L. Guiraud, J.M. Souquet, N. Terrier, A. Ageorges, V. Cheynier, and C. Verries. 2011. Effect of pre- and post-veraison water deficit on proanthocyanidin and anthocyanin accumulation during Shiraz berry development. Aust. J. Grape Wine Res. 17:90–100.

Pastor del Rio, J.L., and J.A. Kennedy. 2006. Development of proanthocyanidins in Vitis vinifera L. cv. Pinot noir grapes and extraction into wine. Am. J. Enol. Vitic. 57:125–132.

Peleg, H., K. Gacon, P. Schlich, and A.C. Noble. 1999. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. J. Sci. Food Agric. 79:1123-1128.

Prieur, C., J. Rigaud, V. Cheynier, and M. Moutounet. 1994. Oligomeric and polymeric procyanidins from grape seeds. Phytochemistry 36:781-784.

Quijada-Morin, N., J.M. Hernandez-Hierro, J.C. Rivas-Gonzalo, and M.T. Escribano-Bailón. 2015. Extractability of low molecular mass flavanols and flavonols from red grape skins. Relationship to cell wall composition at different ripeness stages. J. Agric. Food Chem. 63:7654-7662.

Roby G., J.F. Harbertson, D.A. Adams, and M.A.Matthews. 2004. Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. Aust. J. Grape Wine Res. 10:100-107.

Roggero, J.P., S. Coen, and B. Ragonnet. 1986. High performance liquid chromatography survey on changes in pigment content in ripening grapes of Syrah. Am. J. Enol. Vitic. 37:77–83.

Romero, P., J.I. Fernández-Fernández, and A. Martines-Cutillas. 2010, Physiological thresholds for efficient regulated deficit-irrigation management in winegrapes grown under semiarid conditions. Am. J. Enol. Vitic. 61-300-312.

Spayd S.E., J.M. Tarara, D.L. Mee, and J.C. Ferguson. 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic. 53:171-182

Sun, B., M. De Sa, M. Leonardo, I. Caldeira, F.L. Duarte, and I. Spranger. 2013. Reactivity of polymeric proanthocyanidins toward salivary proteins and their contribution to young red wine astringency. J. Agric. Food Chem. 61:939-926.

Tardaguila, J., F. Martinez de Toda, S. Poni, M.P. Diago. 2010. Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. Am. J. Enol. Vitic. 61:372–381

Vidal, S., L. Francis, S. Guyot, N. Marnet, M. Kwiatkowski, R. Gawel, V. Cheynier, and E.J. Waters. 2003. The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. J. Sci. Food Agric. 83:564–573.

Vidal, S., L. Francis, A. Noble, M. Kwiatkowski, V. Chenyer, and E. Waters. 2004. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. Anal. Chim. Acta 513:57-65.