

Effects of Abiotic Factors on Phenolic Compounds in the Grape Berry – A Review

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Grape berry phenolic compounds are widely described in literature. Phenolics can be divided into two main groups: flavonoids and non-flavonoids, of which the flavonoids are the most important. The two best-known groups of flavonoids are the anthocyanins and condensed tannins (also called proanthocyanidins). Anthocyanins are responsible for the red colour in grapes. The condensed tannins (proanthocyanidins) are responsible for some major wine sensorial properties (astringency, browning, and turbidity) and are involved in the wine ageing processes. This review summarises flavonoid synthesis in the grape berry and the impact of environmental factors on the accumulation rate during ripening of each of the flavonoids. The impact of the accumulated flavonoids in grapes and the resulting impact on the sensorial aspects of the wine are also discussed.

INTRODUCTION

Globally, grapes are one of the most widely cultivated crops. In 2012, grapes covered 7.5 million hectares of arable land (OIV, 2013). Most of the grapes are fermented into wine, or used for table grapes and raisin production. Many researchers have shown that the growing site (climate and soil) and viticultural practices have a direct impact on grape maturity and the phenolic composition of the berry. Flavonoids play an important role in grape and wine quality. Therefore, an in-depth understanding of flavonoid development and composition during berry development under South African climatic conditions is needed.

Grape berry development involves a complex series of physical and biochemical changes. These can be divided into three major phases: green growth (Stage I), the lag phase (Stage II), and the ripening phase (Stage III). During these three phases, primary and secondary metabolites are synthesised under complex gene and enzymatic control. Primary metabolites such as sugars, amino acids and organic acids are involved in normal growth, development, and reproduction of plant species. Secondary metabolites such as phenolics and stilbenoids have ecological functions. These ecological functions include defence against predators, parasites and diseases (Conde *et al.*, 2007; Ali *et al.*, 2010). Phenolic compounds have a diversity of structures and can be divided into two main groups, namely flavonoids and non-

flavonoids (Cheynier *et al.*, 2006). The phenolic compounds of interest in this study are the flavonoids, and they will be discussed in depth in the following paragraphs.

Flavonoid biosynthesis

Flavonoid biosynthesis is the result of the shikimate and phenylpropanoid pathways (Dewick & Haslam, 1969; Heller & Forkmann, 1988). Flavonoids are characterised by two benzene rings (rings A and B) bonded by an oxygenated heterocyclic pyran ring (ring C). They therefore possess a C₆-C₃-C₆ skeleton (Fig. 1) (Somers & Vérette 1988; Ribéreau-Gayon, 2000). The heterocyclic ring is closed in most flavonoids, but remains open in chalcones and dihydrochalcones (Stafford, 1990). Variation in the oxidation state and substitution on ring C define the different classes of flavonoids (Fig. 1).

Flavan-3-ols

Flavan-3-ols are the most abundant class of flavonoid compounds in grape berries (Adams, 2006; Terrier *et al.*, 2009). Flavan-3-ols comprise monomers (catechins), oligomers, and polymers. They are also referred to as proanthocyanidins or condensed tannins (Cheynier & Rigaud, 1986; Ricardo-da-Silva *et al.*, 1991 a, b). The major flavan-3-ol monomers in grape seeds are (+)-catechin,

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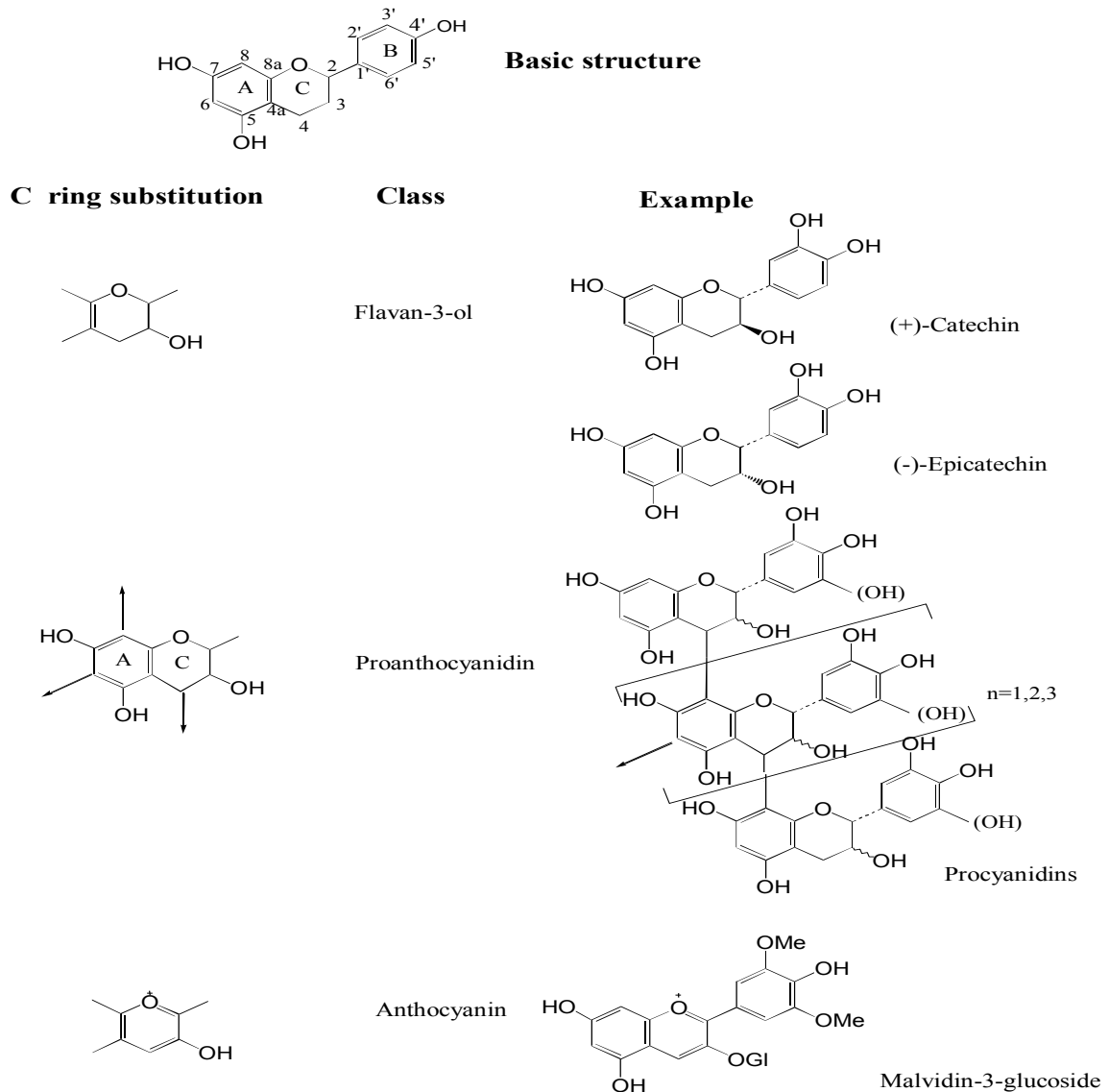


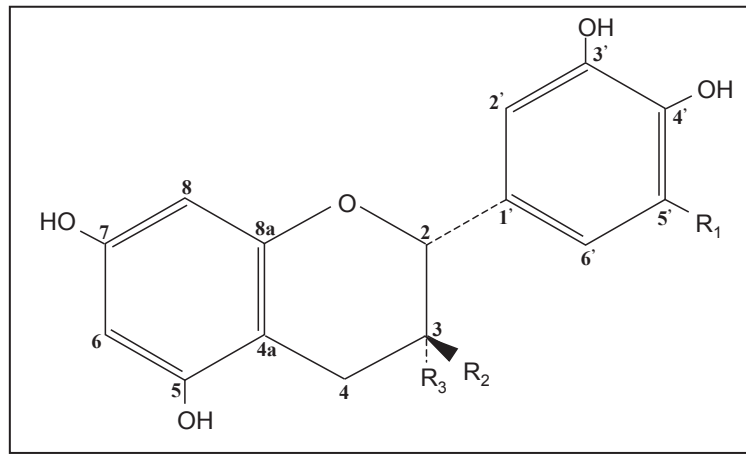
FIGURE 1
Flavonoid nomenclature (Somers & Vérette, 1988).

(-)-epicatechin and a galloylated form of (-)-epicatechin-3-*O*-gallate (Fig. 2). (-)-Epigallocatechin and trace amounts of (+)-gallocatechin are also found in grapes. Flavan-3-ols in grape skins are represented by (+)-catechin, (-)-epicatechin, (-)-epicatechin-3-*O*-gallate and (-)-epigallocatechin. The presence of (+)-gallocatechin in *Vitis vinifera* has been reported while (+)-catechin-2-gallate and (+)-gallocatechin-3-gallate have been detected in some non-*Vinifera* varieties (Piretti *et al.*, 1976; Czochanska *et al.*, 1979; Lee & Jaworski, 1987). Condensed tannins are formed during the polymerisation process and comprise flavan-3-ol subunits connected by interflavan linkages (C₄-C₈ or C₄-C₆) (Fig. 3) (Haslam, 1998).

Proanthocyanidins, or condensed tannins, are mostly situated in the solid parts of the cluster (skins, seeds and stems) and, to a lesser degree, in the pulp (Sun *et al.*, 1999; Jordão *et al.*, 2001; Ó-Marques *et al.*, 2005). Seeds have the highest concentration of procyanidins (Ricardo-da-Silva

et al., 1992a). Within the grape berry, proanthocyanidins or condensed tannins are situated in the hypodermal layers of the skin and the soft parenchyma of the seed between the cuticle and the hard seed coat (Adams, 2006).

Skintannin exhibit a higher degree of polymerisation than seed tannins, expressed as the mean degree of polymerisation (mDP) (Adams, 2006). Kennedy *et al.* (2000a) and Downey *et al.* (2003a) found proanthocyanidin polymers with 25–40 subunits comprising equal proportions (-)-epicatechin and (-)-epicatechin-3-*O*-gallate with (+)-catechin as terminal subunits. The polymer length remained constant until veraison. Polymer length decreased to about 30 subunits four weeks after veraison and to approximately 20 subunits at harvest (Kennedy *et al.*, 2000a; Downey, *et al.*, 2003a). The mDP in seeds varies between three to sixteen subunits comprising (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-*O*-gallate (Cheynier *et al.*, 1998; Downey *et al.*, 2003a; Bogs *et al.*, 2005; Mané *et al.*, 2007). From fruit-set to one



	R ₁	R ₂	R ₃
(+)-catechin	H	OH	H
(+)-gallocatechin	OH	OH	H
(-)-epicatechin	H	H	OH
(-)-epigallocatechin	OH	H	OH

FIGURE 2
Chemical structures of flavanols (Moutounet *et al.*, 1996).

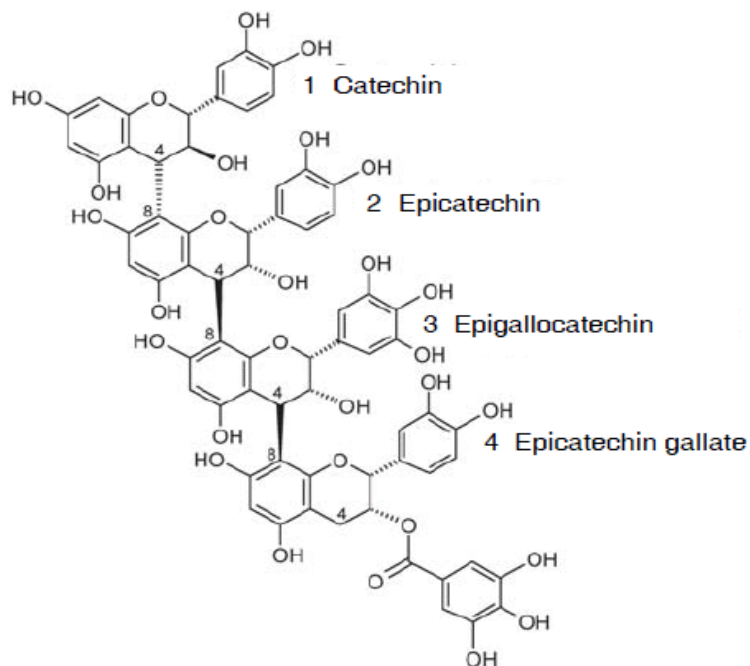


FIGURE 3
Condensed tannin and the four subunits: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin gallate (Adams, 2006).

week pre-veraison, polymer length remained between five and six subunits. An increase in the terminal subunits one week pre-veraison exceeded the accumulation of extension subunits, resulting in a decrease in polymer length to four subunits (Downey *et al.*, 2003a). Various average ranges of mDPs are reported for proanthocyanidins in grape berries. Prieur *et al.* (1994), Moutounet *et al.* (1996) and Labarbe

et al. (1999) reported seed mDP ranging between 8 and 16 units for grape seeds, whilst Mané *et al.* (2007) reported values between 3 and 4 units. Skin mDP ranges between 27 and 45 units on average (Moutounet *et al.*, 1996; Souquet *et al.*, 1996; Mané *et al.*, 2007).

The flavan-3-ols are synthesised as part of the phenylpropanoid pathway (Fig. 4). Other secondary

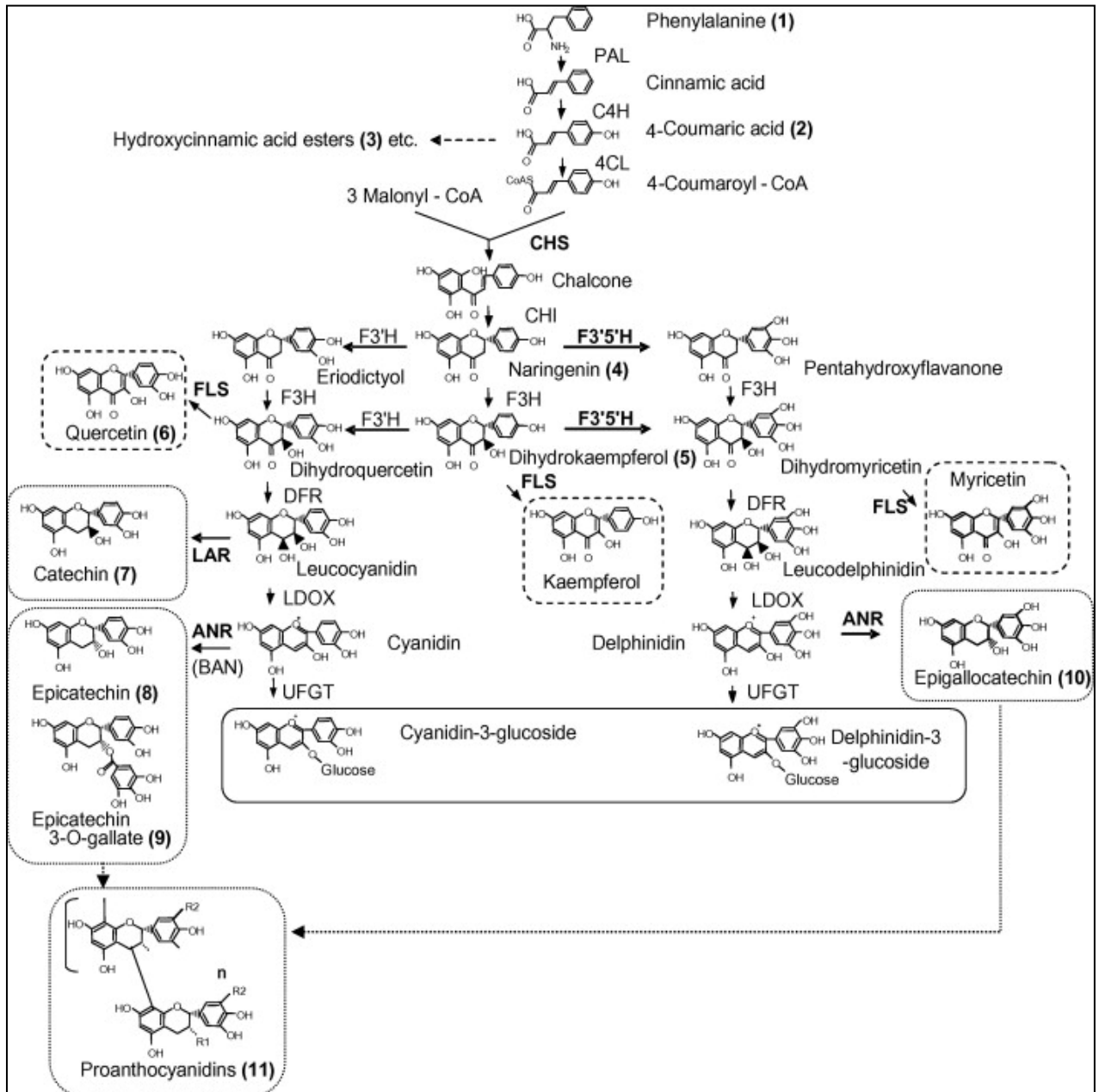


FIGURE 4
Phenylpropanoid pathway in the grape berry (Koyama *et al.*, 2012).

metabolites such as lignins, lignans, stilbenes and hydroxycinnamic acids are also produced in this pathway (Schwinn & Davies, 2004). Phenylalanine (obtained via the shikimate pathway) and malonyl-CoA (derived from citrate produced by the tricarboxylic acid cycle) (Davies & Schwinn, 2006) are the main flavonoid precursors. Phenylalanine is converted into the activated hydroxycinnamic acid p-coumaroyl-CoA by three enzymatic conversions and catalysed by phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase and 4-coumarate-CoA ligase. Malonyl-CoA is required for flavonoid biosynthesis and acts as an “extender” molecule and acid moiety donor for acylation of flavonoid glycosides that form the first flavonoid. The point of entry into the flavonoid pathway is the formation of a chalcone (mostly naringenin). The chalcone is formed from

p-Coumaroyl-CoA and three acetate units from malonyl-CoA through the action of chalcone synthase (CHS). The chalcone then gives rise to a flavonoid with a C15 backbone, which is directly or indirectly converted to a range of other flavonoids in a pathway of intersecting branches with intermediate compounds (Schwinn & Davies, 2004). A flavonoid with a heterocyclic C-ring is isomerised to a flavanone through the activity of chalcone isomerase (CHI) (Schwinn & Davies, 2004). Hydroxylation of flavanones is catalysed by flavanone 3 β -hydroxylase (F3H), which results in dihydroflavonols. The latter is then subjected to catalysis by dihydroflavonol 4-reductase (DFR) resulting in leucocyanidins that are colourless and unstable compounds. Proanthocyanidins are formed through interflavan linkages between the flavan-3-ol building blocks. Flavan-3-ols are

formed via two biosynthetic routes: (i) 2,3-*trans*-flavan-3-ols are produced from leucocyanidins by leucoanthocyanidin reductase (LAR); and (ii) 2,3-*cis*-flavan-3-ols from cyanidin by anthocyanidin reductase (ANR). LAR removes the 4-hydroxyl from leucocyanidins to produce 2,3-*trans*-flavan-3-ols while the ANR converts cyanidin to the corresponding 2,3-*cis*-flavan-3-ols (Tanner *et al.*, 2003; Xie *et al.*, 2003) (Fig. 5). Flavan-3-ols are synthesised in the cytoplasm and transported to the vacuoles where polymerisation occurs and proanthocyanidins accumulate.

Anthocyanins

In the final step of anthocyanidin-3-*O*-glycoside biosynthesis, pigments are formed through the activity of anthocyanidin synthase, also referred to as leucoanthocyanidin dioxygenase (LDOX), which is also an anthocyanidin-3-glycosyltransferase to the corresponding anthocyanin (Fig. 5) (Davies & Schwinn, 2006). Schwinn & Davies (2004) suggested that hydroxylation has a key impact on anthocyanin colour. An increase in hydroxylation of the B-ring results in the shift in colour from red to blue, determining the type of anthocyanin produced.

Flavonols

Flavonols are flavonoids found in higher plants in glycosidic forms developed during the flavonoid biosynthetic pathway (Mattivi *et al.*, 2006). Synthesis of flavonols predominately occurs in the grape skin (Price *et al.*, 1995). Quercetin-3-*O*-glucoside and quercetin-3-*O*-glucuronide have been

identified as the main flavonols within the grape berries (Cheyrier *et al.*, 1986; Price *et al.*, 1995; Downey *et al.*, 2003b). Various researchers have investigated the molecular structure and the expression of the main enzymes, and a general pathway for flavonol biosynthesis has been established (Downey *et al.*, 2003b; Bogs *et al.*, 2006; Castellarin *et al.*, 2006; Mattivi *et al.*, 2006) (Fig. 6).

Changes in flavonoid content with ripening

It is clear that flavonoid biosynthesis is influenced by berry maturation. The grape seed tannin biosynthesis studied in grape cultivars such as Cabernet Sauvignon, Shiraz, and Pinot noir indicated an overall tendency for the maximum concentration to be reached at veraison, decreased thereafter, and to remain constant during maturation (Kennedy *et al.*, 2000a; Kennedy *et al.*, 2000b; Jordão *et al.*, 2001; Downey *et al.*, 2003b; Downey *et al.*, 2006). Other studies reported that the concentration and composition were influenced by grape variety and the vintage (Ricardo-da-Silva *et al.*, 1991c & 1992b; Jordão *et al.*, 2001). Ribereau-Gayon *et al.* (2000) suggested that grape cultivars such as Cabernet franc, Pinot noir, Grenache, and Tempranillo generally have higher levels of seed tannin compared to Cabernet Sauvignon and Merlot noir. As for grape skin tannin, investigators found a higher concentration at fruit set and noted a decrease and then an increase around veraison, followed by another decrease (Kennedy *et al.*, 2001; Kennedy *et al.*, 2002a; Downey *et al.*, 2003a).

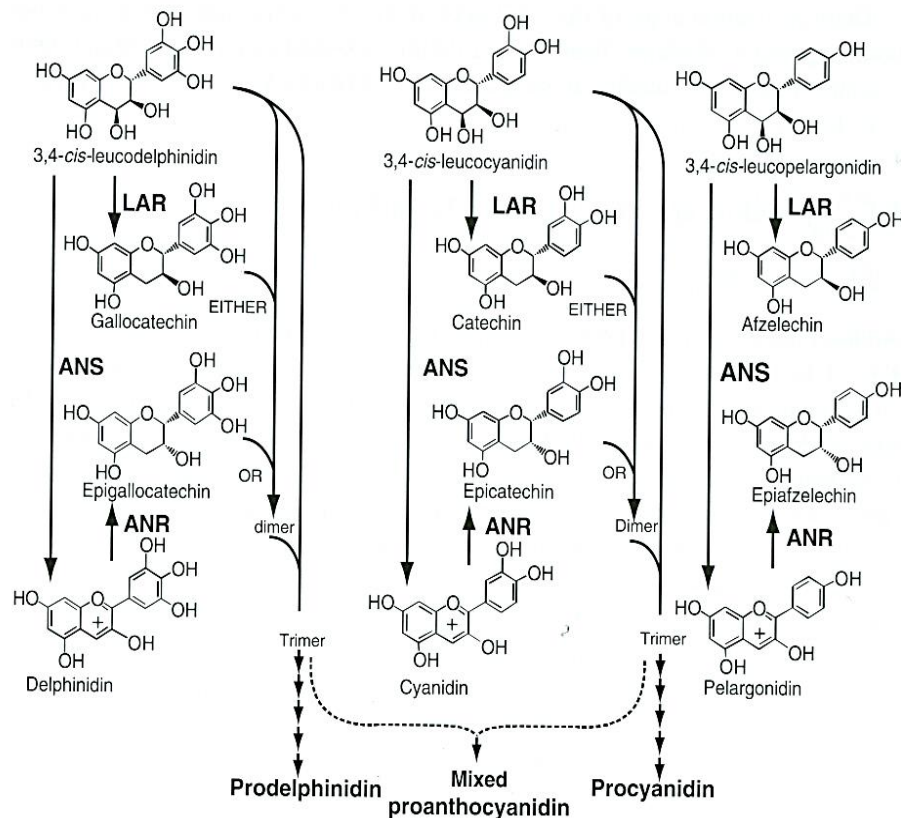


FIGURE 5
Biosynthesis of flavan-3-ols and proanthocyanidins (Tanner, 2006).

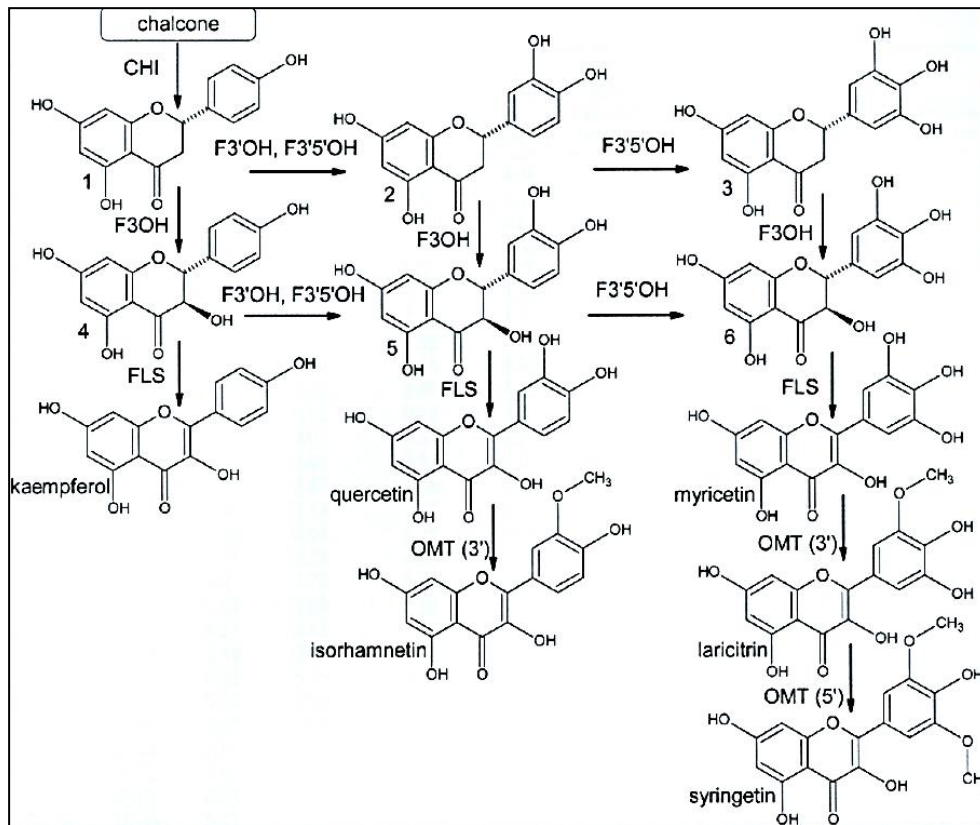


FIGURE 6

General pathway for flavonol biosynthesis (Mattivi *et al.*, 2006).

Various researchers suggested that anthocyanin development and composition are influenced by cultivar, climatic conditions (abiotic factors such as light, temperature, and water), and viticultural practices (Kliwer & Torres, 1972; Jackson & Lombard, 1993; Dokoozlian & Kliwer, 1996; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002; Downey *et al.*, 2004). Downey *et al.* (2003b) reported that the total concentration of flavonols in berries was low pre-veraison, and then increased post-veraison. Flavonoid composition clearly changes with ripening and research indicates potential influences of environmental parameters such as temperature and light.

Environmental factors affecting development of phenolic compounds in grape berries

Cultivar and seasonal variability affecting flavonoid development

In light of global climate change, numerous authors proposed climatic models to forecast the impact on grape and wine quality as well as production (Jones *et al.*, 2000; Jones *et al.*, 2005; Orduña, 2010). These climatic models encompass air temperature as well as relative humidity, precipitation and viticultural records (Stock *et al.*, 2004; Jones *et al.*, 2005). Temperature increases between 2002 and 2049 of 0.42°C per decade and 2.04°C overall is predicted. In both the Northern and Southern hemisphere, authors found that an increase in seasonal temperatures, solar radiation and rainfall had an impact on the length of the season, sugar concentration and the potential alcohol level of the fermented wines (Laget

et al., 2008; Jones *et al.* 2000; Petrie *et al.*, 2008).

Phenolic development is impacted by the above-mentioned environmental parameters as well as the genetic factors (Cohen & Kennedy, 2010; Orduña, 2010; Castellarin *et al.*, 2012). Furthermore, the cultivation practices and resulting microclimate around the developing fruit affect the fruit composition, for example total soluble solids, flavan-3-ol monomers, proanthocyanidins, and pigmented polymers (Cortell *et al.*, 2005). Environmental factors such as sunlight, temperature, ultra-violet (UV) radiation, and plant water status play a role in the accumulation of proanthocyanidins, flavonols, and anthocyanins (Table 1) (Crippen & Morrison, 1986; Kennedy *et al.*, 2002b; Ojeda *et al.*, 2002; Downey *et al.*, 2004; Mori *et al.*, 2005 & 2007; Buchetti *et al.*, 2011; Gregan *et al.*, 2012; Koyoma *et al.*, 2012; Reshef *et al.*, 2018).

Light

Plant metabolism is greatly dependent on solar effects (Cohen & Kennedy, 2010). Zucker (1965) found that the functioning of PAL is affected by white light (visible light spectrum). Dokoozlian and Kliwer (1996) found that exposure of berries during growth stages I (green growth stage), and II (lag phase) resulted in an increased PAL, which resulted in a higher anthocyanin concentration. Investigations into the effect of light on grape composition resulted in varying outcomes, as described below.

Haselgrove *et al.* (2000) and Spayd *et al.* (2002) studied the impact of light on Shiraz and Cabernet Sauvignon

TABLE 1
Grapevine fruit responses to environmental factors (adapted from Cohen & Kennedy, 2010).

Phenolic compounds	Environmental factors	Responses
Anthocyanin	Light intensity	Increase per berry content in sun exposed versus canopy shaded fruit (Crippen & Morrison, 1986). Exposed berries (increase in per berry content) (Downey <i>et al.</i> , 2004).
	UV exposure	Total monomeric skin anthocyanin (TMSA) concentrations were not influenced by UV radiation, but rather by the visible spectrum of light and temperature, which played a crucial role (Spayd <i>et al.</i> , 2002).
	Temperature	Decrease at high temperatures (30°C–35°C) (Mori <i>et al.</i> , 2005 & 2007). Cooler temperatures increased TMSA (Spayd <i>et al.</i> , 2002) More anthocyanins at 20°C than at 30°C (Yamane <i>et al.</i> , 2006).
	Irrigation	Water deficit increased concentration (Koundouras <i>et al.</i> , 2005). Early irrigation/severe deficit: lower concentration and amount per berry (Ojeda <i>et al.</i> , 2002).
Flavonols	Light intensity	Increased concentration with exposure (Price <i>et al.</i> , 1995; Spayd <i>et al.</i> , 2002; Koyama <i>et al.</i> , 2012; Gregan <i>et al.</i> , 2012).
	UV exposure	Exclusion of solar UV radiation remarkably decreased concentration (Koyama <i>et al.</i> , 2012). Increase when UV transmitting barriers were used (Spayd <i>et al.</i> , 2002). Increase in flavonol concentration under high UV light exposure (Reshef <i>et al.</i> , 2018).
	Temperature	No effect (Mori <i>et al.</i> , 2005).
	Irrigation	Deficit increased concentration (between anthesis and veraison, and veraison and harvest) (Ojeda <i>et al.</i> , 2002).
Proanthocyanidins (PAs)	Light intensity	Exposure lead to an increase per berry, whilst shade resulted in an increase in substitution positions within the molecule (Downey <i>et al.</i> , 2004). Exposure resulted in an increase in seeds and skins mDP (Downey <i>et al.</i> , 2004).
	UV exposure	UV exclusion did not affect the concentration and composition of PAs (Koyama <i>et al.</i> , 2012).
	Irrigation	Post-veraison affected total flavan-3-ol monomers in seed tissue, mainly as a result of variations in the catechin amount (Koundouras <i>et al.</i> , 2009). Water deficits over four seasons increased tannin concentration less and only by reducing fruit growth, except in one year where the tannin content was increased (Bucchetti <i>et al.</i> , 2011).
	Temperature	Heating and cooling berries altered the initial accumulation rate (<i>via</i> biosynthesis) pre-veraison (Cohen <i>et al.</i> , 2012).

berries, respectively. According to Haselgrove *et al.* (2000), berries that received high levels of sunlight had high levels of quercetin-3-glucoside and low levels of malvidin-3-glucoside. The total anthocyanin levels varied between treatments and depended on the degree of bunch exposure. Spayd *et al.* (2002) found that berries that were exposed to sunlight had increased total skin monomeric anthocyanins regardless of the ambient temperature. Subsequent investigations showed that low light also reduced colour in Emperor table grapes and in Pinot Noir (Kliewer 1970, 1977). Similar results were later reported in Shiraz (Smart *et al.*, 1985) and Cabernet Sauvignon grapes (Morrison & Noble, 1990; Hunter *et al.*, 1991; Dokoozlian & Kliewer, 1996). Together these results created a strong impression

that light was necessary for colour formation in grapes, an impression reinforced by observations from other plant species, such as apple, where light is an absolute requirement for anthocyanin biosynthesis (Siegelman & Hendricks, 1958; Chalmers & Faragher, 1977; Lancaster, 1992; Dong *et al.*, 1998). Wicks and Kliewer (1983) and Dokoozlian and Kliewer (1996) suggested that low light intensity reduced anthocyanins and some other flavonoids, while enhanced light intensity increased the flavonoid content of grapes. Results of these studies indicate that light is important in the colour formation in grapes. This theory is also supported for anthocyanin biosynthesis in other plant species such as apples (Siegelman & Hendricks, 1958; Lancaster, 1992; Dong *et al.*, 1998).

However, some investigations found contradictory results. Crippen & Morrison (1986) found that there were no significant differences in the anthocyanin concentration and content at harvest between sun-exposed and shaded grapes, although there were differences during berry development. Others reported that high light intensities ($>100 \mu\text{mol m}^{-2}\text{s}^{-1}$) resulted in decreased anthocyanin levels (Bergqvist *et al.*, 2001; Spayd *et al.*, 2002). Ristic *et al.* (2007) found that the anthocyanin content of grapes in a shade box treatment versus the non-shaded treatment did not differ significantly. Only a change in the deoxygenated anthocyanins was observed, as well as an increase in seed tannin and decrease in skin tannin between the shaded and non-shaded treatments, respectively. In a study on vine vigour, Cortell *et al.* (2005) found that seed proanthocyanidin composition in grapes was slightly different between vigour zones identified as high, medium and low, but the total amount was not affected. The total amount of epigallocatechin and mDP values of the skin proanthocyanidins was increased in the low vigour vines (vigour index of 0.09 and 0.44, respectively).

Many explanations have been suggested for the above-mentioned differences in results ranging from differences in cultivar sites, vine vigour, vintage effects, sampling method, and the analytical technique used (Cortell *et al.*, 2005; Downey *et al.*, 2006; Bucchetti *et al.*, 2011). Therefore, it can be deduced that:

- a) controlled conditions (e.g. greenhouse or growth chamber) are desirable to study the effect of abiotic factors (light intensity and quality, temperature and water) on berry phenolic composition; and
- b) data should be presented on a per berry basis and in concentration to understand the dynamic of berry phenolic biosynthesis from berry set to maturation as well as the impact on overall concentration.

Temperature

An increase in plant temperature, either through direct heating by incident radiation or increased air temperatures, will increase the rate of metabolic processes in the plant, with an associated increase in development and metabolite accumulation (Hawker, 1982; Ebadi *et al.*, 1995; Dokoozlian & Kliewer, 1996; Downey *et al.*, 2006). The accumulation/biosynthesis of total soluble solids and organic acids, the biosynthesis of aromatic precursors and colour components, and the process of photosynthesis are all enzyme-driven and therefore regulated by temperature, light, and plant water status (Jackson & Lombard, 1993).

Gladstones (1992) suggests that pigment formation and the optimal physiological ripening of grapes for the synthesis of colour and aroma compounds take place between 20°C and 22°C. When the day temperature is high, low night temperatures are necessary to ensure a low pH and high natural acidity (Jackson & Lombard, 1993). Mori *et al.* (2005) found that metabolic pathways are altered when the ambient temperature reaches 30°C. Martínez-Lüscher *et al.* (2017) found that the use of colour shade nets efficiently palliate temperature a few weeks prior to harvest whilst transmitting enough radiation into the bunch zone.

Chorti *et al.* (2010) and Mori *et al.* (2005) found that high night temperatures resulted in a decrease in the

anthocyanin accumulation within the berry, but there was no change in the flavonol concentration. However, high temperatures inhibited the gene expression of CHS, F3H, dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX), and UDP-glucose. Flavonoid 3-O-glucosyltransferase (UGT) activity decreased at veraison and was followed by an increase after veraison (Mori *et al.*, 2005). Buttrose *et al.* (1971) found that if the day temperatures were constant at 20°C for Cabernet Sauvignon, it was favourable for colour formation, but day temperatures $>30^\circ\text{C}$ resulted in less colour. Recently, Tarara *et al.* (2008) found that low light and high berry temperatures decreased the total skin anthocyanin (TSA). These findings are supported by the findings of Coombe (1987), which suggested that the primary metabolism of the berry is optimal at approximately 30°C (Downey *et al.*, 2006).

Proanthocyanidin accumulation reaches a peak close to veraison and decreases towards harvest. This could be ascribed to its extractability rather than a degradation or turnover (Cheynier *et al.*, 1997; De Freitas & Glories, 1999; Kennedy *et al.*, 2001; Ó-Marques *et al.*, 2005). The amount of seed tannin in berries is related to the number of seeds per berry (Habertson & Adams, 2002). Cohen *et al.* (2008) studied the effect of temperature during the green berry stage and maturation. Proanthocyanidin accumulation was linearly related to the heat summation during the grape development period. Yet, damping of the diurnal temperature by daytime cooling and night-time heating resulted in a reduction in the proanthocyanidin mDP. Downey *et al.* (2004) suggested that shading had no significant effect on the levels of condensed tannins in the skins or seeds of ripe fruit. However, there were noticeable differences in the total condensed tannins over two vintages. These differences were ascribed to changes in the skin tannin content. Cohen *et al.* (2012) found that heating and cooling of berries from 20.5°C by $\pm 8^\circ\text{C}$ altered the initial rates of proanthocyanidin accumulation. However, the total proanthocyanidin accumulation was not related to the thermal time, but is more likely a function of berry development within a particular season.

Water constraint/stress

Irrigation of vineyards is a worldwide practice in arid and semi-arid regions, and it has been found that it affects the biosynthesis of phenolics (Cohen & Kennedy, 2010). Roby *et al.* (2004a; 2004b) found an increase in the skin tannins and anthocyanin amounts per berry, as well as concentrations with an increased water deficit. Ojeda *et al.* (2001; 2002) studied the impact of water deficits during different berry growth stages. Smaller berries and higher skin flavonol concentrations were correlated with water stress during berry green growth stages. Proanthocyanidin and anthocyanin concentrations were also impacted as the skin-to-pulp weight ratio increased due the induced water stress applied before and/or after veraison. The latter findings correlate with other studies (Kennedy *et al.*, 2002b; Petrie *et al.*, 2004; Salon *et al.*, 2005; Koundouras *et al.* 2006). Castellarin *et al.* (2007) found that water deficit before and after veraison resulted in a reduction in berry size, and the flavonol concentrations were affected by the timing of the irrigation. An increase in proanthocyanidin concentration

was noted after veraison, but was similar for all treatments (early and late deficit irrigation) at harvest. In general, the timing of water constraint/water stress (i.e. before or after veraison), the water constraint levels and the duration of the water constraints will affect the concentration of major phenols (Deloire *et al.*, 2004).

Ultra-violet radiation

Fruit composition is affected by photosynthetic, UV, thermal and phytochrome effects (Smart, 1987; Kolb *et al.*, 2001; Kolb *et al.*, 2003; Berli *et al.*, 2010). Light movement occurs through a passage of different tissue layers via light scattering. Therefore, plants can be described as a complex optical system (Smith, 1975). After light passes through the plant surface, the spectral quality and quantity may be altered by wavelength dependent absorption (Smith, 1975).

Plant photosynthesis is sustained by the “visible” range of the spectrum on the earth surface (400–800 nm). However, when the visible spectrum radiation is gathered, plants are also exposed to UV radiation in the wavelength range 290–400 nm. UV radiation can be divided into UV-A (315–400 nm), UV-B (280–320 nm) and UV-C (<280 nm) ranges. Morphogenetic changes in plants have been caused by UV-B radiation (Rozema *et al.*, 1997). Furthermore, Jordan (1996), Rozema *et al.* (1997), Vass (1997), and Hollósy (2002) found damage to lipids, nucleic acids, and proteins. Teramura & Sullivan (1994) reviewed the primary, secondary, and indirect effects of UV-B radiation on photosynthesis. Plant morphogenetic parameters that are changed are plant height, leaf area, leaf thickness, branching, and plant phenology (Tevini & Steinmuller, 1987; Barnes *et al.*, 1990; Ryel *et al.*, 1990; Bornman & Vogelmann, 1991; Teramura & Sullivan, 1994). UV-B radiation affects the secondary metabolism of plants. Secondary metabolite production can be stimulated by UV-B as well as the prevailing abiotic and biotic conditions (Rozema *et al.*, 1997).

UV-B radiation affects some enzymes of the phenylpropanoid pathway. PAL and CHS activity are stimulated by UV-B radiation (Jansen *et al.*, 1998; Pontin *et al.*, 2010). PAL catalyses the deamination of phenylalanine to form trans-cinnamic acid. Hydroxycinnamic acids are particularly effective in screening out UV-B radiation as they absorb effectively in the 300 nm range of the UV-B spectral region, whereas flavonoids absorb at 280 nm. Flavonoids absorb UV-B radiation; and epidermal flavonoids, in particular, act as UV-B screens for interior tissues of leaves and stems. Elevated levels of UV-B radiation are known to cause a limited increase of tannins and lignin (Gehrke *et al.*, 1996).

The impact of UV radiation on grapevine functioning was at the centre of various studies. Kolb *et al.* (2001) found increased levels of hydroxycinnamic acids (coumaric and caffeic acid) in sun-exposed grape leaves. In the berries, however, lower levels of hydroxycinnamic acids were obtained with increased radiation, while similar to grapevine leaves, quercetin and kaempferol increased (Kolb *et al.*, 2003). Spayd *et al.* (2002) studied the effect of UV barriers over the canopy and fruiting zone over a 2-year period. Flavonol biosynthesis was influenced by UV barriers, as individual and total flavonol concentration was significantly reduced

when UV absorbing material barriers were used. Koyama *et al.* (2012) suggested that UV exclusion did not affect the concentration and composition of proanthocyanidins, but confirmed a decrease in flavonol concentration. Gregan *et al.* (2012) suggested that the composition of flavonols in the skins of Sauvignon blanc grapes is determined by UV-B radiation.

Sensory properties of grape and wine phenolics

Phenolic compounds in wine contribute to the wine sensorial properties (wine colour, astringency, bitterness and mouthfeel) and antioxidative properties (Gawel, 1998). Phenolic levels in wine can be affected by several factors such as grape genotypes (Ricardo-da-Silva *et al.*, 1992b; Sun *et al.*, 2001), the winemaking practices and the conditions of wine ageing and storage (Sun *et al.*, 1999; Sun *et al.*, 2001). The conversion of anthocyanins and proanthocyanidins to other polymeric species contributes to the change in colour and taste of a wine. The impact of polymerisation reactions on wine sensory properties is largely unknown. Some researchers suggest a contribution of newly formed polymeric pigments to astringency mouthfeel (Oberholster, 2009), and others suggest a decrease of wine astringency (Weber *et al.*, 2013; Wollmann & Hofmann, 2013).

Monomeric and polymeric flavan-3-ols are the primary contributors to the astringency and bitterness character of red wine (Singleton & Trousdale, 1992). Astringency is a tactile sensation in which drying, puckering, and roughing are produced by the interactions of wine tannins with salivary proteins (Robichaud & Noble, 1990). Bitterness is a taste sensation perceived by each of the several thousand sensors on the tongue (Katsnelson, 2015). Astringency perception is not well understood, but can be caused by (i) an increase in friction, (ii) interaction between tannins and oral epithelial proteins/taste receptors, and (iii) change in salivary viscosity (Gawel, 1998; McRae & Kennedy, 2011). Protein-polyphenol interactions can be divided into (i) hydrophobic interactions, and (ii) hydrogen bonding, which is influenced by the degree of polymerisation, galloylation, and hydroxylation of tannins (Gawel, 1998; Peleg *et al.*, 1999). (+)-Catechin exhibits bitterness and astringency in white wine solutions (Arnold *et al.*, 1980; Robichaud & Noble, 1990). Five concentrations ranging between 0-1200 mg/L was evaluated by Robichaud & Noble (1990), while three concentrations between 160-300 mg/L was studied by Arnold *et al.* (1980) in base wine. Thorngate and Noble (1995) showed that the intensity of astringency and bitterness of two monomeric flavan-3-ols – (-)-epicatechin and (+)-catechin – differs with (-)-epicatechin, having a higher intensity than (+)-catechin. Three concentration levels (0.5, 0.9 and 1.2 g/L of (-)-epicatechin and (+)-catechin) in a model wine solution were assessed.

Chira *et al.* (2008) found a positive correlation between astringency intensity and mean degree of polymerisation (mDP) in grape skins (mDP 23.1 and 20.94 in 2006 and 2007 vintages, respectively). Both astringent and bitterness perception thresholds are influenced by the concentrations. Therefore, a higher concentration results in an increase in the intensity of the sensation. Astringency and bitterness are influenced by the mDP of polymers. With an increased chain

length, both bitterness and astringency increase. However, astringency increases faster than bitterness (Arnold *et al.*, 1980; Lea & Arnold, 1978; Gawel 1998). Peleg *et al.* (1999) found that bitterness is elicited by an interaction with a specific bitter membrane-bound receptor or through surface membrane interactions. Therefore, an increase in molecular size of procyanidins decreases bitterness by limiting the access to a membrane-bound receptor or by direct depolarisation of the taste receptor cell. Lea & Arnold (1978) suggested that the increase in the perceived astringency with the mDP is due to greater capacity of polyphenols to bind the proteins and stimulate astringency. Vidal *et al.* (2003) suggested that the mDP in apple and grape extracts were the most discriminatory structural variable as astringency increased with an increase in polymerisation. An increase in the galloylation can result in an increase in coarseness, while trihydroxylation of the B-ring decreased coarseness (Vidal *et al.*, 2004a). Vidal *et al.* (2004b) suggested that anthocyanins in their glucoside and coumaroylated forms did not influence astringency and bitterness of model wine solutions. However, polysaccharides play an important role in the mouthfeel properties of wine. Acidic polysaccharides significantly decreased the astringency, while neutral polysaccharides had less of an effect in a model wine solution. Other parameters that affect the intensity and duration of astringent and bitter sensations by altering the salivary flow and composition are (i) wine pH, (ii) ion concentration, (iii) temperature, and (iv) ethanol concentration (Gawel, 1998).

CONCLUSIONS

The composition of wine grape berries is affected by genotype, clones, abiotic factors and cultural practices. Therefore, grape quality is a complex concept that depends on berry composition and size. Grape berry composition is affected by abiotic factors (light, temperature, soil water content, wind, as well as air humidity) mainly at the meso- and microclimatic levels.

Plants are complex optical systems that are dependent on the light environment (visible light and UV) amongst other parameters. The light environment is affected by both long-term (row direction, vine spacing, trellising system, etc.) and short-term practices (canopy manipulations, pruning, and trellising that will affect the architecture of the canopy).

Berry temperature is important, as it is affected not only by sunlight exposure, but also by the availability of water to maintain transpiration. Increased exposure to sunlight from an early stage of berry development, as well as the availability of water, has an effect on the fruit growth and composition. Sunlight exposure ($>100 \mu\text{mol m}^{-2}\text{s}^{-1}$), combined with simultaneous high temperatures, lead to a decrease in phenolic compounds such as anthocyanins and total phenolics. Grapevine water deficit is used as a tool to manage the synthesis of secondary metabolites.

It can be said that vine metabolism (and overall performance) is affected by a complex interaction between natural and man-made factors. At the microclimatic level, the management of light quantity and quality is a powerful tool to regulate the quantitative and qualitative performance of the vine. This review contributes to the knowledge around

the effect of sunlight and temperature on grapevine berry responses to bunch and canopy microclimatic changes. By determining, under controlled conditions, the possible effect of these two abiotic factors at the bunch microclimatic level, it should be possible to establish thresholds of light and temperature effect on berry growth and phenolic biosynthesis.

Working in a vineyard, the impact of terroir concept will not be fully comprehended because of the number of variables linked to this notion (soil, meso-climatic, and vines differences). The prevailing conditions within a terroir unit will, therefore, impact the abiotic factors and the threshold values as it has been demonstrated within the literature study. This complicates our understanding of the effects of light, temperature, and vine water status on berry phenolics composition.

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