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Differential tissue-specific accumulation and function of tocochromanols in grape berries

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ABSTRACT

Grape berries have been extensively studied in terms of antioxidant characterization, specifically in anthocyanin, total phenol, and tannin accumulation. However, very little is known about vitamin E composition and contents in this fruit. Aiming to examine the function of vitamin E during grape berries ripening, tocochromanol contents and composition were evaluated in berries and leaves of grapevines (*Vitis vinifera* L. cv. Merlot), from just before *veraison* to commercial harvest. We also determined the time-course evolution of tocochromanol accumulation in various fruit tissues, including the skin, pulp, and seeds, and measured the extent of primary and secondary lipid peroxidation, as well as fruit technological maturity parameters. Vitamin E accumulated at higher levels in leaves than in fruits, although the tissue-specific evaluation of tocochromanol contents revealed that berry skin is also rich in α-tocopherol whereas tocotrienols were present in seeds only. α-Tocopherol content decreased during ripening, more specifically in the skin, and it was accompanied by an increase in the extent of lipid peroxidation. Contents and variations in the levels of α-tocopherol, but not those of the other tocochromanols, were inversely related to changes in lipid peroxidation during fruit ripening, as indicated by tissue-specific variations in malondialdehyde contents. In conclusion, α-tocopherol is more abundant in leaves than fruit, yet it apears to exert a role in the modulation of the extent of lipid peroxidation in grape berries, more specifically in the skin, where α-tocopherol depletion and malondialdehyde accumulation may be related to an adequate progression of fruit ripening.

1. Introduction

Grape berries are since ancient times considered of high relevance for human societies due to the various products human culture has developed from them, being wine the most economically relevant derived product (Terral et al., 2010). Grape quality for winemaking comprises a series of characteristics that influence the chemical composition of the wine and its organoleptic properties. Among these properties there are the typical aromas of each variety, taste sensations, chromatic properties, and antioxidant properties, all highly appreciated by consumers (Arcari et al., 2013). Although this is fundamental in the quality of wine, several factors do not depend solely on the composition of the grapes (Downey et al., 2006), because during wine vinification and aging processes a series of chemical reactions affect the physico-chemical and organoleptic characteristics of the product (Sacchi et al., 2005; Garde-Cerdán and Ancin-Azpilicueta, 2006; Casassa and Harbertson, 2014; Allegro et al., 2021). Grape quality and subsequently wine attributes depend, among other factors, on the correct development of grape ripening, since it leads to the final contents and composition of phenolic compounds and other antioxidants that give fundamental sensory attributes such as colour, astringency, bitterness, and the body of the wine, in addition to improving the wine aging potential (Boss et al., 1996; Vidal et al., 2003; Chira et al., 2015).

Although grape berries, considered as non-climacteric fruit, do not show a large increase in ethylene emission or respiration rates at the onset of ripening (*veraison*), ethylene may still be involved in grape berries ripening together with other hormones (Böttcher et al., 2013). While ABA, ethylene, and brassinosteroids have been proposed to play a role as ripening promoters, auxins, cytokinins, gibberellins, jasmonates, and polyamines may function as ripening inhibitors (Davies and Böttcher, 2009; Deluc et al., 2009; Kuhn et al., 2013; Fortes et al., 2015; Ribalta-Pizarro et al., 2021). Moreover, lipid peroxidation events are essential in triggering grape berries ripening, being the onset of fruit ripening characterized by reactive oxygen species accumulation and

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lipoxygenase-mediated membrane lipid peroxidation, most particularly in the skin (Pilati et al., 2014). Peroxidation of poly-unsaturated fatty acids (PUFAs), such as linolenic (18:3) and linoleic (18:2) acid, can give rise to various oxylipins, which can act as signalling molecules or be precursors of aromatic volatiles (Kalua and Boss, 2009). Unfortunately, however, there is still very little information about the role of antioxidants in modulating the extent of lipid peroxidation in fruits. This is particularly true in studies evaluating temporal variations in antioxidants including tissue separation, although such spatiotemporal approaches have been considered essential to better understand plant physiological processes (Munné-Bosch, 2022).

Vitamin E is a group of eight lipid-soluble compounds that include four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ) that help protect membranes from lipid peroxidation (Falk and Munné-Bosch, 2010). Plastochromanol-8 is an antioxidant that, together with tocopherols and tocotrienols, belongs to the group of tocochromanols, displaying huge similarities in structural terms and playing a very similar antioxidant function. Plastochromanol-8 has been found to occur in several plant species, including mosses, and lichens, but it does not accumulate in all photosynthetic organisms, as it occurs with α-tocopherol (Kruk et al., 2014). Unfortunately, despite the unique role of tocochromanols among antioxidants in inhibiting the propagation of lipid peroxidation (Muñoz and Munné-Bosch, 2019), very little is still known about the role of vitamin E in the modulation of lipid peroxidation during fruit ripening (Muñoz and Munné-Bosch, 2018). Although a recent study investigated the accumulation of tocopherols in the skin and seeds of Alvarinho grape berries, methodological limitations in the separation of tissues did not allow to evaluate tissue-specific changes in the extent of lipid peroxidation during ripening (as not enough skin could be isolated from berries in a mature stage to perform malondialdehyde measurements; Garrido et al., 2021).

In the present study, a possible differential accumulation of tocochromanols in leaves and fruits of grapevines (*Vitis vinifera* L. cv. Merlot) was evaluated. Furthermore, we examined spatiotemporal changes in vitamin E accumulation during grape berries ripening by measuring tocochromanol composition and contents in various tissues of grape berries, including the skin, pulp, and seeds, from just before *veraison* until commercial harvest. Finally, we aimed at linking the observed spatiotemporal changes with variations in the extent of lipid peroxidation and fruit quality attributes.

2. Material and methods

2.1. Plant material and sampling

Grapevines (*Vitis vinifera* L. cv. Merlot) from a commercial orchard in Avinyonet del Penedés, Barcelona, Spain (41°22′06.3″N 1°46′22.9″E) were used for the study from just before *veraison* until ripe, commercial harvest stage. Grapevines were growing under Mediterranean field conditions north-south oriented, planted at 2 m between rows and with 1 m between them with a vineyard plant density of 5000 vines per hectare. Whole berries and tissular berry analysis, including skin, pulp, and seeds, were performed on samples collected two days before *veraison* (August 8th, 2019) and at 5, 12, 27, 40, and 54 days after *veraison* (DAV), The *veraison* was determined by the producer when 50% of berries changed colour (BBCH 85, Lorenz et al., 1995). The last sampling data (54 DAV) corresponds to the commercial harvest date, which occurred on October 3rd, 2019. Leaf sample analysis were performed on samples collected at −2, 12, 40, and 54 DAV. All samplings were performed on clear sunny days at midday (at maximum incident photosynthetically-photon flux density, which decreased progressively over the season from 26 to 18 MJ m^{-2}). Mean daily air temperatures also decreased progressively from *veraison* to harvest from 27 to 18 °C. Accumulated monthly precipitation was 68.4 mm and 18.5 mm during August and September, respectively. For each biological replicate, one hundred berries and four fully expanded leaves per vine were collected, samples were immediately frozen with liquid nitrogen, and then stored at −80 °C upon arrival to the laboratory and until subsequent processing and analyses. Moreover, the skin, pulp, and seeds were separated from at least 20 berries for each vine with the help of liquid nitrogen (to prevent defreezing during tissue separation), and each tissue obtained from different fruits from the same plant were always pooled together to provide sufficient material for subsequent analyses. Samples from a total of twelve vines per sampling time point were collected, so that 12 biological replicates were used. Samples were immediately frozen in liquid nitrogen and kept at −80 °C until analyses. For each biological replicate, at least one-hundred mg of pooled sample was always used for each biochemical analysis.

2.2. Tocochromanols analyses

The quantification of the different tocochromanol forms, including tocopherols (α, β, γ and δ), tocotrienols (α, β, γ and δ), and plastochromanol-8 was performed by HPLC as described (Vincent and Munné-Bosch, 2022). Samples were extracted with methanol containing 0,01% BHT (w/v) using ultrasonication (Bransonic ultrasonic bath 2800, Emerson Industrial, Danbury, CT, USA), centrifuged at 13,000 rpm during 10 min at 4 °C. The same procedure was repeated twice to allow full extraction. Then, supernatants were pooled, and after filtration, tocochromanols were separated by isocratic HPLC using an Inertsil 100A column (5 μm, 0.03 × 0.25 m, GL Sciences Inc., Japan) and the HPLC system consisted of a Waters 600 controller pump (Waters Corporation, Massachusetts, USA), a Waters 717 plus auto-sampler (Waters Corporation, Massachusetts, USA), and a Jasco FP-1520 fluorescence detector (FP-1520, Jasco, Tokyo, Japan). Calibration curves with authentic standards (Sigma) were used for quantification as described (Vincent and Munné-Bosch, 2022).

2.3. Lipid peroxidation analyses

The extent of lipid peroxidation in membranes was assessed by analyzing primary (lipid hydroperoxide) and secondary (malondialdehyde, MDA) lipid peroxidation products, as described (Morales et al., 2021). In short, lipid hydroperoxides content was estimated spectrophotometrically by using the Fox-2 assay in methanolic extracts, while MDA content was determined by HPLC using the thiobarbituric acid-reactive substances assay after extraction with methanol containing 0.01% (w/v) BHT. The $(TBA)_2$-MDA adduct was separated on an Hypersyl ODS-5 μm column (250 × 4.6 mm, Teknokroma, St. Cugat, Spain) and eluted in the mobile phase with 5 mm potassium phosphate buffer (pH 7.0) containing 14% acetonitrile and 0.6% tetrahydrofuran. The $(TBA)_2$-MDA adduct was quantified by its absorbance at 532 nm and coelution with an authentic standard of 1,1,3,3-tetraethoxypropane (Sigma, Steinheim, Germany), which is stoichiometrically converted into MDA during the acid-heating step of the assay (Morales et al., 2021).

2.4. Grape berry technological maturity

The volume of 50 berries, total soluble solids (TSS, °Brix), titratable acidity (TA, g tartaric acid L^{-1}), and TSS/TA were measured following the standardized methodology described by the International Organisation of Vine and Wine (OIV, 2019).

2.5. Statistical analyses

Statistical analyses were performed by applying a one-way ANOVA and Tukey *posthoc* test indicated with different letters when a given parameter differed between sampling times (IBS SPSS Statistics 19; SPSS Inc., Illinois, USA). Spearman correlations were performed in grape berries to describe putative relationships between the technological maturity parameters and antioxidants. Differences were considered

significant when p values were below 0.05 ($p < 0.05$). Considering that Spearman regression coefficients were higher than Pearson coefficients we expected some monotonic, non-linear relationships between the analyzed parameters. To visualize those relationships, we used Sigma Plot Regression Wizard to adjust the best fit in each case.

3. Results

3.1. Tocochromanol composition and contents in leaves, fruits, and berries tissues

A comparative analysis of tocochromanol composition and contents in leaves and berries of grapevines revealed that the major tocochromanol found in both leaves and fruit is α-tocopherol, the most active vitamin E form. The content of this lipophilic antioxidant in leaves increased during the season reaching 4533 μg g DW^{-1} at 54 DAV, representing approximately 60% of the total tocochromanol content (TTC) (Fig. 1A). In berries, α-tocopherol followed an opposite pattern and accumulated less than 10% content to that obtained for leaves, decreasing from 24 μg g DW^{-1} just before *veraison* to 19 μg g DW^{-1} at 54 DAV, but represented about 75%–80% of the total vitamin E forms in grape berries (Fig. 1B).

In leaves, plastochromanol-8 was present at lower levels but showed a similar trend to that observed for α-tocopherol, since it represented approximately 38% of TTC in these organs and increased from 2035 μg g DW^{-1} just before *veraison* to 2885 μg g DW^{-1} at 54 DAV (Fig. 1A). Furthermore, its content in grape berries remained relatively low but still represented between 18% and 25% of the TTC. Plastochromanol-8 content in the fruits varied from 5.6 μg g DW^{-1} just before *veraison* to 6.0 μg g DW^{-1} at the end of the season and followed the same dynamics as α-tocopherol (Fig. 1B).

A tissue-specific quantification of vitamin E in grape berries revealed that α-tocopherol was also the most abundant tocochromanol form (Fig. 1C). α-Tocopherol was also the major form of tocochromanol found in skin, followed by plastochromanol-8 and γ-tocopherol. Tocochromanol dynamics in the pulp showed a similar dynamics to the skin, but in general, accumulated lower contents (Fig. 1C). Seeds were found to be the tissue with the higher diversity regarding tocochromanol composition, since besides α-tocopherol, γ-tocopherol, and plastochromanol-8, high concentrations of α-tocotrienol and γ-tocotrienol were also found to be present (Fig. 1C).

3.2. Stress markers in leaves, fruits, and berries tissues

Stress markers were evaluated in leaves in terms of the maximum quantum yield (F_v/F_m ratio) and the concentration of lipid peroxidation products, such as lipid hydroperoxides and MDA (Fig. 2A). The highest F_v/F_m value of 0.74 was observed just before *veraison*, decreasing progressively during the season to 0.71 at 54 DAV. Lipid hydroperoxide content increased transiently at 12 DAV up to 18.5 μmol H_2O_2·g DW^{-1}, to decrease later progressively throughout the season. Furthermore, MDA content was consistently higher and kept relatively constant over the season, declining only by 10% at 54 DAV (Fig. 2A).

In grape berries, lipid hydroperoxide content remained low and stable over the season without significant differences among sampling dates, whereas MDA content increased progressively from 57.2 nmol g DW^{-1} just before *veraison* to 190.3 nmol g DW^{-1} at 54 DAV, reaching values 4-fold higher than those observed at the beginning of the season (Fig. 2B).

Tissue-specific analysis of lipid peroxidation products revealed that lipid hydroperoxide content in the skin and seeds was kept low and stable throughout the season (Fig. 2C). Meanwhile, lipid hydroperoxide content in the pulp decreased with the progression of ripening. MDA contents showed a different trend between the analyzed tissues, where the highest concentration was found in the skin, boosting sharply at 27 DAV, and kept high until 54 DAV, ending the season with concentrations almost 28-fold higher than those observed just before *veraison*. The lowest content of MDA was found in the seed, with contents representing a thousand fraction of the ones observed in the skin (Fig. 2C).

3.3. Linking tocochromanol composition and contents with fruit quality

General analytical parameters of quality were evaluated in terms of volume, TSS, TA concentration, and TSS/TA ratio (Fig. 3). Berries

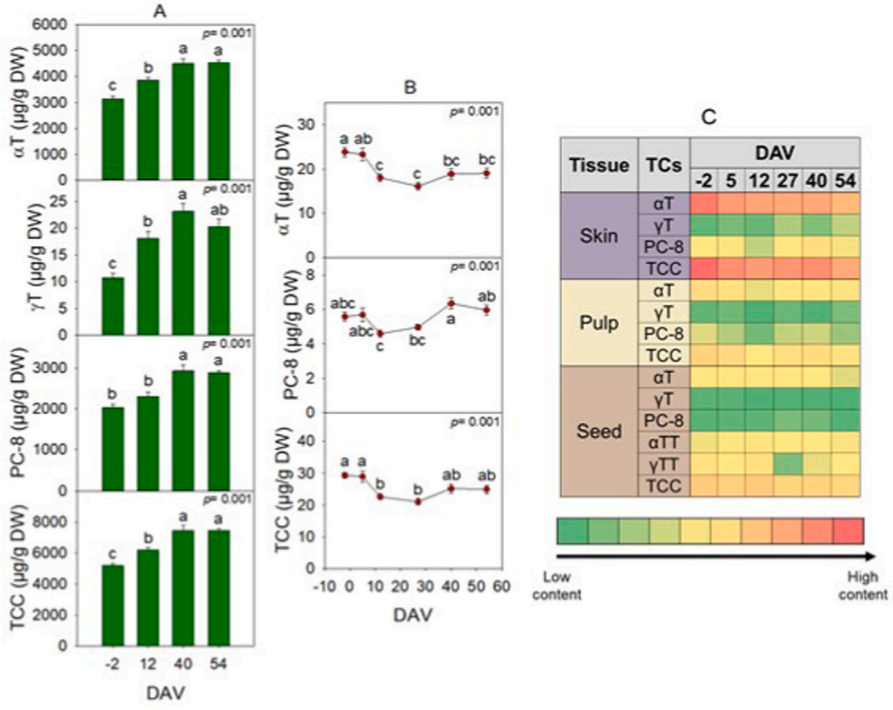


Fig. 1. Time-course evolution of tocochromanol composition and content of leaves (A), grape berries (B), and berries tissues: skin, pulp, and seed (C), from just before *veraison* (−2 DAV) to commercial harvest (54 DAV). Data show the mean and standard error of 12 replicates. Each replicate corresponds to a pool of two leaves. In leaves and berries, different letters show significant differences between sampling points ($p < 0.05$, Tukey *posthoc* test). In berries tissues, the scale of colour denotes tocochromanols concentration, from green (low contents) to red (high contents). DAV, days after *veraison*; αT, α-tocopherol; γT, γ-tocopherol; PC-8, plastochromanol-8; TCC, total tocochromanol content.

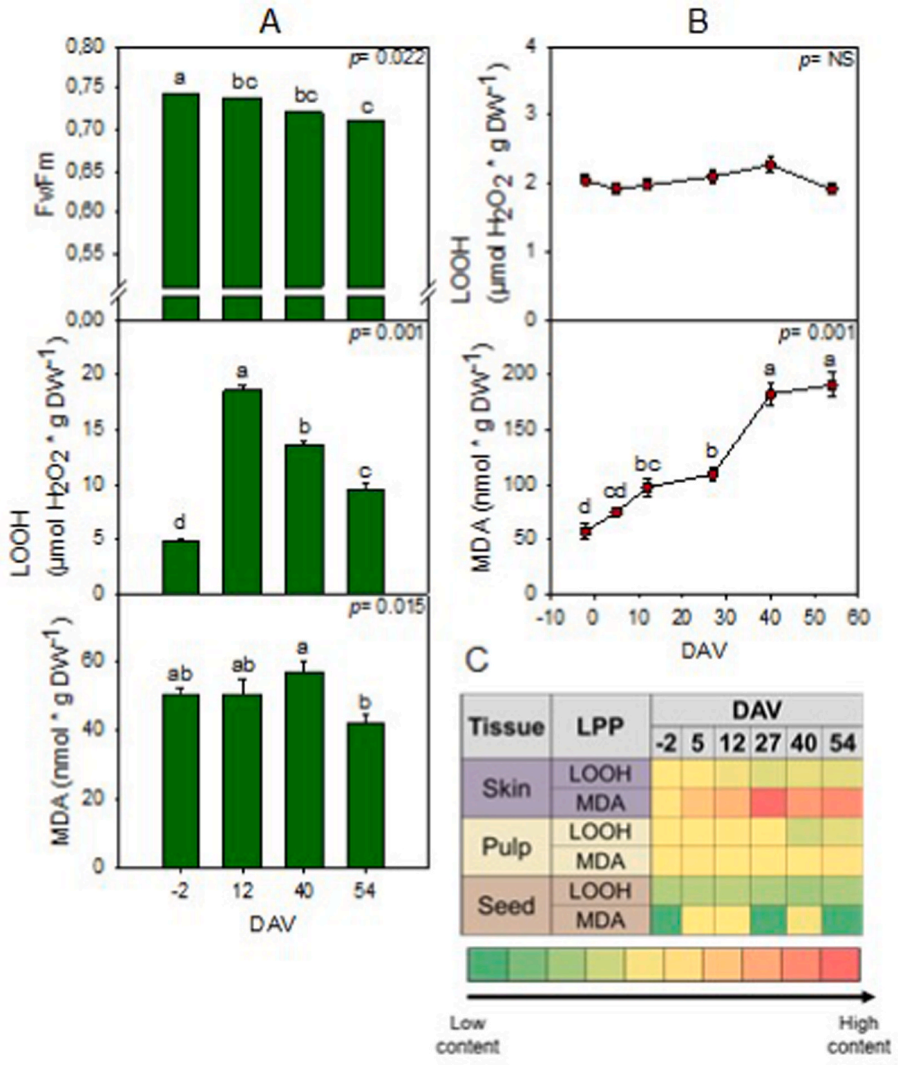


Fig. 2. Time-course evolution of maximum efficiency of PSII photochemistry (or maximum quantum yield, Fv/Fm ratio), and extent of primary and secondary lipid peroxidation in leaves (A), grape berries (B) and grapes tissues: skin, pulp, and seeds (C) from just before *veraison* (−2 DAV) to commercial harvest (54 DAV). Data show the mean and standard error of 12 replicates. Each replicate corresponded to a pool of 2 leaves. Different letters show significant differences between sampling points ($p < 0.05$, Tukey *posthoc* test). In berries tissues, the scale of colour denotes LOOH and MDA concentration, from green (low contents) to red (high contents). DAV, days after *veraison*; LOOH, lipid hydroperoxides; MDA, malondialdehyde; LPP, lipid peroxidation products.

reached their maximum volume at 12 DAV increasing their size by 65%, while chemical changes kept occurring. TA showed a strong reduction between the two first sampling dates, representing half the values from two days before *veraison* to 5 DAV, and gradually decreased with a steady period between 12 and 27 DAV, ending the season with the lowest values. TSS progressively increased up to 50% of its values from the beginning of the experiment to 40 DAV, when the berries ceased their sugar increase until the harvest date. The same dynamics was observed in the TSS/TA ratio, which increased progressively up to 54 DAV.

Spearman regression analysis showed that parameters associated with an adequate progression of grape development and ripening were highly correlated between them, such as the increase in volume, TSS and TSS/TA ratio. α-Tocopherol and MDA contents were also significantly correlated ($p < 0.001$) with the above-mentioned parameters, with particularly strong regression coefficients in the case of MDA. However, while α-tocopherol content negatively correlated with TSS ($R = -0.425$; $p < 0.001$) and TSS/TA ($R = -0.379$; $p = 0.001$) and positively correlated with TA ($R = 0.352$; $p = 0.002$), MDA showed the complete opposite relationship and with a stronger trend, being positively correlated with TSS ($R = 0.773$; $p < 0.001$) and TSS/TA ($R = 0.853$; $p < 0.001$) and negatively correlated with TA ($R = -0.852$; $p < 0.001$). Furthermore, a relatively weak but significant correlation was observed between α-tocopherol and MDA ($R = -0.330$ $p = 0.005$).

When adjusting those relationships into non-linear functions, some of them resulted in higher adjustments (Fig. 4). The best fit for α-tocopherol with quality parameters resulted to be as well with TSS ($R^2 = 0.258$), TA ($R^2 = 0.183$), and TSS/TA ratio ($R^2 = 0.216$) obtained with cubic functions. However, relationships with MDA showed to be stronger and obtained the best fit with sigmoidal (TSS; $R^2 = 0.693$), Weibull (TA; $R^2 = 0.660$), and polynomial inverse (TSS/TA; $R^2 = 0.765$) functions. The association between α-tocopherol and MDA in grape berries resulted in a significant but weak relationship ($R^2 = 0.089$) fitted to a sigmoidal function, while the same association in berries skin was found to be significant and stronger ($R^2 = 0.261$, Fig. 5). In the latter case, both associations between α-tocopherol and MDA in grape berries appeared however to be weaker than that obtained with the aforementioned Spearman regression analysis.

4. Discussion

The production of reactive oxygen species is constantly occurring in aerobic organisms like plants due to photosynthesis, respiration, and other metabolic processes, and although they can be toxic at high concentrations, low levels can induce signalling that is essential in leaf, flower, and fruit development (Muñoz and Munné-Bosch, 2018; He and Ding, 2020). Exposure of leaves or fruits to adverse environmental conditions can induce an uncontrolled and excessive production of reactive oxygen species in chloroplasts causing cellular damage due to lipid peroxidation cascades (Gill and Tuteja, 2010), thus resulting in

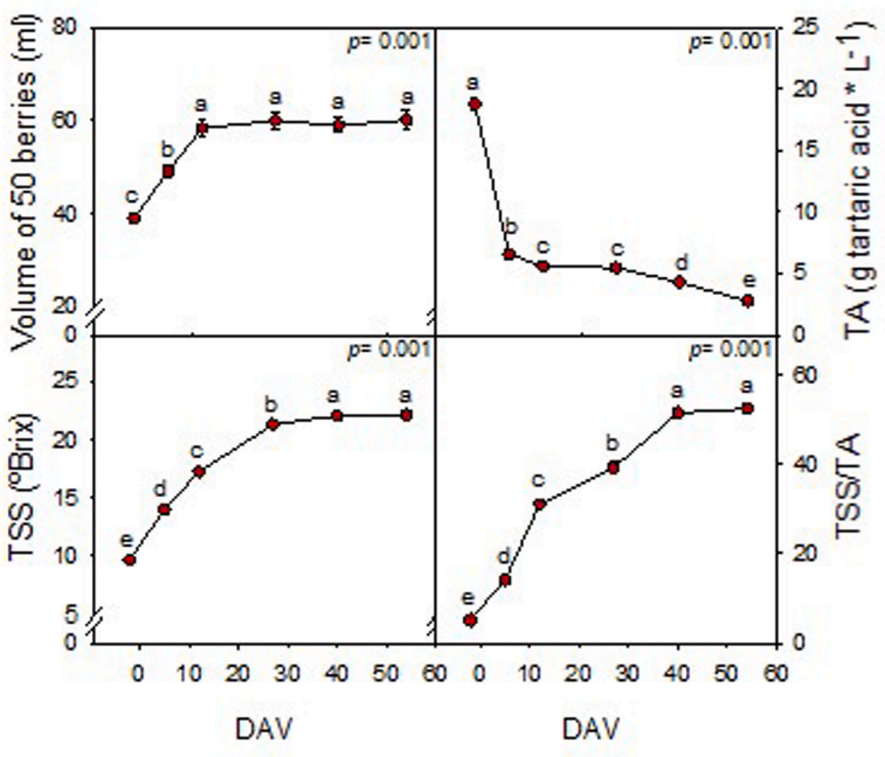


Fig. 3. Time-course evolution of the volume, total soluble sugars (TSS), total acidity (TA), and TSS/TA of grape berries from just before *veraison* (−2 DAV) to commercial harvest (54 DAV). Data show the mean and standard error of 12 replicates. Each replicate corresponded to a pool of 50 berries. Different letters show significant differences between sampling points ($p < 0.05$, Tukey *posthoc* test). DAV, days after *veraison*.

oxidative damage if antioxidant mechanisms are not activated. Here, a typical response to environmental stress could be observed. *Fv/Fm* ratio in leaves ranged between 0.70 and 0.75, decreasing progressively as the season advanced from just before *veraison* to commercial harvest, indicating photoinhibition to the photosynthetic apparatus (Takahashi and Badger, 2011). Noteworthy, summer stress on leaves was very mild and could be well tolerated by the plant, as indicated by slight changes in MDA content in leaves. Interestingly, foliar MDA content kept constant from −2 DAV until 40 DAV to decrease then by 27% just before harvest, most likely due to the less stressful environmental conditions of late September and early October (just before harvest) when air temperature and incident PPFD markedly decreased compared to August. Therefore, despite the slight summer stress observed in leaves, grapevines were able to tolerate summer conditions very well, as it has been shown previously (Correia et al., 1995; Palliotti et al., 2009; Ju et al., 2018). In part, this can be attributed to an enhanced photoprotective and antioxidant response of α-tocopherol in leaves, which increased progressively over the season. It is well known that this antioxidant protects thylakoids from photo-oxidative stress and lipid peroxidation (Havaux et al., 2005), and the observed response fits well with this antioxidant function.

Tocochromanol composition and content, as well as its behaviour over the season, differed markedly in fruits compared to leaves. Both α-tocopherol and PC-8 accumulated much less in fruits than in leaves, with less than 1% of tocochromanols accumulating in fruit compared to leaves. Furthermore, while PC-8 content was comparable to that of α-tocopherol in leaves, PC-8 content in fruit was comparatively lower. Interestingly, tocochromanol concentrations increased sharply in the skin, which may be related to the photosynthetic capacity of this tissue (Fig. 6). The skin keeps some photosynthetic activity until senescent-like processes (such as chloroplast disorganization and cell death) occur during late ripening stages (Ghan et al., 2017). During these stages, chloroplasts are still present but have a lower thylakoidal organization and accumulate very large plastoglobules (Fougère-Rifot et al., 1995; Teixeira et al., 2022). In contrast, the number of chloroplasts in the pulp is much smaller compared to that of the skin. In contrast, seeds contain proplastids (Fougère-Rifot et al., 1995; Choi et al., 2021). Differences in the number of chloroplasts and plastid differentiation between fruit tissues is essential to understand the differential spatiotemporal patterns of tocochromanol accumulation since large plastids with large plastoglobules in the skin are more likely to accumulate tocochromanols (Morelli et al., 2023). Furthermore, concentrations of PC-8 and γ-tocopherol in the skin and pulp were much lower than those of α-tocopherol, thus suggesting α-tocopherol is the major antioxidant among tocochromanols influencing the propagation of lipid peroxidation, especially in the skin, which is the outer barrier integrating environmental changes or pressures that could influence grape ripening.

Lipid peroxidation events are essential in triggering grape berries ripening, so the onset of fruit ripening is characterized by both reactive oxygen species accumulation and lipoxygenase-mediated membrane lipid peroxidation in the skin (Pilati et al., 2014). Lipid peroxidation has been shown to occur at different stages of grape berries ripening, where reactive oxygen species accumulation appears to play a role in developmental responses. In Pinot Noir grape berries, Pilati et al. (2007) described an oxidative burst at *veraison* that involved a rapid accumulation of H_2O_2 until 1–2 weeks after v*eraison*, and the modulation of ROS scavenging enzymes. These processes have gained interest in the grape industry because of their influence on fruit quality (Pilati et al., 2014; Carvalho et al., 2015; Rustioni et al., 2020). In the present study, the content of α-tocopherol in the skin decreased progressively during ripening, which might have led to the inability to prevent lipid peroxidation. While LOOH content remained low and stable, MDA accumulation increased sharply over the season, indicating that lipidic fragmentation processes occurred rapidly and generated oxidative stress in the skin (Farmer and Mueller, 2013). This process may explain the increase in porosity of skin cell walls at ripe stages, allowing fruit

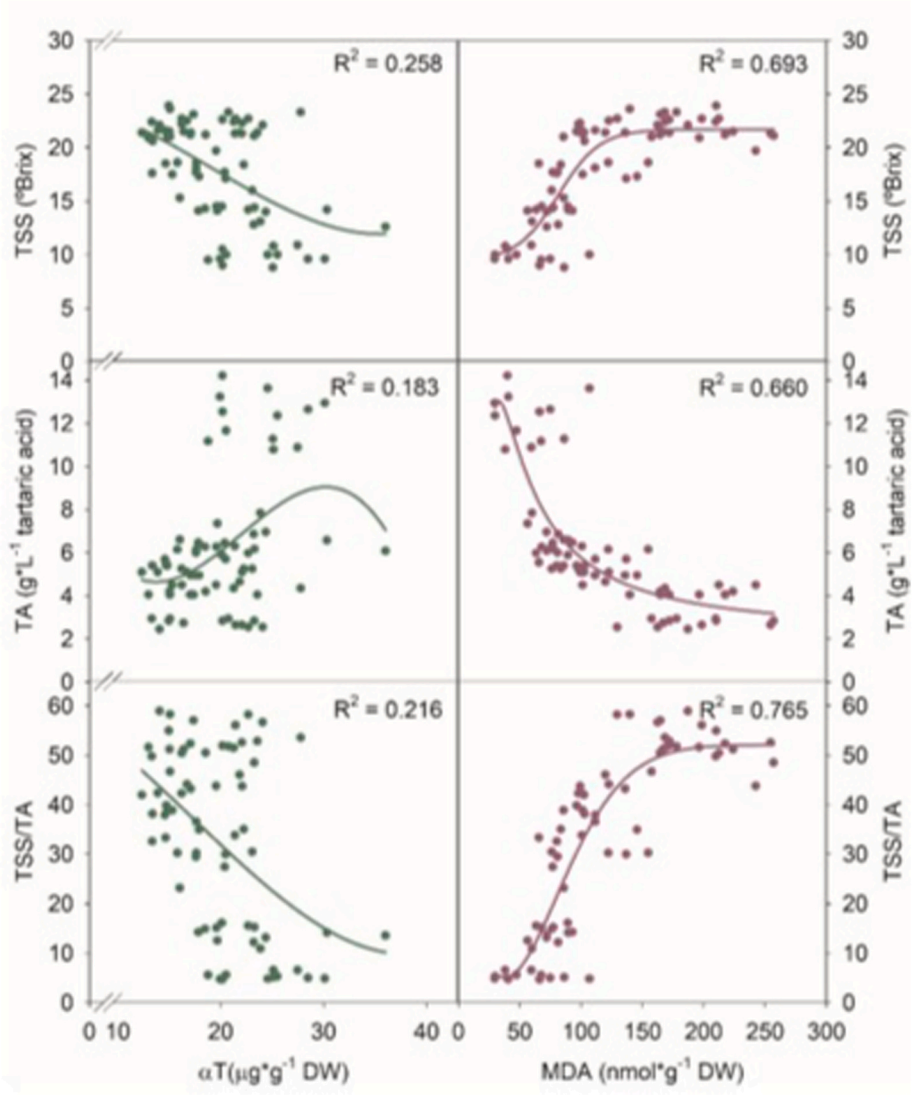


Fig. 4. Adjusted relationships in non-linear functions for α-tocopherol and malondialdehyde with technological maturity parameters: α-tocopherol with TSS, TA, TSS/TA; and MDA with TSS, TA, and TSS/TA. DAV, days after *veraison*; αT, α-tocopherol; MDA, malondialdehyde; TSS, total soluble sugars; TA, total acidity.

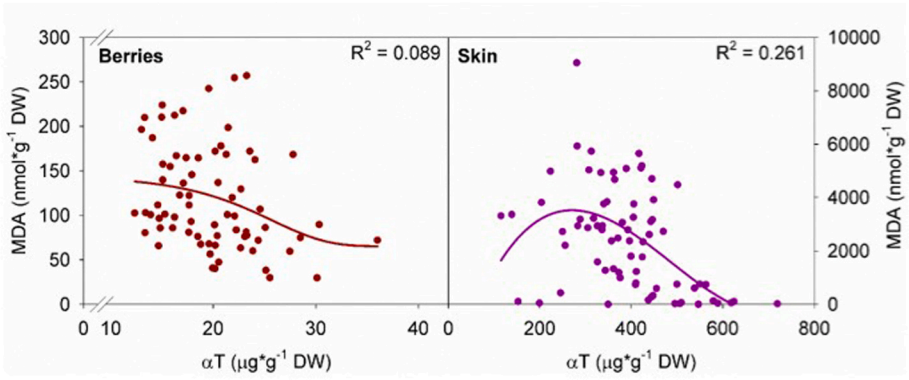
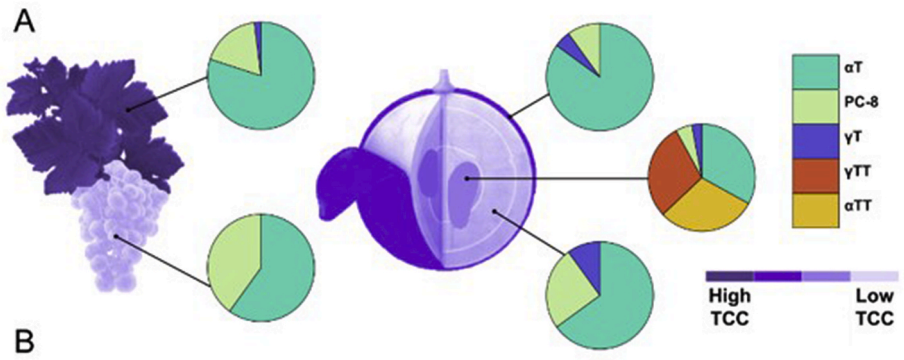


Fig. 5. Adjusted relationships in non-linear functions for α-tocopherol with malondialdehyde in grape berries and berries skin. DAV, days after *veraison*; αT, α-tocopherol; MDA, malondialdehyde.

softening and favoring aroma development (Conde et al., 2007).

Within the winemaking industry, there are two fundamental approaches to describe the maturity of grape berries: technological maturity, which depends on those processes occurring within the pulp, such as the accumulation of sugars and acidity loss; and phenolic maturity, which depends on phenolic composition and concentration in the skin and seeds (Coombe and McCarthy, 2000; Dokoozlian, 2000). In leaves and grape berries, α-tocopherol and MDA correlated with each



Tissue	TCC	LPP
Leaf	↗	↘
Berry	↘	↗
Skin	↘	↗
Pulp	↘	≈
Seed	≈	≈

Fig. 6. **(A)** Tissue-specific composition and concentration of tocochromanols in leaf, grape, skin, pulp, and seed. **(B)** Diagram showing the tissue-specific evolution of total tocochromanol content (TCC) and lipid peroxidation products (LPP) during ripening.

other, and with all technological maturity parameters, and MDA seemed to have a greater influence since its correlations were stronger for all parameters. Distefano et al. (2022) described increases in vitamin E contents when applying a plant-derived biostimulant on greenhouse cherry tomatoes, which was accompanied by a reduction in TSS, suggesting a compromise between antioxidant protection and sugar accumulation. To our knowledge, prior to the present study there was no previous evidence of any relationship between these lipophilic antioxidants and the accumulation of sugars in grape berries. Furthermore, α-tocopherol showed to be the major form of vitamin E in the present study, both in photosynthetic and non-photosynthetic tissues, as earlier observed in strawberry plants (Casadesús et al., 2020). Because α-tocopherol is known to have a fundamental role in preventing the propagation of lipid peroxidation (Munné-Bosch and Alegre, 2002), α-tocopherol in grape berries could be limiting the extent of MDA accumulation to facilitate a correct berry ripening. Interestingly, α-tocopherol content decreased throughout the season within grapes and berries skin, making possible the accumulation of MDA, which highly correlated with technological maturity parameters. In the case of TSS and TSS/TA ratio, the relationship with MDA adjusted better to non-linear functions, observing a clear dose-dependent association, since a saturation point was observed when MDA accumulation reached values of 100–150 nmol g DW^{-1}, which occurred at 27 DAV. Phenolic compounds are known for their antioxidant, nutraceutical, and sensory properties, which are fundamental to grape and wine quality (Conde et al., 2007; Allegro et al., 2018). During the winemaking process, the extractability of these compounds is a key component to ensure wine quality and stability, and this process is highly dependent on skin cell wall morphology and composition, which vary among cultivars, ripening stages, and climatic conditions. The stress conditions observed during the summer season in the presente study made lipid peroxidation more likely to occur, disrupting the fluidity and functionality of the cell membrane, since it was observed a decrease in α-tocopherol within the skin, which is known to enhance membrane rigidity. This could be an oenological advantage at late ripening stages when sugar intake to the berries ceases since oxidative damage in skin cell walls is suggested to increase the easiness of phenolic compounds extractability and therefore, wine quality (Ortega-Regules et al., 2006, 2007; Farmer and Mueller, 2013; Paladines-Quezada et al., 2019).

In conclusion, α-tocopherol is more abundant in leaves than fruit, yet it seems to exert a role in the modulation of the extent of lipid peroxidation in grape berries, more specifically in the skin, where α-tocopherol depletion may be related to an adequate progression of fruit ripening and quality. Although α-tocopherol is the only antioxidant with the capacity of preventing the propagation of lipid peroxidation by eliminating lipid peroxyl radicals, lipid peroxidation can also arise from direct oxidation of fatty acids by singlet oxygen and by enzymatic processes triggered by lipoxygenase. Therefore, although as shown here α-tocopherol is important in controlling the extent of lipid peroxidation, it is not the only factor involved. Further research is needed to establish the relative contribution of α-tocopherol in relation to that of other antioxidants (polyphenols, tannins, carotenoids) in the control of lipid peroxidation events during fruit ripening.

Author contribution statement

C.R.P. and S.M.B. designed the experiments; C.R.P. and P.M. performed experiments; C.R.P. and S.M.B. wrote the manuscript with the help of P.M.; S.M.B. supervised the work.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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