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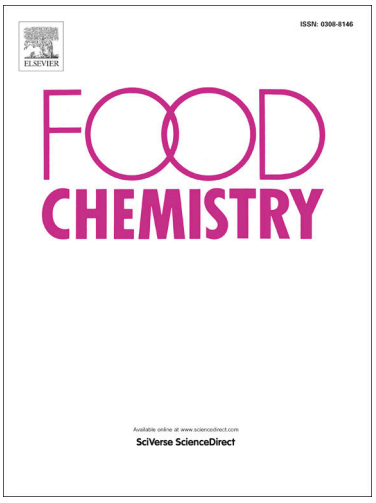

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Role of anthocyanin traits on the impact of oenological tannins addition in the first stage of red winegrape skin simulated maceration

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Abstract

In winemaking, exogenous tannins are added before maceration to improve future wine color characteristics derived from extracted grape anthocyanins. The study aimed to investigate the relation between different grape varieties, selected according to their anthocyanin profile, and the effect of five exogenous tannin formulations differing in origin and chemical features. Anthocyanin content, polymeric pigments, and color traits were assessed during a 72-hour skin simulated maceration. Grape skin-derived tannins increased color intensity (up to one unit) and polymeric pigments formation (up to 6.5%) in malvidin-prevalent Merlot and Cabernet sauvignon, with different extent depending on the anthocyanin richness. Grape seed-derived and ellagic formulations favored the pigment polymerization, the first in Nebbiolo and Sangiovese (up to 8.2%), which are characterized by high ratios of disubstituted anthocyanins, and the latter in malvidin-rich Syrah and Aglianico (up to 5%).

A positive effect of quebracho regarded the defense of anthocyanin forms, particularly in Sangiovese and Nebbiolo.

Keywords: winegrapes, exogenous tannins, anthocyanin composition, skin maceration, wine color, polymerization, HPLC analysis.

1. Introduction

Red wine color is the first characteristic perceived by consumers and therefore identifying its first-sight quality. This sensory perception mainly relies on grape anthocyanins extracted during skin contact maceration in the first steps of winemaking. Anthocyanin content and profile is mainly variety-dependent, although several factors can influence the anthocyanin concentration in grape berry skins, such as edaphoclimatic factors, agronomical practices, seasonal features, and harvest conditions (Mattivi, Guzzon, Vrhovsek, Stefanini, & Velasco, 2006). Nevertheless, monomeric anthocyanins represent only a part of the final wine color, since starting from their extraction they can take part in several reactions with grape and yeasts derived compounds, such as copigmentation and polymerization. The adducts produced, non-covalently the former and covalently the latter, are considered to increase color stability, since these forms are not bleachable by sulfur dioxide. The copigmented and the polymerized forms can correspond to the 8–30% and 35–63% of the wine color, respectively (Versari, Boulton, & Parpinello, 2008).

Several winemaking technologies have been highlighted to enhance anthocyanin extraction from grape skins to juice in order to achieve high anthocyanin contents, based on the modification of temperature, time, solid-liquid contact, and on the use of additives such as enzymes or antioxidants to both enhance the extraction and preserve the extracted anthocyanins, and possibly favor anthocyanin polymerization (Setford, Jeffery, Grbin, & Muhlack, 2017). Among additives, the use of exogenous tannins in winemaking has been proposed as a practice aimed to increase the antioxidant capacity, enhance pigment polymerization, and modify the sensory properties of wines (Versari, Du Toit, & Parpinello, 2013; OIV 2019).

Commercial oenological tannins are usually found as pure or mixed formulations of two main classes of tannins, the hydrolysable and the condensed tannins. Hydrolysable tannins include gallotannins from gallnuts of -mainly- tara (*Caesalpina spinosa*) and oak (*Quercus* spp.), which are composed by glucose esterified in different extent by gallic acid, and the ellagitannins extracted from chestnut

(*Castanea* spp.) and oak (*Quercu*s spp.), characterized by glucose esterified with gallic, ellagic, and hexahydroxidiphenic acids (HDDP) (Hagerman, 2002). Gallotannins are usually monomeric glucose reaching high galloyl substitution on core glucose and the galloyl residues themselves, whereas ellagitannins can be found as monomeric and dimeric structures, where vescalagin and castalagin monomeric forms can represent up to the 50% of total ellagitannins (Jourdes, Pouységu, Deffieux, Teissedre, & Quideau, 2013).

On the other hand, condensed tannins, also called proanthocyanidins, are polymers of flavan-3-ols units, classified depending on the flavan-3-ol subunit nature. Procyanidins (catechin and epicatechin) and prodelphinidins (gallocatechin and epigallocatechin) subunits own a phloroglucinol-type A ring, whereas profisetinidins (fisetinidol and epifisetinidol) and prorobinetinidins (robinetinidol and epirobinetinidol) subunits own a resorcinol-type A ring.

Extracts from exotic wood, i.e. *Acacia* and *Mimosa* spp. (*Mimosaceae* family) and quebracho (*Schinopsi*s spp.) are reported to be mainly composed by resorcinol-type A ring subunits. Prorobinetinidins are the main constituents of *Mimosaceae* family, followed by fisetinidol, catechin, and gallocatechin in a lesser extent, whereas in quebracho only fisetinidol and, in minor extent, catechin units are found (Venter et al., 2012a; Venter, Sisa, van der Merwe, Bonnet, & van der Westhuizen, 2012b).

On the contrary, grape condensed tannins are composed by procyanidins and prodelphinidins, monomer nature and different extent of polymerization differentiating condensed tannins, as well as the degree of galloylation. In grape seeds, prodelphinidins are not present, whereas the presence of the galloylated group is very common. The opposite is found in grape skins (Kennedy & Jones, 2001). Grape skin proanthocyanidins own a higher mean degree of polymerization (up to 50 subunits) with respect to seed proanthocyanidins (up to 10 subunits, determined by phloroglucinolysis) (Rousserie, Rabot, & Geny-Denis, 2019). Regarding exotic wood, the degree of polymerization is very low with respect to their grapes analogues, given by a lower reactivity of the resorcinol-type subunits. Most of

the molecules range from dimers to tetramers in quebracho (Venter et al., 2012b), although traces of molecules up to undecamer have been found in Acacia (Vivas, Nonier, de Gaulejac, Absalon, Bertrand, & Mirabel, 2004; Venter et al, 2012a), leading to a mean polymerization degree of 3-4 units (Venter et al., 2012b).

Tannin concentration and composition are the main factors influencing wine astringency and bitterness. Grape-derived tannin characteristics, such as mean degree of polymerization (mDP), galloylation percentage, stereochemistry, and B-ring tri-hydroxylation, influence the perceived astringency (Ma, Guo, Zhang, Wang, Liu & Li, 2014). Particularly, increased mDP and galloylation are known to enhance astringency sensation, as well as stereochemistry and conformation (i.e. linear or branched structures) influence the magnitude of this sensation. Prodelphinidins are less astringent than procyanidins (Fernandez, Kennedy & Agosin, 2007), whereas a lack of information remains about prorobinetinidins and profisetinidins, even if quebracho-derived tannins have been reported particularly bitter and astringent when compared with the other formulations (Puech, Prida & Isz; 2007). Grape-derived tannins influence also wine bitterness, showing gradual reduction of elicited bitterness with an increased mDP (Ma et al., 2014). Concerning hydrolysable tannins, at an equimolar concentration, ellagitannins have been found to be more astringent than gallotannins. At the same time, these hydrolysable tannins typically show higher perceived astringency than seed-derived tannins (Gombau et al., 2019).

The International Organisation of Vine and Wine (OIV) regulates the use of tannin formulations in wine production. Recently, OIV recognized the use of exogenous tannins in winemaking, at grape must and wine steps, for the purpose of improving the antioxidant activity and color expression of the obtained wines (OIV, 2019). Previously, several researches have been conducted showing the different properties owned by these products, considering their polyphenols composition and the consequent ability to act as antioxidant, oxygen scavenger, copigmentation cofactors, polymerization enhancers, antimicrobial agents, as well as their contribution to the wine sensory properties

(Harbertson, Parpinello, Heymann, & Downey, 2012; Magalhães, Ramos, Reis, & Segundo 2014; Gombau, Vignault, Pascual, Canals, Teissedre, & Zamora, 2016; Pascual et al., 2017; Vignault et al., 2018; Gombau et al., 2019; Vignault et al., 2019).

In addition to the differences determined by the origin, tannin extracts present on the market are very heterogeneous, since the purity of the source, the extraction protocol, and the purification procedure can lead to very different final products (Versari et al., 2013; Vignault et al., 2018). Moreover, the efficacy of exogenous tannins in winemaking not only is attributable to their chemical characteristics but also to the step in which they are added. Therefore, there is the exigence to adapt their use taking into account several parameters, such as the variety features in terms of polyphenols content and relative abundance, as well as the winemaking technique and phase (Versari et al., 2013).

Although several research works have been published on the effect of oenological tannins on the phenolic composition and chromatic characteristics of the resulting wines, the variety impact on the effectiveness of their addition has not yet been studied. Therefore, the aim of this study was to evaluate the effect of different exogenous tannin formulations in the first steps of grape skins maceration emphasizing on the possible relation with the grape anthocyanin profile. To this purpose, more than twenty red grape varieties were grouped according to their anthocyanin profile, and five groups were identified. Seven varieties, including at least one variety for each group, were then selected as a reference, and subjected to simulated berry skin macerations in presence of each exogenous tannin, tested at the dosage commonly employed for wine production. The evaluation of different parameters involved in the wine color was carried out, in particular the evolution of extracted grape anthocyanins, in terms of content, polymerization, and chromatic characteristics. The knowledge of relationships between the efficiency of the tannin addition and the anthocyanin traits will enable tailoring the tannin use on anthocyanin features of the grape variety.

2. Materials and Methods

2.1 Reagents and Standards

Chemicals of analytical reagent grade, solvents for HPLC, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), iron (III) chloride hexa-hydrate, and Folin-Ciocalteu reagent were acquired from Sigma-Aldrich (St. Louis, MO, USA). The standard of malvidin-3-O-glucoside chloride was obtained from Extrasynthese (Genay, France). The solutions were prepared in deionized water produced by a Milli-Q system (Merck Millipore, Darmstadt, Germany).

2.2 Grape varieties selection

Twenty-six red winegrape (*Vitis vinifera* L.) varieties were selected on general parameters such as crop area, worldwide diffusion, and relevance for renowned wine production, as well as local relevance. A pool of data regarding anthocyanins traits were then collected on the basis of previous literature (Mateus, Machado, & De Freitas, 2002; Cabrita, Silva, & Laureano, 2003; Mattivi et al., 2006; Vasile Simone et al., 2013; Muñoz et al., 2014).

Then, the obtained anthocyanin data as percentage of the five unacylated glucoside, the sum of acetylglucoside, and the sum of *p*-coumaroylglucoside forms were subjected to a hierarchical cluster analysis. The outcome of this elaboration is shown in **Figure 1**, including a heat map representation of the relative abundance of the selected variables. It is possible to well distinguish five groups (G1 to G5) that can differentiate varieties according to their main anthocyanin profile features: malvidin-3-glucoside prevalent and equilibrated acylation ratio (range of cinnamoyl and acetyl derivatives: 8-25%) in G1, malvidin-3-glucoside prevalent and high ratio of coumaroyl-derivatives (>25%) in G2, high ratio of malvidin-3-glucoside (>45%) and its derivatives in G3, relatively high percentage of

peonidin-3-glucoside (>15%) and low or absent acylation in G4, and a disubstituted derivatives prevalent sector (peonidin forms >45%) in G5. Thus, the experiment was conducted with at least one variety representative of these groups: Syrah, Aglianico, Sangiovese, and Nebbiolo were selected for G2, G3, G4, and G5, respectively; for G1 it was decided to choose three varieties, namely Montepulciano, Merlot, and Cabernet Sauvignon because of the number of varieties of high enological interest included in this group.

2.3 Grape samples and density sorting

Vitis vinifera L. cv. Aglianico, Cabernet sauvignon, Merlot, Montepulciano, Nebbiolo, Sangiovese, and Syrah grapes were collected at ripeness (about 24 °Brix soluble solids content) from the CNR-IPSP ampelographic collection of Grinzane Cavour (Cuneo province, north-west Italy, 44.651 N, 7.995 E). Fifteen kilograms of berries were harvested for each grape cultivar and transported to the laboratory for the study. Grape material was manually destemmed and sampled for the assessment of the grape must/juice compositional parameters ("unsorted" sample). Then, the remaining obtained berries were sorted by flotation in sodium chloride solutions with different densities (from 1087 to 1125 kg/m^3) (Fournand, Vicens, Sidhoum, Souquet, Moutounet, & Cheynier, 2006). For this experiment, only the berries belonging to the most represented density class within each variety were taken, which corresponded to 1100 kg/m^3 for Merlot, 1106 kg/m^3 for Cabernet sauvignon, Nebbiolo, and Sangiovese, 1110 kg/m^3 for Montepulciano, and 1114 kg/m^3 for Aglianico and Syrah.

2.4 Standard chemical parameters

The grape must parameters were determined for each variety and sorting combination (sorted/unsorted). Two replicates of about 50 grape berries were crushed, and the liquid must was centrifuged at 3000 × g, 20 °C, 15 min using a Heittich 32R (Tuttlingen, Germany) centrifuge to obtain the supernatant for analysis. Titratable acidity and pH determinations were conducted using

OIV (2016) methods. Organic acids (malic and tartaric acid) and sugars (glucose and fructose) quantifications were performed using an HPLC (Agilent Technologies, Santa Clara, USA) equipped with a UV detector set to 210 nm and a refractive index detector, respectively (Giordano, Rolle, Zeppa, & Gerbi, 2009).

2.5 Berry skin total anthocyanins

For each variety, three repetitions of ten berries (sorted by density) were used to evaluate the anthocyanin maximum extraction and to calculate the extraction yield during the further skin macerations. The berries were peeled, and their skins once separated from the pulp were placed in 25 mL of a 14% v/v ethanol buffer solution adjusted to pH 3.40 containing 5 g/L tartaric acid and 2 g/L sodium metabisulfite (Di Stefano & Cravero, 1991; Río Segade et al., 2014). Then, the sample was homogenized using an Ultra-Turrax T25 high-speed homogenizer (IKA Labortechnik, Staufen, Germany) at 8000 rpm for 1 min. Finally, solutions were centrifuged for 15 min at 3000 × g at 20 °C, using the supernatant for total anthocyanins analysis as indicated below.

2.6 Oenological tannin formulations

The following oenological grade tannin formulations from AEB (Brescia, Italy) were selected to represent the principal product types used during skin maceration: one hydrolysable (ellagitannins from *Quercus* spp., ELQ), one proanthocyanidin from exotic wood (quebracho, QBR), and three proanthocyanidin preparations from grapes, in particular two obtained from grape seeds (SER and SEW) and one from grape skins (SKW). The specifications of the formulations used in this experiment are listed in **Table S1**. The selected oenological tannin formulations were characterized using spectrophotometric analytical methods for the estimation of phenolic content and antioxidant capacity, after dissolution of the 1 g/L of tannin preparation in a wine-like solution (12% v/v ethanol, 4 g/L tartaric acid, brought to pH 3.5 with NaOH 1 mol/L; Vignault et al., 2018). Total phenolics were

determined through the Folin-Ciocalteu assay and by the measurement of absorbance at 280 nm in water and expressed as g of gallic acid/100 g of tannin formulation using external calibration curves of gallic acid (Di Stefano & Cravero, 1991; Vignault et al., 2018). Antioxidant capacity was investigated throughout 2,2-diphenyl-1-picrylhydrazyl (DPPH) and *Ferric Reducing Antioxidant Potential* (FRAP) assays following the method modified by Miliauskas, Venskutonis, & Van Beek (2004) and Benzie & Strain (1996), respectively. An external calibration with Trolox standards was carried out for both antioxidant assays, and the results were expressed as mg of Trolox equivalent/g of exogenous tannin.

2.7 Simulated skin macerations with oenological tannins

Simulated macerations were carried out in a buffer solution at pH 3.40, prepared by the addition of 5 g/L tartaric acid in water, and adjusted to the final pH using 1 mol/L NaOH. Each tannin formulation was firstly dissolved in 100 mL of warm (40 °C) buffer solution enriched with 2% v/v ethanol to help solubilization, then 10 mL of the dissolved tannin solution was added to 90 mL of the buffer solution (without ethanol) for each macerating replicate to obtain a buffer solution containing the selected tannin dosage, that is 4/5 of the maximum recommended dose (i.e. 8, 40, 16, 20, and 24 g/hL for ELQ, QBR, SER, SEW, and SKW, respectively; Table S1) and represents a dose commonly added during maceration phase in industrial winemaking. Therefore, initial berry skin macerating solution contains 0.2% v/v ethanol. The buffer solution for the control experiment was prepared in the same way with the exception of the exogenous tannin addition.

For each grape variety and tannin formulation combination, three independent repetitions of 20 berries each were used for the simulated skin maceration. The berries were weighed, peeled and the skins were quickly introduced in a glass bottle containing 100 mL of the buffered tannin solution. Macerations were carried out at a 25 °C temperature, and samples were taken from all macerations after 6, 24, 48, and 72 hours: for each withdrawal a slight homogenization was performed before

taking the 2.4 mL sample, then an addition of 2.4 mL of 96% v/v ethanol was done to increase the alcohol strength simulating the ethanol production occurring during red wine maceration/fermentation. Therefore, this led to an alcohol strength of 2.50, 4.80, and 7.10 % v/v after 6, 24, and 48 h, respectively.

2.8 Anthocyanin composition and chromatic characteristics of the skin extracts

Total anthocyanins and color parameters were determined in the aliquots sampled throughout the simulated skin maceration by using a spectrophotometer (UV-1800, Shimazdu, Kyoto, Japan). In particular, total anthocyanins index (TAI), expressed as mg of malvidin-3-glucoside chloride/kg of berries, was determined by measuring absorbance at 540 nm after dilution of the sample with an ethanol:water: 37% hydrochloric acid (70:30:1, v/v) solution (Di Stefano & Cravero, 1991). Color intensity ($A_{420\ nm}+A_{520\ nm}+A_{620\ nm}$ on an optical path of 10 mm) and tonality ($A_{420\ nm}/A_{520\ nm}$) were calculated according to OIV (2016) methods after acquisition of the visible spectra of undiluted samples using 1 mm optical path cuvettes. The visible spectra were also used to obtain the CIEL*a*b* parameters at the end of maceration, namely lightness (L*), red/green colour coordinate (a*), yellow/blue colour coordinate (b*), hue angle (H*), and chroma (C*), calculated according to the OIV (2016) method. Furthermore, the ΔE* differential parameter was calculated according to the same method to compare, for each variety, each one of the tested tannin formulations with the respective control.

After 72 hours of maceration, anthocyanin polymeric forms and the anthocyanin profile were assessed. To investigate the occurrence of polymeric forms contributing to color, total polymeric pigments (TPP) were estimated according to bisulfite bleaching as described by Harbertson, Picciotto, & Adams (2003). Individual anthocyanin forms were quantified throughout a HPLC-DAD method (Río Segade et al., 2014) as follows: each skin maceration extract was diluted 1:1 with HCl solution at pH 0.5, filtered using 0.45 μm PTFE membrane filters, and injected (50 μL) in an Agilent 1260

HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a RP-18 column (5 μm, 25 × 0.4 cm; Merck, Darmstadt, Germany), and a diode array detector (DAD). Mobile phase was composed of formic acid/water (10:90, v/v) and formic acid/methanol/water (10:50:40, v/v). The gradient adopted started with 28% of solvent B, increased up to 45% of B in 15 min, to 70% in 20 min, and 90% in 10 min. Then, the column was cleaned with 99% of solvent B for 3 min, and re-equilibrated for 10 min at initial conditions before the next injection. Acquisition and processing were performed using the Agilent ChemStation software (Agilent Technologies, Santa Clara, CA, USA). Individual anthocyanins were quantified, and the results were expressed as mg of malvidin-3-glucoside chloride/kg of berries using an external standard calibration from peak area at 520 nm wavelength.

2.9 Statistical analysis

Data treatment was conducted using R statistic software. Hierarchical cluster analysis and heat map were done using R package 'pheatmap' from the anthocyanins data gathered in the literature (subsection 2.2). On investigated variables, the Analysis of Variance (ANOVA) using Tukey HSD post-hoc test was applied. Levene's and Shapiro-Wilk's tests were used for assessing the homogeneity of variance and normality of ANOVA residuals, respectively. When distribution did not respect homoscedasticity and normality assumption, ANOVA with Welch's correction and Games-Howell post-hoc, and non-parametric Kruskal-Wallis and Pairwise-Conover tests were conducted, respectively. A Principal Component Analysis (PCA) using R package 'factoextra' was performed on individual anthocyanin profile data after 72 h of maceration: individual glucosides, sum of acetylglucosides, and sum of *p*-coumaroylglucosides were used as variables. To compare varieties with different anthocyanins content, each value of treated samples (i.e. different exogenous tannins addition) was subtracted to the control samples and then normalized as *z*-scores.

3. Results and Discussion

3.1 Composition and extraction from untreated berries

Grape must composition at harvest based on technological parameters of unsorted and sorted berries is reported in **Table S2**. Sorted berries were used to conduct the experiment in order to reduce the variability due to heterogeneous ripening, therefore obtaining berries with similar characteristics in terms of phenolic compounds and extractability (Fournand et al., 2006; Río Segade, Giacosa, Gerbi, & Rolle, 2011). The study was performed on the berries of the most representative density class. Sugar contents ranged from 260 to 272 g/L, except for Merlot (239 g/L) and Nebbiolo (254 g/L). Nevertheless, acidity traits are variable as related to the variety, either in sorted or unsorted samples.

The sorted berries were then used to evaluate the grape anthocyanin potential. The results reported in **Figure 2A** confirm that the varieties identified by cluster analysis are different not only in terms of anthocyanin profile, but also in the total richness of these pigments. Montepulciano, belonging to G1 group, showed the highest TAI value with respect to the other varieties (1795 ± 27 mg/kg), followed by Syrah which represented G2 group (1317 ± 83 mg/kg), and Aglianico for G3 (1112 ± 74 mg/kg). Cabernet sauvignon and Merlot (both G1) reported 861 ± 32 mg/kg and 572 ± 23 mg/kg of TAI, respectively, highlighting a significant variability for the total amount among varieties even for similar anthocyanin profile. Sangiovese, which is characterized by the absence of acylated anthocyanins (G4), showed a TAI value of 602 ± 27 mg/kg at harvest, whereas peonidin-prevalent Nebbiolo (G5) had a significantly lower TAI value of 415 ± 26 mg/kg. These values were useful to estimate the extraction yield during the maceration phase (**Figure 2B**), without the impact of exogenous tannin addition. The extraction yield was quite different among varieties: notably, in G4 and G5 varieties (Sangiovese and Nebbiolo, respectively) the extraction peak was reached at 48 h, as these two varieties are characterized by high amounts of disubstituted anthocyanins. It is well known that these anthocyanin forms diffuse earlier in the must due to their conformation (González-Neves,

Gil, & Barreiro, 2008). On the contrary, a longer extraction was found for the G1, G2, and G3 varieties, and, in particular, Montepulciano anthocyanins diffusion had the slowest kinetic. Generally, anthocyanins are quickly diffused from the beginning of the fermentation, despite the low ethanol content due to their hydrophilicity, and mainly depending on their substitution. Nevertheless, some previous studies have underlined other relevant factors to be taken into account besides the anthocyanin substitution patterns. Among them, skin hardness and thickness, cell wall composition, and skin integrity have been recognized as parameters influencing anthocyanin extractability (Ortega-Regules, Romero-Cascales, Ros-García, López-Roca, & Gómez-Plaza, 2006; Río Segade et al., 2011; 2014; Hernández-Hierro et al., 2014).

In general terms, taking into account the total concentration in grape skins and the extracted anthocyanins after 72 h of simulated maceration, an extraction yield ranging from 55 to 73 % was found for the varieties studied (**Figure 2B**), meaning the importance of the first maceration steps in obtaining these colored compounds (Setford et al., 2017). **Figures 2C** and **2D** showed that extracted anthocyanins and color intensity of the macerating solutions are not always following the same trend due to several factors, including the variety anthocyanin characteristics, the extraction of other phenolic compounds, and the participation in several chemical reactions, that could influence these two parameters (Fernandes, Oliveira, Teixeira, Mateus, & De Freitas, 2017).

3.2 Maceration experiments in presence of oenological tannins

In the previous section, control simulated skin macerations of the different grape varieties in increasingly higher alcohol content showed that in the first 72 hours the extraction yield was up to the 73% of total anthocyanins, although differences in extraction kinetics and extracted anthocyanins content have been highlighted. Therefore, the preservation of these compounds in the first hours of maceration is of fundamental importance for the winemaking practice and, with this aim, the use of oenological tannins has been proposed due to their antioxidant and complexation features (Canuti,

Puccioni, Giovani, Salmi, Rosi, & Bertuccioli, 2012; Venturi, Andrich, Serni, Taglieri, & Sanmartin, 2015; Vignault et al., 2018). Color intensity, tonality, total extracted anthocyanins during the first maceration steps (6 and 72 h), as well as individual anthocyanins and polymeric pigments at 72 h were evaluated in wine-like solutions added with five tannin formulations differing in origin and characteristics (**Table S1**): oak (ELQ), quebracho (QBR), white and red winegrapes seeds (SEW and SER, respectively), and white winegrapes skins (SKW).

3.2.1 Color parameters

Color intensity (CI) takes into account the sum of different color fractions (absorbance at 420, 520, and 620 nm, corresponding to yellow-orange, red, and purple hue, respectively; OIV, 2016). This chromatic index considers the pigmented material content, the presence of phenolic compounds acting as copigments, and, among them, the added oenological tannins that can enhance color intensity depending on their constituents (Gombau et al., 2016; Vignault et al., 2019). On the other hand, tonality (T) refers to the relationship between yellow-orange and red hues, giving an indication on the fractions composing the overall color. The degree and nature of substitution on the B-ring of anthocyanins affects their polymerization abilities, and influences directly the color stability and hue of solutions (Heredia, Francia-Aricha, Rivas-Gonzalo, Vicario, & Santos-Buelga, 1998; Leydet et al., 2012; Fernandes et al., 2017). Indeed, a high tonality value can give an information on the stability of pigmented material, since some polymerized pigments own a lower absorption wavelength, shifting the tonality towards orange hues. Nevertheless, excessive unbalance towards orange-yellow hue can be given by undesirable phenolic compounds oxidation (Bradshaw, Prenzler, & Scollary, 2001).

In our experimental conditions, a general increase of CI and T values was observed when tannin formulations were added, however these differences were not always significant (p<0.05), and the selected varieties evidenced a different behaviour when a tannin formulation was added (**Table 1**).

Regarding G1 varieties, different effects were found among the three varieties chosen: in Merlot both CI and T values were increased after 6 h of maceration by SKW (+47.9%, $p < 0.01$; and +23.3%, $p < 0.001$; respectively) with respect to control, although no differences in TAI (**Table 2**) were found during macerations. This can lead to hypothesize a copigmentation effect together with an increase in polymerization because a significant increase of TPP with the addition of SKW was observed at 72 h (**Table 2**). The highest tonality for Merlot was found with SEW (+31.6%), followed by SKW, SER, and QBR tannins (+25.6%, 15.2% and 16.2%, respectively) after 6 h of maceration. Nevertheless, at 72 h no significant differences in CI and T values were found for Merlot with the tannin treatments. Instead, in Cabernet sauvignon macerations CI values increased at 72 h with respect to control when SKW and QBR tannins were present (+33.1% and +30.4%, respectively, $p < 0.05$), which agreed with TAI increases in these samples (**Table 2**). On the contrary, tannin addition during Montepulciano macerations did not lead to significant changes for these two parameters (CI and T), which can be explained by both the different extraction kinetics and the higher content of grape total anthocyanins with respect to Merlot and Cabernet sauvignon (**Figures 2B** and **2C**). Besides Montepulciano, in G1 varieties proanthocyanidin-type based tannin formulations, namely grape skins followed by quebracho, had a significant effect on color, even if in Merlot this was hided at 72 h, probably due to the lower amount of newly-extracted anthocyanins during maceration. CIEL*a*b* data at 72 h (**Table S3**) confirmed for Merlot and Cabernet sauvignon a displacement towards yellow color components for QBR, SEW, and SKW formulations, which own higher values of b* coordinate and hue angle (H*), even if in different extent depending on the formulation and the variety. The detection by the human eye of color differences through a glass requires a ΔE* threshold higher than 3 units (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001) and therefore the use of these three tannin formulations could lead to a perceived increase of wine color when compared to control (ΔE* = 5.06–10.52). However, in Montepulciano no significant differences ($p>0.05$) were found in CIEL*a*b* coordinates, and ΔE* values of tannin-added solutions *vs* control were found below 3 units.

The shift towards orange hues caused by grape tannins addition was reported also in G2 and G3 varieties. In particular, SEW formulation led to a significant increase in tonality for Syrah after 72 h (G2, +9.2%, $p<0.01$) and for Aglianico (G3) either after 6 or 72 h (+20.7%, $p<0.001$; and +5.3%, $p<0.05$; respectively), even though no differences in polymerization percentage (TPP) occurred with respect to control (**Table 2**). Previous studies on Syrah reported variable results. Seed tannin addition led to lower tonality values compared to tannins from other origins (doses of 4 and 8 g/hL) as well as to the control (Chen et al., 2016). Nevertheless, in agreement with the results of the present study, Syrah vinifications performed using 20 g/hL of grape seed tannins were previously found to not give any significant difference in color (Parker et al., 2007). Although the botanical origin of these last formulations was the same (grape seeds-derived tannins), the phenolic composition and content could be different depending on the formulation, which may affect the effectiveness.

In Aglianico, SER addition also caused a significant increase in tonality with respect to the control after 6 and 72 h of maceration (+9.8, $p<0.001$ and +5.1%, $p<0.05$, respectively), confirming the influence of seeds proanthocyanidins formulations on the shift of the absorption wavelength towards lower values. In general, Aglianico tonality seems to be sharply influenced by tannin addition, since after 6 h all the tannins formulations used, with the exception of ELQ tannin, resulted in significantly higher tonality values, therefore leading to increased orange hue (from +9.8% up to +22.0%, $p<0.001$). This increasing trend was found also after 72 h of maceration for all tannins, except for ELQ, although the differences were significant only for the seed tannins tested (SER and SEW). These values are in agreement also with TPP values after 72 h (**Table 2**), with the exception of ELQ, which increased TPP and not T, and SEW, which did not affect polymerization but shifted towards lower wavelength. In the first case, although ellagitannins favour polymerization (Vivas & Glories, 1996), this increase could be counter parted by newly-anthocyanin extracted. On the other hand, using grape formulations (SER, SEW, and SKW) higher tonality values are not always in line with a polymerization increase: a possible explanation could be given by the oxidation of proanthocyanidins, in particular the monomeric constituents produce yellow products (Vivas & Glories, 1996; Bradshaw

et al., 2001), as it could have occurred in the case of SEW seed tannin formulation for Aglianico. Finally, hue angle (H*) and b* color component values were significantly higher only for SKW addition in Aglianico (p<0.05; **Table S3**), and in this variety the ΔE* differences achieved by the tannin-added samples were in most cases over the threshold of 3 units.

In Sangiovese simulated macerations (G4), significant differences were found after 6 h for both CI and T values, in particular SKW increased both these values with respect to control (+30.2% and +9.3%, for CI and T respectively; both p<0.01), whereas SEW and QBR increased tonality values (+7.0%, and +4.7%, respectively). These differences were reduced for longer maceration time, possibly given by the higher amount of the extracted phenolic compounds, being it favoured by ethanol. Nevertheless, color differences can be visually perceived *vs* control according to the ΔE* values (higher than 3 units for all tannins; **Table S3**). QBR and SKW-added macerations also evidenced an increased b* color component after 72 h with respect to control, and the former tannin addition influenced also the CIEL*a*b* hue angle (p<0.01; **Table S3**). Previous results on Sangiovese showed that tannins from different origin positively influenced color parameters after 6 months of bottle storages, and in particular the authors pointed out how the maceration steps are crucial to extract and stabilize Sangiovese pigments, with grape derived tannins positively influencing the color of Sangiovese wines (Canuti et al., 2012). Venturi et al. (2015) found, as well, a positive effect of ellagic tannins in pure formulations or mixed with quebracho tannins when added in the first days of Sangiovese maceration. Specifically, higher total anthocyanins contents and in particular polymerized forms were reported. Although in our case oak tannins (ELQ) did not enhance significantly color parameters, an increasing effect was found in the first hours of maceration for quebracho (QBR) formulation. A possible explanation of the remarkable increase of yellow hue, as tonality or b* coordinate, observed in samples added with grape-derived tannins in most of the previously described varieties, may be anthocyanin-flavanol direct condensation. Colorless, yellow, or red colored derivatives have been shown to be formed as intermediate in the polymeric pigment formation (Es-Safi, Cheynier, & Moutounet, 2003). These compounds may be involved in protecting anthocyanins

from degradation during the first stages of maceration through the formation of stable pigments, which can justify the improved color previously reported in wines added with exogenous tannins (Canuti et al., 2012; Chen et al., 2016). Nevertheless, the extent of the tannin formulation effect depended also on its compositional features such as the tannin richness, polymers size and structure, as well as subunits composition.

In Nebbiolo (G5), tannins formulation addition resulted in all cases in a non-significant (p>0.05) increasing trend, either for CI or T at 6 and 72 h. Regarding CIEL*a*b* parameters at 72 h (**Table S3**), not significant trends for L* (decrease) and a* (increase) were observed for all tannin additions with respect to control. On the other hand, QBR, SEW, and SKW showed higher b* and H* values (p<0.001), and for QBR formulation also a higher C* value was found (p<0.05). Indeed, according to the ΔE* values, color differences can be visually perceived using these three tannin formulations *vs* control. The behavior found for the color characteristics on Nebbiolo can be related to the peculiar anthocyanin profile of this variety, which is mainly composed by cyanidin and peonidin derivatives (>50 %). These anthocyanin forms are known to be first extracted in skins maceration and more prone to oxidation phenomena (Sarni, Fulcrand, Souillol, Souquet, & Cheynier, 1995; González-Neves et al., 2008).

3.2.2 Total anthocyanins and polymeric pigments

Table 2 reports total anthocyanin contents (TAI) of the different macerating solutions added with tannin formulations *vs* control, after 6 and 72 h of maceration, as well as the contribution of total polymeric pigments (TPP) at the last point in order to establish if the use of tannins in the beginning of maceration can enhance the anthocyanin-flavanol adducts formation. As previously mentioned for the color intensity and tonality parameters, the addition of the selected exogenous tannins in the macerating media caused a general increasing trend of TAI values after 72 h, but the differences were not significant in most cases (p>0.05). Instead, TPP percentages for tannins addition *vs* control

macerations showed significant differences (p<0.05) for five varieties out of the seven tested in the experiment, as described below.

Different results were found in G1 varieties when tannin formulations were added. In accordance with color parameters, in Montepulciano no significant differences were detected neither in extracted anthocyanins nor in polymerized fractions. In Merlot, QBR and SKW tannins increased the TPP fractions (+6.0% and +6.5%, respectively, p<0.05), while the increase in TAI content after 72 h was not significant (+11.0% and +12.7%, respectively, p>0.05). Instead, significantly higher TAI content was found in Cabernet sauvignon treated with QBR (+15.7%, p<0.05), even if the polymerized fraction was not modified with this tannin formulation. However, TPP percentage was significantly increased by SKW tannin (+3.2%, p<0.01). Therefore, for the varieties belonging to group G1 higher values of TAI and TPP were usually obtained by adding both proanthocyanidins from grape skins and quebracho, thus enhancing both anthocyanins concentration and their stability, even though in different extent depending on the tannin formulation and variety combination.

Syrah (G2) macerations after 72 h evidenced an increased polymerization percentage with the use of QBR and ELQ tannins (+4.4% and +5.0%, respectively, p<0.01) with respect to the control. Aglianico (G3) showed the greatest influence of the tannin addition on TPP value because this parameter was positively influenced for all tannin formulations (+2.6-3.1%, p<0.001), except for SEW. After 72 h of maceration, a significantly higher polymerization combined with a non-significantly higher TAI content was evidenced for all tannin formulations except SEW.

The two varieties belonging to G4 and G5, namely Sangiovese and Nebbiolo, did not show significant differences in terms of TAI content across samples. However, the highest TAI values after 72 h of maceration were found for QBR and SKW samples. Regarding TPP, while Sangiovese (G4) showed limited and non-significant variations with the exogenous tannin addition, in Nebbiolo (G5) TPP was interested by a particular behavior. First of all, the standard deviation found in control macerations (17.0 ± 5.0%) was quite high and evidences a high maceration variability, then TPP increased with

respect to the control only when SER tannins were used (+8.2%, $p<0.05$). Several authors pointed out that polymeric pigments are formed from the beginning of maceration, becoming responsible for 60% of young wine color (Versari et al., 2008), and the improved polymerization reactions given by tannin addition are mainly due to two different mechanisms. On the one hand, flavan-3-ols extraction from grapes skins requires longer time than that of anthocyanins, since they are mostly entangled in the cell wall of grape skins. In addition, the flavan-3-ols extraction from the seeds requires a higher amount of alcohol to be released from the seed coat (Rousserie et al., 2019). The lack of endogenous flavan-3-ols at the early stages of anthocyanin release can be compensated by a direct addition of exogenous grape-derived tannins from the beginning of maceration, which would speed up the polymerization reactions through the availability of the reaction substrate. On the other hand, tannin formulations own antioxidant capacity depending on the total phenolic content and the phenolic composition, which are related to the tannin origin and the formulation purity (Magalhaes et al., 2014; Pascual et al., 2017; Vignault et al., 2018). The antioxidant capacity varies with the tannin type: in particular, ellagitannins have the highest capability for direct oxygen consumption, showing up to three-fold faster consumption than quebracho tannins and then followed by other tannins from different origins, due to the richness in hydroxyl exchanging groups (Pascual et al., 2017). These antioxidant properties can preserve anthocyanins against oxidation, even if, in the case of grape derived flavan-3-ols, their oxidized forms and polymerized products are reported to be colorless or yellow colored, and may lead to lower hue values (Vivas & Glories, 1996; Bradshaw et al., 2001).

3.2.3 Individual anthocyanin forms

After 72 h of maceration, individual anthocyanins forms were investigated to explore the effects of tannins addition in relation with the profile features. The contents of extracted individual anthocyanins (expressed as mg of malvidin-3-O-glucoside chloride/kg of berries) are reported in **Table 3**. Regarding G1 varieties, in Montepulciano significantly higher cyanidin-3-glucoside

contents were found with respect to the control when QBR was used (+23.1%, p<0.05). In Cabernet sauvignon, SER formulation reported higher delphinidin-3-glucoside contents when compared to the control samples (+35.4%, p<0.05). In contrast, no differences were found in unacylated glucoside anthocyanins in Merlot (G1), Syrah (G2), and Aglianico (G3) simulated macerations with exogenous tannins.

Sangiovese (G4) and Nebbiolo (G5) own a different ratio of disubstituted/trisubstituted anthocyanins, where disubstituted relative abundance (i.e. cyanidin and peonidin derivatives sums higher than 30% and 50%, respectively; Mattivi et al., 2006) is higher with respect to G1, G2, and G3 varieties. In these conditions, the tannin addition effects were remarkable. In general, the use of tannins in processing varieties with a high content of cyanidin and peonidin derivatives (G4 and G5 groups) helped in preserving these forms, leading to a more incisive action than that revealed analyzing total anthocyanins and color parameters (**Tables 1 and 2**). In particular, QBR showed an effective ability in preserving glucoside forms of delphinidin, cyanidin, and peonidin, giving significantly higher results (+26.7-84.4%, p<0.01) with respect to the control for both Sangiovese and Nebbiolo varieties, and for the latter also for petunidin (+31.2%, p<0.05; **Table 3**). Furthermore, significantly higher contents for Nebbiolo delphinidin and cyanidin forms were also evidenced when the tested SKW and ELQ tannin formulations were present in the macerating media, with respect to control. In our experimental condition, the tannin addition in these two varieties leaded to the greatest increases of unacylated cyanidin (from +37.5% to +84.4%).

Disubstituted anthocyanins are easily extracted in the juice/buffer solution thanks to the weak interaction occurring with the cell wall material (Fernandes et al., 2017) but, on the other hand, *o*-diphenols such as cyanidin, petunidin, and delphinidin are more susceptible to chemical oxidation, owning two and, the latter, three hydroxyl groups in the B-ring that lead to higher sensitivity to oxidant compounds (Sarni et al., 1995). In grape juice, this oxidation phenomenon is enhanced by the activity of oxidative enzymes and by the higher content of substrates –mainly hydroxycinnamic

acids– present in grape pulps that are susceptible to generate quinones (Cheynier, Souquet, Kontek, & Moutounet, 1994). Therefore, as a consequence of these effects of conformational susceptibility and enzymatic activity, the oxygen present in the medium before alcoholic fermentation negatively contributes to the preservation of easily-extracted anthocyanins. By contrast, although malvidin remains susceptible to oxidation phenomena, from a conformational point of view it is considered the most stable anthocyanidin, as the B-ring methoxylated groups protect the hydroxyl ones (Cheynier et al., 1994). Furthermore, also due to this conformation, malvidin is the most difficult form to be diffused in the medium (González-Neves et al., 2008), since stronger interactions are presumably formed between skin material and its functional groups.

The protective impact of exogenous tannins in the first phases of simulated maceration, found in our experimental conditions, could be a useful knowledge to be adapted for real winemaking conditions when both enzymatic and chemical oxidation occur competing with grape polyphenols. In fact, even in high-malvidin content varieties, such as Montepulciano and Cabernet sauvignon, a protective trend of the tested QBR tannin emerged from data for cyanidin-3-glucoside or delphinidin-3-glucoside, respectively. On the other hand, malvidin-3-glucoside content was not affected regardless of the variety, probably due to the limited maceration time (72 h) and to the fact that it is slower extracted and slightly less sensitive to oxidation.

Grape variety features are strictly connected with the tannin addition efficacy in skins simulated maceration conditions. Even if a general antioxidant capacity for QBR formulation in the tested conditions was evident for all varieties, those rich in disubstituted anthocyanins showed a higher tannin effect. Quebracho tannins have been found to have generally higher both antioxidant capacity and oxygen consumption rate with respect to other condensed tannins like grape-derived proanthocyanidins (Pascual et al., 2017; Vignault et al., 2018). This characteristic was also observed in the exogenous tannin formulations tested in the present study (**Table S1**). In contrast, the higher efficiency in protection against oxidation and for polymeric pigments formation has been previously

reported for ellagitannins (Vivas & Glories, 1996; Pascual et al., 2017; Vignault et al., 2018). However, in a model solution (12% v/v ethanol) added with pure anthocyanins (malvidin) and ellagitannins, this capability showed a side effect, resulting in a faster decrease of the pigment (Jordão, Ricardo-Da-Silva, Laureano, Mullen, & Crozier, 2008).

Considering the variability of tannin effect found in individual anthocyanin content depending on the formulation used, and in particular some general trends among the varieties, a PCA (**Figure 3**) was performed to understand if tannin formulations may lead to the same effect on individual anthocyanins despite the variety features. In order to minimize the variety effect, differences with respect to control sample were considered. Component 1 (Comp1) accounted for the 43.4% of the explained variance (**Figure 3A**), whereas component 2 (Comp2) explained the 28.8%, leading to 72.2% of total explained variance in the first two components. Comp1 was positively correlated with all the variables, and well explained by *o*-diphenol anthocyanins, petunidin-3-glucoside, delphinidin-3-glucoside, and cyanidin-3-glucoside (correlation coefficient of 0.956, 0.930, and 0.676, respectively, $p<0.001$), followed by sum of *p*-coumaroyl-derivatives and malvidin-3-glucoside (0.615 and 0.609, respectively, both $p<0.001$). Regarding Comp2, positive correlation with peonidin-3-glucoside and acetylated derivatives was found (0.956 and 0.930, $p<0.001$), followed by cyanidin-3-glucoside (0.516, $p<0.01$); whereas it was negatively correlated with *p*-coumaroyl-derivatives (–0.540, $p<0.001$). When looking at the scores map (**Figure 3B**), even if no clear differences among the different tannin-added samples were found, some trends can be highlighted. In the left quadrants, samples with lower amounts of petunidin-3-glucoside and delphinidin-3 glucoside are found, and the most of SER treated samples seems to be characterized by this peculiarity. On the contrary, samples located in the top-right quadrant are characterized by higher contents of peonidin-3-glucoside and cyanidin-3-glucoside, together with acetylated derivatives: Nebbiolo, Sangiovese, and Montepulciano QBR samples are situated in this area, indicating the trend of this tested exogenous tannin to protect the disubstituted anthocyanin forms in the first phase of maceration, in agreement with the results previously reported in the present study for some of the analyzed varieties (**Table 3**).

4. Conclusions

The study examined the influence of the exogenous tannin addition on the first phases of grape skin simulated maceration. The selected tannin formulations from different botanical origin were characterized prior to the experiment and used according to the general winemaking practice. Tannin formulations had an impact on the quantity and quality of pigments found in the macerating media after 72-hour simulated maceration. As general observations, in G1 varieties (Merlot, Cabernet sauvignon, and Montepulciano type), skin-derived proanthocyanidin formulations modified color parameters and increased polymeric pigments concentration with respect to the control, but the effect varied with the cultivar according to the varietal anthocyanin content. Seed-derived tannins, as well, seemed to increase the pigments polymerization in the first step of maceration in Aglianico (G3) and Nebbiolo (G5), although increased tonality value has been found in some cases, such as Syrah (G2), Aglianico, and Sangiovese (G4). Ellagic formulation increased the polymeric pigments in Syrah (G2) and Aglianico (G3), characterized by high quantities of malvidin derivatives. Quebracho formulation was effective for Merlot and Cabernet sauvignon, Syrah, and Aglianico, in different extent, in reaching higher anthocyanin content and polymerization. An increase of color intensity was also found for Cabernet sauvignon and Aglianico.

An investigation of anthocyanins at an individual level showed the decisive effect of some tested formulations (QBR in particular) on the defense of di-substituted anthocyanin forms, particularly for Sangiovese and Nebbiolo where the relative richness of these compounds is high.

In our experimental conditions, the effectiveness of exogenous tannin formulations addition is related to the grape anthocyanin profile, highlighting a greater impact on certain anthocyanin forms. The protection of *o*-diphenol anthocyanins and peonidin was particularly relevant in varieties characterized by a high ratio of cyanidin- and peonidin-3-glucosides (G4 and G5). These findings may help in better tailoring the tannin addition to the variety.

Further studies may be conducted in winemaking conditions in order to advance these findings in a more complex matrix. In fact, the presence of grape seeds and pulps and their derived compounds may lead to further modifications in terms of copigmentation and polymerization reactions. Indeed, it is well-known that yeast-derived metabolites, such as pyruvic acid, acetaldehyde, and several minor compounds, contribute to the red color stabilization in real winemaking conditions through the formation of pyranoanthocyanins and flavanol-anthocyanins ethyl-linked adducts.

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Figure captions

Figure 1. Cluster analysis and heat map of the percentage contribution of each anthocyanin form to the berry skin anthocyanin content of the selected varieties. Elaboration conducted on anthocyanin data gathered from: [a] Mattivi et al. (2006); [b] Vasile Simone et al. (2013); [c] Mateus et al. (2002); [d] Muñoz et al. (2014); [e] Cabrita et al. (2003).

Figure 2. Grape skin total anthocyanin index (TAI) of berries sorted by flotation, expressed in mg/kg berries as malvidin-3-O-glucoside chloride from total extraction (A), extraction yield (%) obtained from the total grape content (B), extracted anthocyanin content expressed in mg/kg berries as malvidin-3-O-glucoside chloride (C), and color intensity (D) of the wine-like solution during grape skin simulated maceration for72 h with no exogenous tannin addition (control). Data are expressed as average value ± standard deviation (n = 3). Different letters in Figure 2A indicate significant differences according to the Tukey HSD test (p<0.05).

Figure 3. Principal component analysis (PCA) of the individual anthocyanin forms after 72 h of maceration: loadings map (A) and scores map (B). “Contrib”: contribution of the single variables to the principal component. The central point of each ellipse represents the mean of the group. Ellipses represent the Euclidean distance from the central points of the group, with equal radius for all groups. DelphG=delphinidin-3-glucoside, CyaG=cyanidin-3-glucoside, PetG=petunidin-3-glucoside, PeoG=peonidin-3-glucoside, MalvG=malvidin-3-glucoside, AcetylG=sum of acetylglucosides, CouG=sum of *p*-coumaroylglucosides. Mo=Montepulciano, Me=Merlot, Cs=Cabernet sauvignon, Sy=Syrah, Ag=Aglianico, Sa=Sangiovese, Ne=Nebbiolo.

Table 1. Color intensity (CI, 10 mm optical path) and color tonality (T) of the macerating solutions after 6 and 72 h of maceration.

Grape variety	Parameter	Time	Control	ELQ	QBR	SER	SEW	SKW	Sign.
Montepulciano	CI (A.U.)	6 h	2.76 ± 0.50	2.63 ± 0.28	3.13 ± 0.61	3.01 ± 0.36	2.99 ± 0.54	3.03 ± 0.58	ns
		72 h	13.08 ± 0.24	13.31 ± 0.78	14.00 ± 0.26	13.55 ± 0.97	12.54 ± 0.70	12.95 ± 0.60	ns
	T	6 h	0.41 ± 0.01	0.42 ± 0.01	0.43 ± 0.03	0.42 ± 0.01	0.45 ± 0.04	0.44 ± 0.02	ns
		72 h	0.36 ± 0.00	0.36 ± 0.00	0.37 ± 0.00	0.36 ± 0.00	0.37 ± 0.01	0.37 ± 0.00	ns
Merlot	CI (A.U.)	6 h	1.44 ± 0.20 b	1.50 ± 0.41 b	1.99 ± 0.14 ab	1.80 ± 0.23 ab	2.04 ± 0.10 ab	2.13 ± 0.10 a	**
		72 h	2.89 ± 0.23	3.63 ± 0.43	3.62 ± 0.05	3.26 ± 0.43	3.56 ± 0.29	3.73 ± 0.34	ns
	T	6 h	0.43 ± 0.01 c	0.46 ± 0.01 c	0.51 ± 0.01 b	0.50 ± 0.01 b	0.57 ± 0.01 a	0.54 ± 0.01 b	***
		72 h	0.49 ± 0.03	0.50 ± 0.01	0.52 ± 0.01	0.52 ± 0.02	0.54 ± 0.02	0.53 ± 0.00	ns
Cabernet sauvignon	CI (A.U.)	6 h	1.31 ± 0.25	0.98 ± 0.55	1.65 ± 0.22	1.25 ± 0.20	1.37 ± 0.17	1.41 ± 0.27	ns
		72 h	2.97 ± 0.20 b	3.04 ± 0.29 b	3.88 ± 0.66 a	3.63 ± 0.48 ab	3.25 ± 0.25 ab	3.96 ± 0.41 a	*
	T	6 h	0.46 ± 0.04	0.52 ± 0.07	0.51 ± 0.04	0.51 ± 0.04	0.54 ± 0.06	0.51 ± 0.05	ns
		72 h	0.46 ± 0.01	0.46 ± 0.01	0.48 ± 0.01	0.47 ± 0.01	0.49 ± 0.03	0.49 ± 0.01	ns
Syrah	CI (A.U.)	6 h	2.41 ± 0.34	2.31 ± 0.21	2.41± 0.22	2.27 ± 0.11	2.20 ± 0.21	2.18 ± 0.46	ns
		72 h	6.79 ± 0.15	6.87 ± 0.29	7.25 ± 0.47	7.61 ± 0.58	6.99 ± 0.27	7.15 ± 0.24	ns
	T	6 h	0.43 ± 0.02	0.47 ± 0.03	0.47 ± 0.01	0.46 ± 0.01	0.43 ± 0.01	0.43 ± 0.02	ns
		72 h	0.40 ± 0.01 b	0.41 ± 0.02 ab	0.40 ± 0.00 b	0.42 ± 0.01 ab	0.43 ± 0.01 a	0.43 ± 0.01 ab	**#
Aglianico	CI (A.U.)	6 h	1.56 ± 0.25 b	1.67 ± 0.13 ab	1.98 ± 0.11 a	1.60 ± 0.11 ab	1.85 ± 0.17 ab	1.86 ± 0.16 ab	*
		72 h	5.31 ± 0.48	5.65 ± 0.34	5.83 ± 0.22	5.54 ± 0.33	5.39 ± 0.22	5.86 ± 0.22	ns
	T	6 h	0.41 ± 0.01 c	0.43 ± 0.01 c	0.47 ± 0.01 b	0.45 ± 0.00 b	0.50 ± 0.00 a	0.47 ± 0.00 b	***
		72 h	0.39 ± 0.01 b	0.39 ± 0.01 ab	0.41 ± 0.01 ab	0.41 ± 0.01 a	0.41 ± 0.00 a	0.40 ± 0.00 ab	*
Sangiovese	CI (A.U.)	6 h	2.42 ±0.17 b	2.50 ±0.19 b	2.96 ±0.39 ab	2.52 ±0.19 ab	2.99 ±0.27 ab	3.15 ±0.16 a	**
		72 h	5.39 ± 0.26	5.22 ± 0.28	5.85 ± 0.62	5.05 ± 0.33	5.58 ± 0.60	5.56 ± 0.27	ns
	T	6 h	0.43 ±0.01 b	0.42 ±0.01 b	0.45 ±0.00 a	0.42 ±0.01 b	0.46 ±0.01 a	0.47 ±0.00 a	**
		72 h	0.47 ± 0.01	0.47 ± 0.01	0.47 ± 0.01	0.48 ± 0.00	0.49 ± 0.01	0.48 ± 0.01	ns
Nebbiolo	CI (A.U.)	6 h	0.81 ± 0.03	0.99 ± 0.06	1.01 ± 0.08	0.97 ± 0.10	1.00 ± 0.21	1.13 ± 0.09	ns
		72 h	1.34 ± 0.10	1.53 ± 0.21	1.69 ± 0.15	1.37 ± 0.04	1.55 ± 0.20	1.63 ± 0.13	ns
	T	6 h	0.49 ± 0.04	0.53 ± 0.06	0.55 ± 0.03	0.53 ± 0.02	0.54 ± 0.05	0.54 ± 0.03	ns

	72 h	0.48 ± 0.02	0.52 ± 0.04	0.53 ± 0.03	0.52 ± 0.01	0.55 ± 0.02	0.50 ± 0.01	ns

Data are expressed as average value ± standard deviation ($n = 3$). Sign.: *, **, ***, and "ns" indicate significant differences at $p < 0.05$, 0.01, 0.001, and not significant, respectively, among values within the same row according to ANOVA or #Welch's ANOVA. Different Latin letters within the same row indicate significant differences among treatments according to Tukey HSD or Games-Howell tests ($p < 0.05$) for ANOVA and Welch's ANOVA, respectively.

Table 2. Total anthocyanins index (TAI) and total polymeric pigments (TPP) found in the macerating media after 6 and 72 h of maceration.

Grape variety	Parameter	Time	Control	ELQ	QBR	SER	SEW	SKW	Sign
Montepulciano	TAI (mg/kg)	6 h	229 ± 28	218 ± 25	244 ± 43	259 ± 29	234 ± 44	242 ± 34	ns
	TAI (mg/kg)	72 h	991 ± 46	981 ± 72	1021 ± 37	984 ± 11	931 ± 53	918 ± 37	ns
	TPP (%)	72 h	10.8 ± 0.8	11.6 ± 0.8	10.9 ± 0.6	9.5 ± 0.5	10.3 ± 1.9	9.1 ± 1.9	ns
Merlot	TAI (mg/kg)	6 h	206 ± 21	192 ± 45	215 ± 11	216 ± 7	219 ± 13	235 ± 9	ns[#]
	TAI (mg/kg)	72 h	418 ± 27	451 ± 28	464 ± 8	421 ± 53	437 ± 21	471 ± 25	ns
	TPP (%)	72 h	15.1 ± 1.3 b	18.8 ± 0.4 ab	21.1 ± 1.2 a	18.0 ± 2.1 ab	20.2 ± 2.0 ab	21.6 ± 3.8 a	*
Cabernet sauvignon	TAI (mg/kg)	6 h	222 ± 32	157 ± 85	233 ± 29	196 ± 27	210 ± 32	214 ± 36	ns
	TAI (mg/kg)	72 h	547 ± 18 b	546 ± 27 b	633 ± 59 a	557 ± 26 ab	548 ± 6 b	602 ± 15 ab	*[#]
	TPP (%)	72 h	20.5 ± 0.2 b	20.2 ± 0.2 b	21.9 ± 0.8 ab	22.0 ± 2.1 ab	22.4 ± 0.4 ab	23.7 ± 1.1 a	**
Syrah	TAI (mg/kg)	6 h	319 ± 45	279 ± 13	276 ± 19	282 ± 14	286 ± 18	278 ± 37	ns
	TAI (mg/kg)	72 h	786 ± 16	782 ± 15	777 ± 12	807 ± 21	788 ± 53	785 ± 32	ns[#]
	TPP (%)	72 h	13.3 ± 0.3 b	18.3 ± 0.7 a	17.7 ± 0.9 a	18.4 ± 1.6 ab	13.3 ± 0.8 b	14.8 ± 3.4 ab	**[#]
Aglianico	TAI (mg/kg)	6 h	225 ± 24	227 ± 16	227 ± 10	213 ± 11	233 ± 15	242 ± 13	ns
	TAI (mg/kg)	72 h	687 ± 44	730 ± 33	731 ± 7	686 ± 5	700 ± 26	735 ± 40	ns
	TPP (%)	72 h	17.3 ± 0.6 b	20.3 ± 1.1 a	20.0 ± 0.7 a	20.4 ± 0.6 a	17.2 ± 0.3 b	19.9 ± 0.5 a	***[#]
Sangiovese	TAI (mg/kg)	6 h	226 ± 14	226 ± 13	243 ± 25	229 ± 14	245 ± 8	248 ± 8	ns
	TAI (mg/kg)	72 h	389 ± 21	390 ± 19	412 ± 33	378 ± 25	404 ± 14	406 ± 16	ns
	TPP (%)	72 h	22.3 ± 2.8	24.9 ± 1.0	23.3 ± 2.1	24.7 ± 3.0	25.0 ± 1.4	23.8 ± 1.2	ns
Nebbiolo	TAI (mg/kg)	6 h	142 ± 10	169 ± 14	152 ± 11	163 ± 14	156 ± 25	179 ± 18	ns
	TAI (mg/kg)	72 h	258 ± 16	274 ± 23	289 ± 23	252 ± 24	269 ± 28	278 ± 22	ns
	TPP (%)	72 h	17.0 ± 5.0 b	21.1 ± 0.7 ab	18.5 ± 0.6 ab	25.2 ± 3.6 a	22.1 ± 0.1 ab	23.0 ± 0.9 ab	*[##]

Data are expressed as average value ± standard deviation (n = 3) in mg of malvidin-3-O-glucoside chloride/kg of berries for TAI parameter, and in percentage for TPP parameter. Sign.: *, **, ***, and "ns" indicate significant differences at $p < 0.05$, 0.01, 0.001, and not significant, respectively, among values within the same row according to ANOVA, [#]Welch's ANOVA, or [##]Kruskal-Wallis. Different Latin letters within the same row indicate significant differences among treatments according to Tukey HSD, Games-Howell, or Conover's tests ($p < 0.05$) for ANOVA, Welch's ANOVA, and Kruskal-Wallis, respectively.

Table 3. Individual anthocyanins content in the macerating media at the end of the monitored period (72 h).

Grape variety	Parameter	Control	ELQ	QBR	SER	SEW	SKW	Sign.
Montepulciano	Delph	105.0 ± 23.1	116.9 ± 14.2	142.2 ± 6.4	114.4 ± 18.3	113.5 ± 9.6	122.7 ± 5.9	ns
	Cya	28.1 ± 3.1 b	30.3 ± 1.9 ab	34.6 ± 1.3 a	25.8 ± 3.4 b	30.4 ± 1.5 ab	31.0 ± 1.8 ab	*
	Pet	130.5 ± 24.1	145.5 ± 14.8	170.1 ± 6.2	141.7 ± 19.8	141.1 ± 11.2	152.9 ± 6.5	ns
	Peo	85.0 ± 4.8	92.8 ± 10.1	98.8 ± 6.4	76.5 ± 8.2	92.1 ± 7.2	94.3 ± 9.2	ns
	Malv	567.2 ± 61.9	641.2 ± 45.8	684.2 ± 20.3	618.8 ± 61.9	602.2 ± 36.6	652.2 ± 18.5	ns
	Acetyl	143.4 ± 13.1 abc	160.1 ± 12.2 a	157.2 ± 12.4 ab	132.3 ± 16.0 abc	119.8 ± 6.2 c	128.3 ± 5.2 bc	**
	p-Coumaroyl	133.9 ± 16.2	157.3 ± 11.1	172.3 ± 14.1	139.6 ± 23.7	141.3 ± 9.6	158.1 ± 8.1	ns
Merlot	Delph	31.2 ± 5.1	37.4 ± 2.3	42.4 ± 3.8	35.7 ± 8.5	33.7 ± 5.7	38.5 ± 5.2	ns
	Cya	13.6 ± 4.4	15.8 ± 0.1	17.5 ± 1.0	12.5 ± 2.8	14.7 ± 4.5	15.2 ± 3.2	ns[#]
	Pet	35.9 ± 4.3	40.5 ± 2.8	43.6 ± 3.4	39.7 ± 7.4	35.9 ± 4.5	39.7 ± 3.6	ns
	Peo	61.4 ±13.3	65.3 ± 2.8	73.8 ± 1.4	58.5 ± 8.5	62.0 ± 10.9	63.3 ± 8.1	ns
	Malv	256.9 ± 13.4 a	268.3 ± 18.5 a	274.2 ± 8.6 a	275.7 ± 22.5 ab	237.9 ± 24.9 c	252.5 ± 8.0 bc	ns
	Acetyl	73 .0 ± 2.1 a	75.3 ± 6.9 a	68.1 ± 3.0 a	62.2 ± 4.1 ab	48.1 ± 7.7 c	50.9 ± 2.5 bc	***
	p-Coumaroyl	57.0 ±0.3 b	65.5 ± 3.6 ab	72.6 ± 5.4 a	62.7 ± 5.3 ab	57.3 ± 12.2 b	62.7 ± 3.0 ab	*[#]
Cabernet sauvignon	Delph	18.9 ± 1.5 b	23.9 ±7.4 ab	36.9 ± 10.0 ab	25.6 ± 1.7 a	28.6 ± 6.6 ab	36.5 ± 9.4 ab	*[#]
	Cya	3.2 ± 1.3	4.2 ± 1.3	5.9 ± 1.7	4.4 ± 0.4	6.4 ± 4.4	5.2 ± 0.4	ns[#]
	Pet	23.0 ± 2.5	26.4 ± 5.9	34.9 ± 6.6	26.9 ± 0.7	28.3 ± 3.7	35.2 ± 8.8	ns[#]
	Peo	24.4 ± 2.0	24.9 ± 4.0	29.6 ± 4.4	25.5 ± 1.0	25.9 ± 4.6	29.5 ± 4.3	ns
	Malv	286.0 ± 27.1	304.9 ± 40.3	342.7 ± 39.3	305.5 ± 16.0	326.1 ± 15.8	363.8 ± 52.8	ns
	Acetyl	136.5 ± 6.0 ab	141.9 ± 20.0 ab	139.4 ± 6.0 a	114.8 ± 5.3 b	114.1 ± 8.4 ab	126.8 ± 15.2 ab	*[#]
	p-Coumaroyl	23.7 ± 4.1	25.3 ± 8.4	37.5 ± 4.3	27.3 ± 3.3	30.2 ± 7.5	39.9 ± 8.8	ns
Syrah	Delph	25.0 ± 0.6	25.4 ± 2.5	28.4 ± 5.9	26.9 ± 4.0	29.1 ± 5.7	29.2 ± 0.5	ns
	Cya	5.0 ± 0.2	5.0 ± 0.6	6.1 ± 0.6	4.9 ± 0.8	5.5 ± 0.6	5.8 ± 1.1	ns
	Pet	41.1 ± 0.7	40.0 ± 3.4	42.7 ± 9.1	42.4 ± 4.7	44.2 ± 5.8	44.9 ± 1.7	ns
	Peo	59.7 ± 3.3	58.2 ± 4.6	67.3 ± 7.4	58.5 ± 7.2	63.0 ± 3.9	65.1 ± 7.6	ns
	Malv	383.3 ± 38.4	353.3 ± 24.3	363.3 ± 49.8	383.5 ± 40.8	377.9 ± 24.5	375.0 ± 18.9	ns
	Acetyl	127.2 ± 12.2 a	117.7 ± 117 ab	109.6 ± 8.7 ab	108.6 ± 13.0 ab	96.8 ± 8.5 b	93.5 ± 4.5 b	*

	p-Coumaroyl	125.4 ± 12.4	130.2 ± 29.9	147.5 ± 10.2	137.2 ± 16.8	133.4 ± 12.5	146.0 ± 24.6	ns
Aglianico	Delph	39.5 ± 9.0	43.6 ± 7.7	44.3 ± 8.9	42.2 ± 3.8	43.3 ± 2.6	50.2 ± 6.3	ns
	Cya	2.5 ± 0.5	2.8 ± 0.5	2.7 ± 0.6	2.5 ± 0.3	2.7 ± 0.1	3.2 ± 0.5	ns
	Pet	54.7 ± 9.5	61.0 ± 8.2	58.4 ± 9.9	58.4 ± 3.9	61.1 ± 2.6	66.3 ± 6.2	ns
	Peo	19.4 ± 2.5	21.4 ± 1.8	21.1 ± 3.5	20.3 ± 1.8	22.1 ± 1.0	23.9 ± 2.8	ns
	Malv	583.5 ± 52.6	634.2 ± 41.0	617.2 ± 42.7	618.9 ± 24.4	638.2 ± 2.1	639.9 ± 36.2	ns
	Acetyl	31.6 ± 4.3 ab	33.5 ± 1.3 a	30.4 ± 1.0 ab	30.5 ± 1.7 ab	27.5 ± 0.4 ab	26.0 ± 3.0 b	*
	p-Coumaroyl	102.6 ± 9.2	113.4 ± 6.4	124.1 ± 13.1	119.0 ± 4.9	119.8 ± 6.2	114.6 ± 10.2	ns
Sangiovese	Delph	51.6 ± 3.7 b	57.4 ± 11.4 ab	69.1 ± 8.2 a	51.5 ± 4.33 b	58.3 ± 3.6 ab	60.2 ± 0.3 ab	**
	Cya	86.3 ± 3.9 b	93.3 ± 11.4 b	123.6 ± 13.4 a	90.3 ± 2.9 b	98.2 ± 5.3 b	101.7 ± 1.1 b	**#
	Pet	70.2 ± 6.5 ab	77.0 ± 5.7 ab	84.7 ±7.8 a	68.9 ± 5.0 b	76.7 ± 3.2 ab	78.9 ± 1.1 ab	*
	Peo	74.2 ± 4.9 b	77.9 ± 5.3 b	94.0 ± 9.5 a	75.0 ± 1.6 b	83.2 ± 1.5 ab	85.2 ± 1.5 ab	**#
	Malv	176.9 ± 23.0	191.0 ± 11.7	193.5 ± 11.9	176.2 ± 9.2	192.2 ± 9.6	197.9 ± 2.9	ns
	Acetyl	-	-	-	-	-	-	
	p-Coumaroyl	-	-	-	-	-	-	
Nebbiolo	Delph	9.3 ± 1.0 c	11.0 ± 1.5 b	14.6 ± 0.7 a	9.8 ± 0.6 bc	11.6 ± 1.6 bc	12.3 ± 0.0 ab	***
	Cya	12.8 ± 3.1 c	17.6 ± 1.5 b	23.6 ± 0.8 a	12.6 ± 0.8 c	19.0 ± 1.6 ab	19.5 ± 1.2 ab	***
	Pet	12.5 ± 0.9 b	13.7 ± 1.3 ab	16.4 ± 0.7 a	13.1 ± 1.1 b	13.9 ± 1.7 ab	14.7 ± 0.1 ab	*
	Peo	120.5 ± 10.1 b	137.5 ± 8.3 ab	159.7 ± 4.7 a	121.9 ± 10.4 b	139.1 ± 16.4 ab	139.6 ± 3.1 ab	**
	Malv	143.2 ± 10.9	137.2 ± 11.3	145.4 ± 12.2	141.0 ± 12.9	135.6 ± 7.5	149.0 ±17.5	ns
	Acetyl	10.8± 4.0 ab	15.8 ± 0.3 a	15.1 ± 1.8 ab	13.9 ± 1.9 ab	11.2 ± 0.9 b	9.9 ± 0.8 b	**#
	p-Coumaroyl	5.1 ± 2.0 b	12.7 ± 1.2 ab	17.2 ± 5.3 a	10.6 ± 3.7 ab	11.5 ± 2.5 ab	13.2 ± 1.6 ab	**

Data are expressed as average value ± standard deviation (n = 3) in mg of malvidin-3-O-glucoside chloride equivalents /kg of berries. Sign.: *, **, ***, and "ns" indicate significant differences at $p < 0.05$, 0.01, 0.001, and not significant, respectively, among values within the same row according to ANOVA or # Welch's ANOVA. Different Latin letters within the same row indicate significant differences among treatments according to Tukey HSD or Games-Howell tests ($p < 0.05$) for ANOVA and Welch's ANOVA, respectively. Delph=delphinidin-3-glucoside, Cya=cyanidin-3-glucoside, Pet=petunidin-3-glucoside, Peo=peonidin-3-glucoside, Malv=malvidin-3-glucoside, Acetyl=sum of acetylglucosides, *p*-Coumaroyl=sum of *p*-coumaroylglucosides.

Highlights

- Grape-derived tannins enhanced polymer pigment formation in first maceration stage
- Quebracho-derived tannins increased disubstituted anthocyanin content
- Tannin addition modified color parameters depending on the variety
- Exogenous tannins addition can be tailored on the varietal anthocyanin profile

CRediT Author Statement

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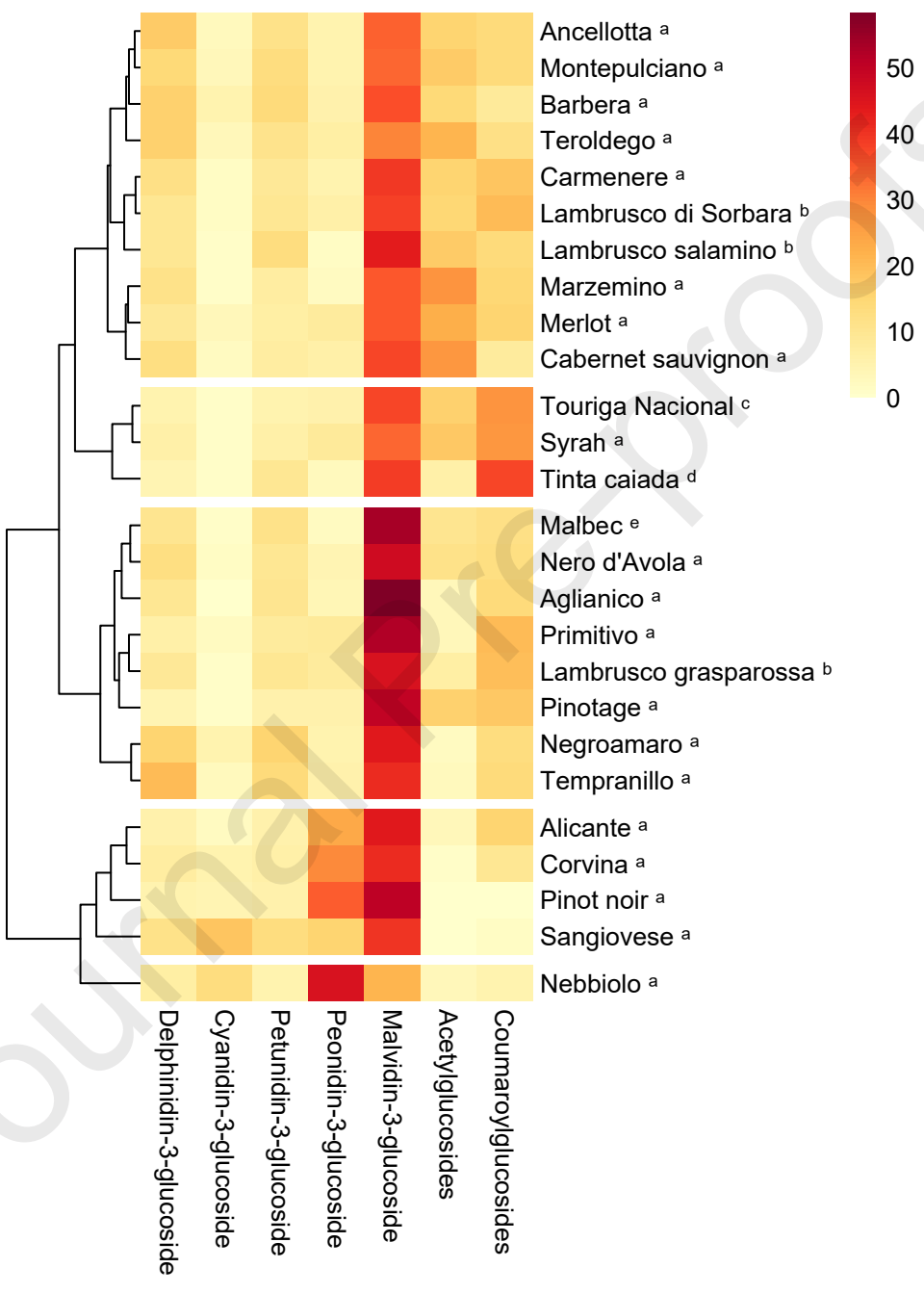

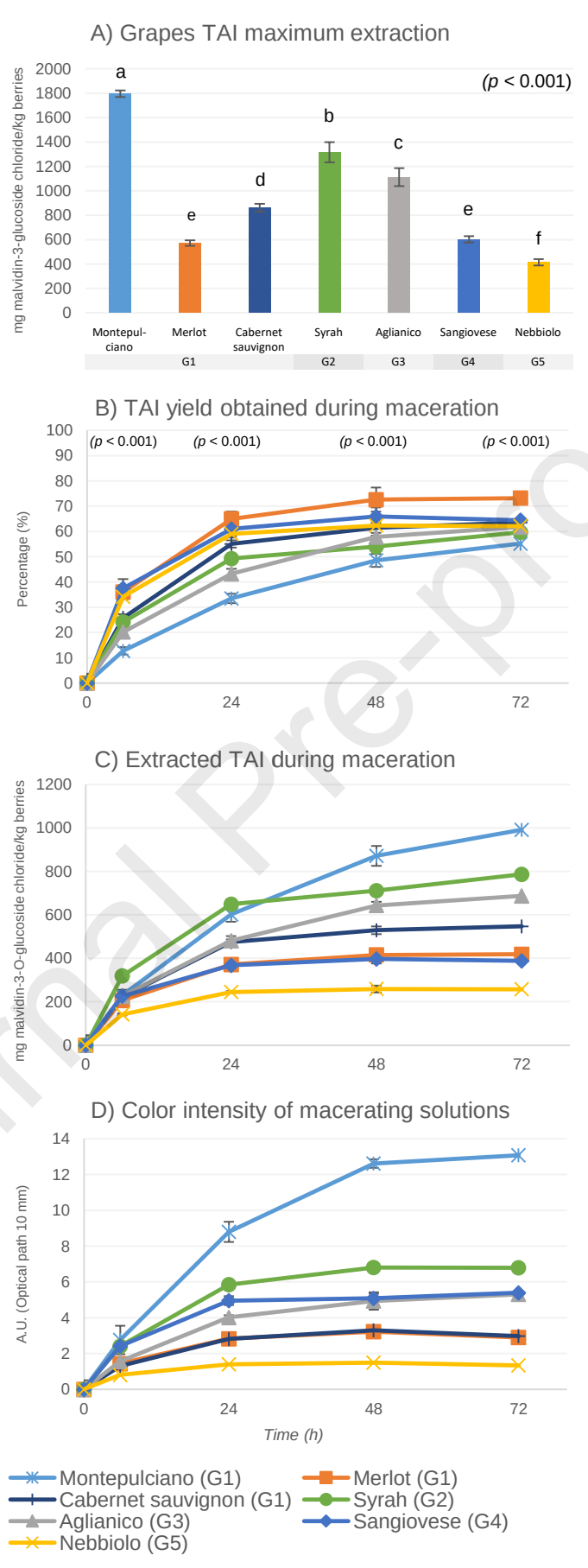
A) Grapes TAI maximum extraction
mg malvidin-3-glucoside chloride/kg berries
(p < 0.001)
a
b
c
d
e
e
f
Montepul-ciano
Merlot
Cabernet sauvignon
Syrah
Aglianico
Sangiovese
Nebbiolo
G1
G2
G3
G4
G5
B) TAI yield obtained during maceration
Percentage (%)
(p < 0.001)
(p < 0.001)
(p < 0.001)
(p < 0.001)
C) Extracted TAI during maceration
mg malvidin-3-O-glucoside chloride/kg berries
D) Color intensity of macerating solutions
A.U. (Optical path 10 mm)
Time (h)
Montepulciano (G1)
Merlot (G1)
Cabernet sauvignon (G1)
Syrah (G2)
Aglianico (G3)
Sangiovese (G4)
Nebbiolo (G5)