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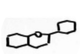

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Influence of Grape Maturity and Maceration Length on Color, Polyphenolic Composition, and Polysaccharide Content of Cabernet Sauvignon and Tempranillo Wines

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ABSTRACT: The aim of this paper was to study how maturity and maceration length affect color, phenolic compounds, polysaccharides, and sensorial quality of Cabernet Sauvignon and Tempranillo wines at three stages of grape ripening. Ripeness increased color extractability, phenolic compounds, and polysaccharide concentrations. Moreover, the proanthocyanidin mean degree of polymerization (mDP) and the percentage of prodelphinidins also increased with maturity, whereas the percentage of galloylation decreased. In general, wines from riper grapes contain higher proportions of skin proanthocyanidins. Color and anthocyanin concentration decreased when the maceration was longer, whereas polysaccharide and proanthocyanidin concentrations did the opposite. It was also detected that the mDP and the percentage of prodelphinidins decreased when the maceration was extended, whereas the percentage of galloylation increased. These data seem to indicate that proanthocyanidin extraction from seeds is clearly increased throughout the maceration time.

KEYWORDS: *wine, grape maturity, maceration length, color, phenolic compounds, polysaccharides*

■ INTRODUCTION

Phenolic compounds are generally considered to be major determinants of the quality of red wines. Most of the main sensory attributes such as color, body, mouthfeel, bitterness, and astringency are directly associated with the composition of wine in anthocyanins, proanthocyanidins, and other phenolic compounds.[1−3] Other compounds such as polysaccharides have also been associated with texture sensations, and it has been proposed that their presence can smooth wine bitterness and astringency.[4,5]

During winemaking, anthocyanins are released from grape skins, whereas proanthocyanidins, also known as condensed tannins, are released from both skins and seeds.[6,7] The composition of proanthocyanidins depends on their origin. Thus, seed proanthocyanidins are made up of (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-gallate,[8] whereas skin proanthocyanidins also contain (−)-epigallocatechin and have a much lower proportion of (−)-epicatechin-3-gallate.[9,10] Therefore, skins release procyanidins and prodelphinidins, whereas seeds only release procyanidins with a higher proportion of galloylation.

On the other hand, the mean degree of polymerization (mDP) of seed proanthocyanidins is lower than that of skin proanthocyanidins.[11] It has been reported that molecular sizes, and especially the monomeric composition of proanthocyanidins, have a considerable influence on the perception of astringency.[12] More specifically, a greater degree of polymerization and a greater percentage of galloylation cause a greater sensation of astringency.[1,12,13]

Polysaccharides are components of cell walls that cover and protect the plasma membrane of plant cells (grape berries)[14] and the microorganisms involved in the winemaking process (yeasts and lactic acid bacteria).[15,16] Moreover, fungal grape diseases can increase the polysaccharide content of wine, which can cause technological problems.[17] Furthermore, the use of such enological additives as arabic gum or carboxymethylcellulose can also alter the composition of wine polysaccharides.[18] Hence, wine polysaccharides can be classified on the basis of their origin in grape polysaccharides, microbial polysaccharides, or additive polysaccharides.

There are several types of grape polysaccharides, but many of them are enzymatically degraded or precipitated during alcoholic fermentation, so wine contains appreciable amounts of only arabinogalactan proteins (AGP) and type II rhamnogalacturonans (RG-II).[19,20] The other major source of wine polysaccharides is yeasts, which can release significant amounts of mannoproteins (MP).[21]

It is generally considered that grape ripeness strongly influences the phenolic and polysaccharide composition of its respective red wines.[22,23] The synthesis of anthocyanins starts during veraison and remains active throughout grape ripening,[24] which causes a gradual accumulation in the skins.[25] In contrast, proanthocyanidin concentration is highest at veraison and subsequently decreases until just before complete ripeness, after which time it remains relatively constant.[26] Simultaneously, the mDP increases throughout ripening.[26−28]

Moreover, the progressive enzymatic degradation of the walls of skin cells during ripening[29] augments the presence of soluble polysaccharides[30,31] in the grape juice and also increases the

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extractability of phenolic compounds.[32−34] By contrast, the extractability of proanthocyanidins from seeds behaves in quite the opposite fashion, probably because oxidation phenomena and gradual seed lignification[35] prevent them from dissolving. For this reason, it is generally considered that grapes that are not well ripened may produce more astringent wines because their seeds can release more proanthocyanidins, which are highly galloylated.

Grape maturity can also exert an indirect but non-negligible effect on polyphenol and polysaccharide solubilization. In particular, higher ethanol levels, usually present in wines from well-ripened grapes, seem to favor polyphenol extraction[10,36] but diminish polysaccharide concentration by precipitation.[20]

Nowadays, deeply colored and full-bodied red wines are highly valued by consumers. For this reason, winemakers try to produce this kind of wine, which is necessarily very tannic. Many techniques have been proposed to improve color and phenolic compound extraction such as the use of pectolytic enzymes,[37] cold prefermentative maceration,[38] thermovinification,[39] flash expansion,[40] and greater volume and frequency of pumping over and pigeage (punchdown) or delestage (rack and return).[41] Nevertheless, the length of time the wine is in contact with skins and seeds is probably the main factor.[7,42]

All of these procedures have proved to be useful for increasing the color and polyphenol concentration of wine, but they can sometimes extract an excess of proanthocyanidins, which makes the wine too astringent and bitter,[43] especially when grapes are not completely ripened.[44]

Numerous studies have investigated the changes in anthocyanin,[24] proanthocyanidin,[45] and polysaccharide[23] composition during berry development and maturation. Other studies have focused on the extraction of these compounds into wine[46] with regard to maceration time.[47,48] However, very few papers have simultaneously studied the influence of grape maturity and maceration length on the extractability of polyphenols[7,36] and, to our knowledge, none have studied the extractability of polysaccharides.

The aim of this paper was to study how grape maturity and maceration length affect the color, polyphenolic composition, and polysaccharide content of Cabernet Sauvignon and Tempranillo wines.

■ MATERIALS AND METHODS

Chemicals and Equipment. Methanol, acetonitrile, formic acid, and acetic acid were of HPLC grade and were purchased from Panreac (Barcelona, Spain). Acetaldehyde, phloroglucinol, ascorbic acid, sodium acetate, and ammonium formate were purchased from Sigma-Aldrich (Madrid, Spain). Absolute ethanol and hydrochloric acid were purchased from Panreac. Malvidin-3-*O*-glucoside chloride (≥95%), proanthocyanidin dimer B2 (≥90%), (+)-catechin (≥99%), (−)-epicatechin (≥99%), (−)-epigallocatechin (≥98%), and (−)-epicatechin-3-*O*-gallate (≥97.5%) were purchased from Extrasynthese (Genay, France). A pullulan calibration kit Shodex P-82 (P-5, M_w = 5.9 kDa; P-10, M_w = 11.8 kDa; P-20, M_w = 22.8 kDa; P-50, M_w = 47.5 kDa; P-100, M_w = 112 kDa; P-200, M_w = 212 kDa; P-400, M_w = 404 kDa; P-800, M_w = 788 kDa) was obtained from Waters (Barcelona, Spain), whereas a pullulan 1.3 kDa and four dextrans BioChemika (12, 25, 50, and 80 kDa) were obtained from Fluka (St. Louis, MO, USA). The polysaccharides used as external standards for quantification were pectins from citrus fruit (≥90%) and dextrans synthesized by *Leuconostoc mesenteroides* (≥99.9%) purchased from Sigma-Aldrich (St. Louis, MO, USA). The HPLC analyses were performed using an Agilent 1200 series liquid chromatograph equipped with a G1362A refractive index detector (RID), a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1316A column oven, and a G1329A autosampler (Agilent Technologies, Santa Clara, CA, USA). All of the spectrophotometric measurements were performed using a Helios Alpha UV−vis spectrophotometer (Thermo Fisher Scientific Inc., Waltman, MA, USA).

Grapes and Wines. This study was carried out with grapes from two *Vitis vinifera* cultivars of the 2009 vintage: Tempranillo, from the experimental vineyard of the Oenology Faculty (Rovira i Virgili University) at Constantí (AOC Tarragona), and Cabernet Sauvignon, from the Juvé & Camps estates at Mediona (AOC Penedes). Both cultivars were harvested at three maturity levels (around 3, 5, and 7 weeks after veraison). Thirty-six microvinifications were carried out for each cultivar in an attempt to study the influence of grape maturity and maceration length on wine composition and quality. At each maturity level, 80 kg of grapes was harvested and carefully destemmed. Subsequently, the berries were randomly distributed in 12 groups of 6 kg each, crushed with a semiautomatic crusher (Gual, Villafranca del Penedès, Spain), sulfited (100 mg $K_2S_2O_5$/kg), and placed in 8 L tanks equipped with a submerged cap system according to the winemaking method described by Sampaio et al.[49] All tanks were immediately inoculated with 200 mg/kg of selected yeast (EC1118, Lallemand Inc., Montreal, Canada) and maintained at a room temperature of 25 ± 1 °C. All of these microvinifications were controlled daily by measuring the temperature and the density of the juice. Two mechanical punchdowns of the cap were made around 1060 and 1020 density units to improve color extraction. After 1, 2, 3, and 4 weeks of maceration, the wines from three of the tanks were racked. Once alcoholic fermentation had completely finished, wines were sulfited (100 mg $K_2S_2O_5$/L) and kept at 4 °C for 1 month for tartaric stabilization. Malolactic fermentation was inhibited to prevent any possible variations in the rhythm of this transformation that could affect each wine differently. Finally, wines were bottled and stored in a dark cellar at 15 °C until analysis. The analyses started 2 months after bottling and were finished 3 weeks later.

Standard Grape Juice Analysis. The analytical methods recommended by the International Organization of Vine and Wine (OIV) were used to determine the sugar concentration and titratable acidity of the grape juices.[50]

Standard Wine Analysis. Ethanol content (% v/v) was analyzed with a FTIR spectrometer BACCHUS II (TDI, Gavà, Spain). pH values were determined by a pH-meter Basic-20 (CRISON, Barcelona, Spain). The total polyphenol index (TPI) was determined by measuring the 280 nm absorbance of a 1:100 dilution of wine with a spectrophotometer, using a 10 mm quartz cuvette and multiplying the absorbance value by 100 as described by Ribéreau-Gayon et al.[44] The total anthocyanin content was determined by spectrophotometry using the method described by Niketic-Aleksic et al.[51]

Color Parameters. Ten microliters of a 10% (v/v) acetaldehyde solution was added to 1 mL of wine sample 20 min before color measurement to avoid sulfite interferences. The color intensity (CI) was estimated using the method described by Glories.[44] The CIELAB coordinates, lightness (L^*), chroma (C^*), hue (h^*), red-greenness (a^*), and yellow-blueness (b^*), were determined according to the method of Ayala et al.,[52] and data processing was performed with MSCV software.[53]

HPLC Anthocyanidin Analysis. Reversed-phase HPLC analyses of the anthocyandins were carried out by injecting 40 μL of wine into an Agilent 1200 series liquid chromatograph (HPLC-DAD) and using an Agilent Zorbax Eclipse XDBC18, 4.6 × 250 mm, 5 μm column (Agilent Technologies). The solvents used were 10% aqueous formic acid (solvent A) and a mixture of 45% methanol, 45% water, and 10% formic acid (solvent B) in accordance with the method described by Valls.[54] Chromatograms were recorded at 530 nm, and anthocyanin standard curves were made using malvidin-3-*O*-glucoside chloride. Compounds were identified by recording their UV spectra with the diode array detector and comparing these with the UV spectra reported in the literature. The five anthocyanidin-3-monoglucosides of wine (delphinidin, cyanidin, peonidin, petunidin, and malvidin) and their respective acetylated and *p*-coumarylated anthocyanins were quantified.

Wine Proanthocyanidin Analysis. Acid-catalyzed depolymerization of proanthocyanidin in the presence of an excess of phloroglucinol was used to analyze the content of proanthocyanidins, their monomeric composition, and their mDP, as described by Kennedy and Jones.[55] A 10 mL sample of wine was evaporated under a low-pressure vacuum (Univapo 100 ECH, Uni Equip, Germany). Subsequently, it was resuspended in 6 mL of distilled water and then applied to Set Pak Plus tC18 Environmental cartridges (Waters, Milford, MA, USA) that had previously been activated with 10 mL of methanol and 15 mL of water. The samples were washed with 15 mL of distilled water, and then the proanthocyanidins were eluted with 12 mL of methanol, immediately evaporated under a vacuum, and redissolved in 2 mL of methanol. Finally, 100 μL of this sample was reacted with a 100 μL phloroglucinol solution (0.2 N HCl in methanol, containing 100 g/L phloroglucinol and 20 g/L ascorbic acid) at 50 °C for 20 min. The reaction was stopped by adding 1000 μL of 40 mM aqueous sodium acetate. Reversed-phase HPLC analysis (Agilent series 1200 HPLC-DAD) was carried out with an Agilent Zorbax Eclipse XDBC18, 4.6 × 250 mm, 5 μm column (Agilent Technologies) as described below, and the injection volume was 30 μL. The solvents used were 1% aqueous acetic acid (solvent A) and methanol (solvent B) at a flow rate of 1 mL/min. The elution conditions were 1.0 mL/min. Elution was performed with a gradient starting at 5% B for 10 min, a linear gradient from 5 to 20% B in 20 min, and a linear gradient from 20 to 40% B in 25 min. The column was then washed with 90% B for 10 min and re-equilibrated with 5% B for 5 min before the next injection. The monomers (+)-catechin, (−)-epicatechin, and (−)-epicatechin-3-*O*-gallate were identified by comparing their retention times with those of the pure compounds. The phoroglucinol adducts of (+)-catechin, (−)-epicatechin, (−)-epigallocatechin, and (−)-epicatechin-3-*O*-gallate were identified by their retention time (described in the literature) and confirmed through an HPLC-MS analysis. Analyses were performed with the Agilent 1200 series HPLC using an Agilent 6210 time-of-flight (TOF) mass spectrometer equipped with an electrospray ionization system (ESI). Elution was carried out under the same HPLC analysis conditions described below. The capillary voltage was 3.5 kV. Nitrogen was used both as a dry gas at a flow rate of 12 L/min at 350 °C and as a nebulizer gas at 60 psi. Spectra were recorded in positive ion mode between m/z 50 and 2400. This assay was also carried out without the addition of phloroglucinol to measure the flavan-3-ol monomers that are naturally present in wine. The number of terminal subunits was considered to be the difference between the total monomers measured in normal conditions (with phoroglucinol and acid) and that obtained when the analysis was performed without phloroglucinol and acid addition. The number of extension subunits was considered as the addition of all the phloroglucinol adducts. The mDP was calculated by adding the terminal and extension subunits (in moles) and dividing by the terminal subunits. Because acid catalysis with phloroglucinol is not completely efficient, the real yield of the reaction was measured using a pure B2 proanthocyanidin dimer [(−)-epicatechin-(4→8)-(−)-epicatechin]. This yield was used to calculate the total proanthocyanidin concentration from wine.

Polysaccharide Analysis. Wine samples were processed using the methodology described by Ayestarán et al.[37] Briefly, 10 mL of wine was centrifuged (8500 rpm, 20 min) by a Biofuge Primo centrifuge (Heraeus, Hanau, Germany), and the supernatant was concentrated to a final volume of 2 mL using a vacuum evaporator (Univapo 100ECH, Uniequip, Martinsried, Germany). Total soluble polysaccharides were precipitated by adding 10 mL of cold acidified ethanol (0.3 M HCl in absolute ethanol) and kept for 24 h at 4 °C. Then, the samples were centrifuged (8500 rpm, 10 min, 4 °C), the supernatants were discarded, and the pellets were washed four times with cold ethanol to remove the interference materials. Finally, the precipitates were dissolved in 1 mL of ultrapure water, frozen to −80 °C, and freeze-dried using a lyophilizer Christ Alpha 1-4 (Martin Christ, Osterode am Harz, Germany). To determine the molecular distribution and quantify the polysaccharides obtained from wines, the soluble fractions were analyzed by high-resolution size exclusion chromatography (HRSEC) using a refraction index detector (RID). The lyophilized samples were resuspended in 1 mL of 30 mM ammonium formate and filtered through a 0.45 μm pore size nylon membrane, after which 100 μL was injected onto the column. Separation was carried out at 20 °C using two Shodex OHpak SB-803 HQ and SB-804 HQ columns connected in series (300 mm × 8 mm i.d.; Showa Denko, Japan). The mobile phase consists of an aqueous solution of 30 mM ammonium formate, applied with a constant flow of 0.6 mL/min for 60 min, and a RID cell temperature of 35 °C. The molecular weight distribution of the wine fractions was followed by calibration with pullulan and dextran standards of different molecular weights (see above). The polysaccharides were quantified on the basis of the peak area for each fraction, using the external standard method with pectin and dextran commercial standards. The calibration curve was obtained by injecting standard solutions, under the same conditions as for the samples analyzed, in the range between 0 and 2 g/L.

Sensory Analysis. All of the wines were tasted by a group of eight expert enologists from the Rovira i Virgili University 6 months after bottling. A sensory training session was held beforehand so that the experts could homogenize criteria. They all took part in two descriptive trials in which they evaluated each wine for six sensorial attributes on a scale from 1 to 10: fruitiness, vegetal, acidity, astringency, bitterness, and mouthfeel. The values indicate the intensity of the sensation for each attribute. The first trial was focalized to maturity employing wines of 2 and 3 weeks of maceration, and the data correspond to the average of both maceration times. The second trial was focalized to maceration length, and all samples were tasted.

Statistical Analysis. All of the data are expressed as the arithmetic average of three replicates. Two- and one-factor ANOVA tests were carried out with SPSS software.

RESULTS AND DISCUSSION

Table 1 shows the changes in sugar concentration and titratable acidity of the grape juices of both cultivars at the three different ripening stages. As expected, sugar concentration increased throughout the maturation time, whereas titratable acidity decreased. All of these data confirm that the grapes had ripened correctly and that the three crops were different from one another.

Table 1. Changes in Sugar Concentration and Titratable Acidity of the Grape Juices of Both Cultivars at the Three Different Ripening Stages[a]

maturity level	sugar content (g/L)	titratable acidity (g/L)
	Cabernet Sauvignon	
1	192.1 ± 1.7 a	9.9 ± 0.3 c
2	217.6 ± 1.7 b	7.1 ± 0.5 b
3	236.3 ± 1.7 c	5.9 ± 0.7 a
	Tempranillo	
1	192.1 ± 3.4 a	6.1 ± 0.3 c
2	204.0 ± 1.7 b	5.1 ± 0.1 b
3	217.6 ± 1.7 c	3.8 ± 0.1 a

[a]Results are expressed as the average values of 12 replicates ± standard deviation (n = 12). Different letters indicate statistical differences (p < 0.05) for an ANOVA test.

Tables 2 (Cabernet Sauvignon) and 3 (Tempranillo) show the evolution of ethanol content, pH, total anthocyanin concentration (measured by spectrophotometry), and total phenolic index (TPI) of different wines as a function of the level of maturity and the length of maceration. The ethanol content of wines increased throughout ripening in both cultivars, and their values were, in general terms, in agreement with the corresponding sugar concentration observed in grapes. As expected, the length of maceration seems not to have any influence on this parameter with the only exception of the Cabernet Sauvignon wines from the latest harvest. This may be due to the presence of some dried grapes, which delay their sugar release and are therefore responsible for the increased ethanol content observed after the second week of maceration.

Table 2. General Analytic Parameters: Cabernet Sauvignon Wines[a]

parameter	maturity level	maceration length: 1 week	2 weeks	3 weeks	4 weeks	global
ethanol content (% v/v)	1	12.1 ± 0.1 α, A	12.1 ± 0.1 α, A	12.1 ± 0.0 α, A	12.1 ± 0.1 α, A	**12.1 ± 0.0 a**
	2	13.7 ± 0.0 β, A	13.7 ± 0.0 β, A	13.8 ± 0.1 β, A	13.8 ± 0.1 β, A	**13.8 ± 0.0 b**
	3	14.0 ± 0.3 β, A	14.6 ± 0.0 γ, B	14.6 ± 0.1 γ, B	14.6 ± 0.2 γ, B	**14.4 ± 0.0 c**
	global	**13.3 ± 0.0 a**	**13.5 ± 0.0 b**	**13.5 ± 0.0 b**	**13.5 ± 0.0 b**	***p*-interaction value = 0.0022**
pH	1	3.41 ± 0.05 α, A	3.47 ± 0.04 α, A	3.51 ± 0.04 α, AB	3.57 ± 0.12 α, B	**3.49 ± 0.01 a**
	2	3.73 ± 0.04 β, AB	3.71 ± 0.03 β A	3.76 ± 0.01 β, B	3.76 ± 0.04 α, B	**3.73 ± 0.01 b**
	3	3.78 ± 0.01 β, AB	3.78 ± 0.01 γ, A	3.79 ± 0.01 β, AB	3.85 ± 0.07 β, B	**3.80 ± 0.01 c**
	global	**3.64 ± 0.01 a**	**3.65 ± 0.01 ab**	**3.69 ± 0.01 bc**	**3.72 ± 0.01 c**	***p*-interaction value = 0.0158**
total anthocyanins (mg/L)	1	653 ± 29 α, C	615 ± 32 α, C	553 ± 32 α, B	462 ± 36 α, A	**571 ± 10 a**
	2	810 ± 66 β, C	738 ± 16 β, C	630 ± 62 α, B	606 ± 7 β, A	**696 ± 10 b**
	3	920 ± 10 γ, C	929 ± 16 γ, C	823 ± 12 β, B	737 ± 31 γ, A	**852 ± 10 c**
	global	**794 ± 11 d**	**761 ± 11 c**	**669 ± 11 b**	**602 ± 11 a**	***p*-interaction value = 0.2244**
TPI	1	40.0 ± 1.7 α, A	47.4 ± 1.7 α, B	51.8 ± 3.1 α, BC	52.3 ± 2.8 α, C	**47.9 ± 0.6 a**
	2	49.7 ± 3.5 β, A	56.3 ± 0.9 β, B	61.5 ± 2.9 β, C	62.7 ± 0.3 β, C	**57.6 ± 0.6 b**
	3	54.4 ± 1.6 β, A	66.2 ± 0.4 γ, B	67.6 ± 1.8 γ, B	68.4 ± 2.0 γ, B	**64.2 ± 0.6 c**
	global	**48.0 ± 0.7 a**	**56.6 ± 0.7 b**	**60.3 ± 0.7 c**	**61.1 ± 0.7 c**	***p*-interaction value = 0.4611**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). Total anthocyanins are expressed as mg/L of malvidin-3-*O*-glucoside. TPI corresponds to the total phenolic index. Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

Table 3. General Analytic Parameters: Tempranillo Wines[a]

parameter	maturity level	maceration length: 1 week	2 weeks	3 weeks	4 weeks	global
ethanol content (% v/v)	1	12.1 ± 0.4 α, A	11.9 ± 0.4 α, A	11.9 ± 0.3 α, A	11.9 ± 0.0 α, A	**12.0 ± 0.1 a**
	2	12.7 ± 0.1 β, A	12.8 ± 0.0 β, A	12.9 ± 0.1 β, A	12.7 ± 0.1 β, A	**12.8 ± 0.1 b**
	3	14.1 ± 0.3 γ, A	14.2 ± 0.1 γ, A	14.0 ± 0.3 γ, A	13.8 ± 0.2 γ, A	**14.0 ± 0.1 c**
	global	**13.0 ± 0.1 a**	**13.0 ± 0.1 a**	**12.9 ± 0.1 a**	**12.8 ± 0.1 a**	***p*-interaction value = 0.7711**
pH	1	3.40 ± 0.02 α, A	3.40 ± 0.02 α, A	3.42 ± 0.02 α, A	3.40 ± 0.01 α, A	**3.40 ± 0.01 a**
	2	3.65 ± 0.03 β, A	3.68 ± 0.01 β, AB	3.67 ± 0.01 β, A	3.67 ± 0.02 β, B	**3.69 ± 0.01 b**
	3	3.67 ± 0.05 β, A	3.73 ± 0.01 γ, AB	3.80 ± 0.11 γ, BC	3.89 ± 0.01 γ, C	**3.77 ± 0.01 c**
	global	**3.57 ± 0.01 a**	**3.60 ± 0.01 ab**	**3.63 ± 0.01 b**	**3.69 ± 0.02 c**	***p*-interaction value = 0.0150**
total anthocyanins (mg/L)	1	458 ± 47 α, B	405 ± 37 α, B	270 ± 18 α, A	282 ± 36 α, A	**354 ± 12 a**
	2	655 ± 63 β, C	582 ± 24 β, BC	531 ± 21 β, AB	473 ± 11 β, A	**561 ± 12 b**
	3	622 ± 68 β, B	581 ± 28 β, B	552 ± 24 β, AB	483 ± 13 β, A	**560 ± 12 b**
	global	**578 ± 13 d**	**523 ± 13 c**	**451 ± 13 b**	**414 ± 14 a**	***p*-interaction value = 0.1823**
TPI	1	45.8 ± 1.8 α, A	48.7 ± 1.9 α, A	46.0 ± 2.1 α, A	49.6 ± 2.4 α, A	**47.5 ± 0.7 a**
	2	57.4 ± 2.7 β, A	59.1 ± 1.4 β, A	59.8 ± 1.5 β, A	59.9 ± 1.8 β, A	**59.0 ± 0.7 b**
	3	56.8 ± 4.5 β, A	59.9 ± 3.3 β, AB	63.0 ± 1.7 β, B	62.8 ± 1.5 β, B	**60.6 ± 0.7 b**
	global	**53.3 ± 0.8 a**	**55.9 ± 0.8 b**	**56.3 ± 0.8 b**	**57.4 ± 0.9 b**	***p*-interaction value = 0.3567**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). Total anthocyanins are expressed as mg/L of malvidin-3-*O*-glucoside. TPI corresponds to the total phenolic index. Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

As expected, ripening had a clear effect on wine pH (the riper the grape, the higher the pH). In general terms, maceration length also affected wine pH. The longer the maceration time, the higher the pH. This effect was clearer when the grapes were riper.

Total anthocyanin content also increased throughout ripening in both cultivars, which indicates that grape skins have also ripe. These data are in agreement with data from other studies[22,36] and confirm the previously described influence of ripening on

Table 4. Color Parameters: Cabernet Sauvignon Wines[a]

parameter	maturity level	maceration length: 1 week	2 weeks	3 weeks	4 weeks	global
CI	1	11.2 ± 0.6 α, A	12.2 ± 0.3 α, A	11.9 ± 0.4 α, A	12.0 ± 0.9 α, A	**11.8 ± 0.2 a**
	2	14.1 ± 1.4 β, A	14.8 ± 0.4 β, A	14.1 ± 0.9 β, A	14.0 ± 0.2 β, A	**14.3 ± 0.2 b**
	3	14.1 ± 0.5 β, A	15.1 ± 0.4 β, A	14.7 ± 0.1 β, A	14.6 ± 1.0 β, A	**14.7 ± 0.2 b**
	global	**13.1 ± 0.2 a**	**14.1 ± 0.2 b**	**13.6 ± 0.2 ab**	**13.5 ± 0.2 ab**	***p*-interaction value = 0.9550**
*C**	1	56.1 ± 2.4 α, B	56.1 ± 1.9 α, B	54.4 ± 1.2 α, AB	49.9 ± 4.0 α, A	**54.1 ± 0.6 a**
	2	56.9 ± 2.2 α, A	57.2 ± 0.6 α, A	54.7 ± 2.3 α, A	54.1 ± 0.8 α, A	**55.7 ± 0.6 a**
	3	55.3 ± 0.7 α, B	55.8 ± 0.3 α, B	54.9 ± 0.1 α, B	52.4 ± 2.5 α, A	**54.6 ± 0.6 a**
	global	**56.1 ± 0.6 b**	**56.4 ± 0.6 b**	**54.6 ± 0.6 b**	**52.1 ± 0.6 a**	***p*-interaction value: 0.5815**
*L**	1	51.5 ± 3.4 β, B	47.0 ± 0.7 β, A	47.3 ± 1.0 β, A	46.5 ± 2.3 β, A	**48.1 ± 0.51 b**
	2	43.2 ± 3.1 α, A	41.1 ± 0.9 α, A	42.4 ± 1.9 α, A	42.6 ± 0.1 α, A	**42.3 ± 0.51 a**
	3	42.3 ± 1.1 α, B	39.9 ± 0.7 α, A	41.0 ± 0.2 α, AB	40.9 ± 2.1 α, AB	**41.0 ± 0.51 a**
	global	**45.7 ± 0.6 b**	**42.7 ± 0.6 a**	**43.6 ± 0.6 a**	**43.3 ± 0.6 a**	***p*-interaction value = 4540**
*H**	1	5.14 ± 2.64 α, A	6.11 ± 0.72 α, A	7.26 ± 0.97 α, AB	9.69 ± 0.85 α, B	**7.05 ± 0.36 a**
	2	8.37 ± 1.04 αβ, A	10.18 ± 0.77 β, AB	12.56 ± 2.07 β, BC	13.34 ± 0.88 β, C	**11.11 ± 0.36 b**
	3	8.85 ± 0.86 β, A	9.78 ± 0.62 β, A	11.87 ± 0.77 β, B	13.30 ± 1.13 β, B	**10.95 ± 0.36 b**
	global	**7.45 ± 0.42 a**	**8.69 ± 0.42 b**	**10.56 ± 0.42 c**	**12.11 ± 0.42 d**	***p*-interaction value = 8694**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). CI, color intensity; C^*, chroma; L^*, lightness; and H^*, hue. Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

anthocyanin concentration.[24,25] In contrast, total anthocyanin concentration diminished when the maceration time was longer. This observation may be due to different causes. On the one hand, anthocyanins may be degraded and/or absorbed by yeasts and the tank surface,[56] and, on the other hand, anthocyanins can be transformed in new pigments with a different maximum wavelength.

Maturity and maceration length also influence the total phenolic content of wines in both cultivars. Specifically, TPI was higher when the grapes were riper and when the maceration length was longer. These results are quite logical and coincide with those of previous studies.[36,47]

The changes in the color parameters of wines from both cultivars are shown in Tables 4 (Cabernet Sauvignon) and 5 (Tempranillo). In general terms, the wine color intensity (CI) of both cultivars increased and lightness (L^*) decreased with maturity, especially between the first and second harvests. In contrast, neither chroma (C^*) nor hue (H^*) showed a clear trend. These data confirm that wines from riper grapes show a deeper red color.

The effect of the length of maceration, however, seemed to depend on the cultivar. Therefore, the behavior of CI and C^* was not well-defined for Cabernet Sauvignon wines, but both parameters decreased significantly with maceration length in Tempranillo wines. L^* did not show a clear trend during maceration in either of the cultivars. However, it increased significantly in the third harvest of Tempranillo and decreased significantly between the first and the second weeks of Cabernet Sauvignon from the first harvest. Finally, H^* increased significantly with maceration time in Cabernet Sauvignon wines, whereas no clear behavior was found in Tempranillo wines.

Tables 6 and 7 show the quantification of anthocyanins by HPLC-DAD in Cabernet Sauvignon and Tempranillo wines, respectively. Cabernet Sauvignon wines had higher anthocyanin concentrations than Tempranillo wines at similar maturation stages and maceration times. Moreover, Cabernet Sauvignon wines had a significantly higher proportion of acetylated anthocyanins and a lower proportion of coumarylated anthocyanins than their corresponding Tempranillo wines. These differences in the proportion of acetylated and coumarylated anthocyanins have previously been described by other authors and are currently used as parameters to distinguish varieties.[57]

As a general rule, the total anthocyanin concentrations determined by HPLC-DAD were similar to, although somewhat lower than, the total anthocyanin concentrations measured by spectrophotometry. This is logical because spectrophotometric analysis includes the contribution from other pigments in the measurement and, therefore, overestimates the total anthocyanin concentration, whereas the HPLC-DAD methods detect only free anthocyanins.[58] The total anthocyanin concentration of wines from both cultivars tended to increase significantly with maturity,[22] although in some cases a slight decrease between the first and second harvestswas detected. In contrast, total anthocyanin concentration decreased significantly with maceration length.[41,56] In general terms, this behavior was observed in both nonacylated and acylated anthocyanins (acetylated and coumarylated). As has been mentioned above,these results are in agreement with previously published data.

The results of analyzing wine proanthocyanidins obtained by acid depolymerization in the presence of excess phloroglucinol are shown in Tables 8 (Cabernet Sauvignon) and 9 (Tempranillo). The total proanthocyanidin concentration of Cabernet Sauvignon wines was affected by maturity and maceration length and, in general, was higher when the grapes were riper and the maceration longer. These data are completely logical and agree with the data available in the literature.[41,46,47] Moreover, maturity seems to affect proanthocyanidin extractability.

Table 5. Color Parameters: Tempranillo Wines[a]

parameter	maturity level	maceration length: 1 week	2 weeks	3 weeks	4 weeks	global
CI	1	12.4 ± 0.8 α, B	12.3 ± 0.6 α, B	11.9 ± 0.5 α, AB	11.1 ± 0.6 α, A	**11.9 ± 0.2 a**
	2	13.7 ± 0.2 $\alpha\beta$, A	14.0 ± 0.1 β, A	13.3 ± 1.3 α, A	12.8 ± 1.2 β, A	**13.5 ± 0.2 b**
	3	14.7 ± 1.2 β, B	14.3 ± 0.8 β, B	12.9 ± 1.3 α, AB	11.6 ± 0.2 $\alpha\beta$, A	**13.4 ± 0.2 b**
	global	**13.6 ± 0.3 c**	**13.5 ± 0.3 c**	**12.7 ± 0.3 b**	**11.8 ± 0.3 a**	***p*-interaction value: 0.3864**
C^*	1	57.1 ± 1.0 α, B	55.1 ± 1.2 α, B	47.8 ± 1.3 α, A	48.4 ± 2.5 α, A	**52.1 ± 0.5 a**
	2	57.9 ± 0.2 α, C	55.2 ± 0.5 α, B	53.9 ± 0.5 β, B	49.6 ± 2.6 α, A	**54.2 ± 0.5 b**
	3	58.3 ± 0.8 α, C	55.9 ± 0.7 α, CB	52.9 ± 3.6 β, B	48.5 ± 0.4 α, A	**53.9 ± 0.5 b**
	global	**57.8 ± 0.5 d**	**55.4 ± 0.5 c**	**51.5 ± 0.5 b**	**48.8 ± 0.5 a**	***p*-interaction value = 0.0398**
L^*	1	44.5 ± 2.2 β, A	44.0 ± 1.6 β, A	43.1 ± 1.3 α, A	46.1 ± 1.5 β, A	**44.4 ± 0.6 b**
	2	41.9 ± 0.4 $\alpha\beta$, A	40.3 ± 0.2 α, A	41.6 ± 3.7 α, A	42.1 ± 2.8 α, A	**41.5 ± 0.6 a**
	3	39.9 ± 2.5 α, A	40.4 ± 1.7 α, A	42.9 ± 2.8 α, AB	46.3 ± 0.5 β, B	**42.4 ± 0.6 a**
	global	**42.1 ± 0.7 a**	**41.5 ± 0.7 a**	**42.6 ± 0.7 a**	**44.8 ± 0.7 b**	***p*-interaction value = 0.1827**
H^*	1	363.4 ± 1.0 β, B	362.3 ± 2.1 β, AB	359.4 ± 0.6 α, A	360.8 ± 3.3 α, AB	**361.5 ± 0.5 b**
	2	358.5 ± 0.7 α, A	357.7 ± 0.1 α, A	357.9 ± 2.0 α, A	358.2 ± 2.5 α, A	**358.1 ± 0.5 a**
	3	356.6 ± 1.3 α, A	355.6 ± 0.7 α, A	357.6 ± 1.5 α, A	357.3 ± 0.3 α, A	**356.8 ± 0.5 a**
	global	**359.5 ± 0.5 a**	**358.5 ± 0.5 a**	**358.3 ± 0.5 a**	**358.8 ± 0.5 a**	***p*-interaction value = 0.1234**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). CI, color intensity; C^*, chroma; L^*, lightness; and H^*, hue. Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

Table 6. Anthocyanin Quantification by HPLC-DAD (Milligrams Malvidin-3-*O*-glucoside per Liter): Cabernet Sauvignon Wines[a]

parameter	maturity level	maceration length: 1 week	2 weeks	3 weeks	4 weeks	global
total anthocyanins	1	306.2 ± 19.9 α, D	250.9 ± 29.9 α, C	207.6 ± 18.9 α, B	139.4 ± 20.7 α, A	**226.1 ± 5.2 a**
	2	288.0 ± 11.9 α, B	253.3 ± 5.4 α, B	195.8 ± 36.5 α, A	185.3 ± 3.3 β, A	**230.6 ± 5.2 a**
	3	414.1 ± 11.7 β, D	380.1 ± 7.0 β, C	315.0 ± 8.8 β, B	266.4 ± 5.8 γ, A	**343.9 ± 5.2 b**
	global	**336.1 ± 6.0 d**	**294.8 ± 6.0 c**	**239.5 ± 6.0 b**	**197.1 ± 6.0 a**	***p*-interaction value = 0.0744**
nonacylated anthocyanins	1	209.4 ± 11.6 α, C	173.5 ± 22.2 α, B	144.2 ± 13.4 α, B	96.6 ± 14.9 α, A	**155.9 ± 3.6 a**
	2	210.0 ± 4.5 α, C	183.4 ± 3.3 α, B	148.0 ± 25.4 α, A	139.1 ± 3.5 β, A	**170.1 ± 3.6 b**
	3	296.1 ± 7.0 β, D	276.0 ± 4.7 β, C	230.6 ± 5.1 β, B	197.8 ± 3.9 γ, A	**250.1 ± 3.6 c**
	global	**238.5 ± 4.1 d**	**211.0 ± 4.1 c**	**174.3 ± 4.1 b**	**144.5 ± 4.1 a**	***p*-interaction value = 0.0804**
acetylated anthocyanins	1	84.1 ± 7.5 β, D	67.2 ± 6.5 β, C	55.4 ± 4.4 β, B	37.9 ± 5.2 α, A	**61.1 ± 1.5 b**
	2	63.4 ± 6.1 α, B	58.0 ± 1.5 α, B	40.0 ± 9.0 α, A	38.7 ± 1.5 α, A	**50.0 ± 1.5 a**
	3	100.9 ± 4.5 γ, D	89.0 ± 2.1 γ, C	72.7 ± 3.5 γ, B	80.5 ± 1.5 β, A	**80.5 ± 1.5 c**
	global	**82.8 ± 1.7 d**	**71.4 ± 1.7 c**	**56.1 ± 1.7 b**	**45.3 ± 1.7 a**	***p*-interaction value = 0.0346**
p-coumarylated anthocyanins	1	12.7 ± 1.0 α, D	10.3 ± 1.1 α, C	8.1 ± 1.2 α, B	4.9 ± 0.9 α, A	**9.0 ± 0.3 a**
	2	14.6 ± 1.6 α, C	11.9 ± 0.6 β, B	7.8 ± 2.2 α, A	7.5 ± 0.2 β, A	**10.4 ± 0.3 b**
	3	17.2 ± 0.4 β, D	15.1 ± 0.1 γ, C	11.7 ± 0.4 β, B	9.2 ± 0.2 γ, A	**13.3 ± 0.3 c**
	global	**14.8 ± 0.3 d**	**12.4 ± 0.3 c**	**9.2 ± 0.3 b**	**7.2 ± 0.3 a**	***p*-interaction value = 0.3105**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

In the first harvest, when the grapes were very unripe, no changes were observed in the proanthocyanidin concentration between the first and second weeks of maceration, and it was necessary to wait until the third week of maceration to observe any significant increase. In the second harvest, when grapes were more or less ripe, the proanthocyanidin concentration increased significantly until the third week of maceration, when the values stabilized. Finally, in the third harvest, when the grapes were very ripe, the proanthocyanidin concentration increased significantly until the second week of maceration, which indicates that

Table 7. Anthocyanins Quantification by HPLC-DAD (Milligrams Malvidin-*O*-3-glucoside per Liter): Tempranillo Wines[a]

		maceration length				
parameter	maturity level	1 week	2 weeks	3 weeks	4 weeks	global
total anthocyanins	1	168.5 ± 32.7 α, B	134.7 ± 24.7 α, B	30.3 ± 11.6 α, A	47.5 ± 22.5 α, A	**95.3 ± 5.6s**
	2	194.4 ± 8.6 α, C	138.8 ± 10.9 α, B	118.6 ± 22.7 β, AB	87.4 ± 16.9 β, A	**134.8 ± 6.0 b**
	3	267.5 ± 22.8 β, C	229.5 ± 20.9 β, B	201.4 ± 14.9 γ, B	162.1 ± 7.4 γ, A	**215.1 ± 5.6 c**
	global	**210.2 ± 6.5 c**	**167.7 ± 6.5 b**	**116.8 ± 6.5 a**	**99.0 ± 7.0 a**	***p*-interaction value = 0.0159**
nonacylated anthocyanins	1	131.3 ± 27.7 α, B	95.7 ± 26.5 α, B	16.3 ± 5.2 α, A	31.5 ± 17.0 α, A	**68.7 ± 4.9 a**
	2	162.8 ± 6.0 α, C	117.9 ± 9.2 α, B	101.5 ± 198 β, AB	75.2 ± 14.7 β, A	**14.4 ± 5.2 b**
	3	208.2 ± 20.7 β, C	180.1 ± 17.7 β, BC	158.7 ± 12.2 γ, B	127.2 ± 5.7 γ, A	**168.5 ± 4.9 c**
	global	**167.4 ± 5.7 c**	**131.2 ± 5.7 b**	**92.2 ± 5.7 a**	**77.9 ± 6.1 a**	***p*-interaction value = 0.0291**
acetylated anthocyanins	1	25.0 ± 4.1 β, B	29.6 ± 2.6 β, B	12.6 ± 6.0 α, A	13.8 ± 6.0 $\alpha\beta$, A	**20.3 ± 0.9 b**
	2	15.0 ± 1.5 α, C	10.3 ± 0.7 α, B	8.5 ± 0.9 α, AB	6.7 ± 0.9 α, A	**10.1 ± 1.0 a**
	3	31.1 ± 1.0 γ, C	28.1 ± 1.8 β, BC	24.9 ± 1.7 β, B	20.4 ± 1.6 β, A	**26.1 ± 0.9 c**
	global	**23.7 ± 1.0 b**	**22.7 ± 1.0 b**	**15.3 ± 1.0 a**	**13.7 ± 1.1 a**	***p*-interaction value = 0.0078**
p-coumarylated anthocyanins	1	12.3 ± 3.4 α, B	9.4 ± 0.8 α, B	1.4 ± 0.4 α, A	2.2 ± 1.1 α, A	**6.3 ± 0.5 a**
	2	16.6 ± 1.4 α, C	10.6 ± 1.2 α, B	8.5 ± 1.9 β, AB	5.5 ± 1.2 β, A	**10.3 ± 0.6 b**
	3	28.2 ± 34 β, C	21.2 ± 1.9 β, B	17.8 ± 1.5 γ, AB	14.6 ± 0.3 γ, A	**20.4 ± 0.5 c**
	global	**19.0 ± 0.6 c**	**13.7 ± 0.6 b**	**9.2 ± 0.6 a**	**7.4 ± 0.7 a**	***p*-interaction value = 0.1277**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

Table 8. Proanthocyanidin Analysis by Phloroglucinolysis: Cabernet Sauvignon Wines[a]

		maceration length				
parameter	maturity level	1 week	2 weeks	3 weeks	4 weeks	global
total PA (mg/L)	1	1007 ± 72 α, A	1116 ± 91 α, A	1830 ± 87 α, B	1751 ± 82 α, B	**1426 ± 50 a**
	2	1162 ± 151 α, A	1574 ± 25 $\alpha\beta$, B	2135 ± 83 α, C	2101 ± 170 $\alpha\beta$, C	**1743 ± 52 b**
	3	1281 ± 125 α, A	2088 ± 332 β, B	2066 ± 267 α, B	2171 ± 192 β, B	**1902 ± 52 c**
	global	**1150 ± 59 a**	**1593 ± 63 b**	**2010 ± 59 c**	**2008 ± 53 c**	***p*-interaction value = 0.0434**
mDP	1	8.34 ± 0.49 α, C	6.90 ± 0.29 α, B	6.31 ± 0.31 α, AB	6.14 ± 0.42 α, A	**6.93 ± 0.13 a**
	2	8.92 ± 0.5 α, C	6.90 ± 0.27 α, B	6.52 ± 0.28 α, AB	6.03 ± 0.25 α, A	**7.09 ± 0.14 a**
	3	10.50 ± 0.53 β, B	12.15 ± 0.83 β, C	8.93 ± 0.16 β, A	8.26 ± 0.68 β, A	**9.96 ± 0.14 b**
	global	**9.25 ± 0.16 c**	**8.65 ± 0.16 b**	**7.26 ± 0.16 a**	**6.81 ± 0.15 a**	***p*-interaction value = 0.0000**
%PD	1	30.8 ± 0.2 α, C	24.8 ± 0.8 α, B	23.1 ± 0.9 α, A	23.1 ± 0.6 α, A	**25.4 ± 0.2 a**
	2	33.6 ± 0.8 β, C	27.3 ± 0.7 β, B	25.2 ± 0.7 β, A	24.2 ± 0.8 α, A	**27.6 ± 0.2 b**
	3	34.4 ± 0.6 β, C	29.4 ± 0.2 γ, B	27.5 ± 1.2 γ, A	26.7 ± 0.2 β, A	**29.5 ± 0.2 c**
	global	**32.9 ± 0.2 c**	**27.1 ± 0.2 b**	**25.3 ± 0.2 a**	**24.7 ± 0.2 a**	***p*-interaction value = 0.2941**
%Gal	1	3.9 ± 0.3 α, A	6.3 ± 0.5 β, B	6.3 ± 0.2 α, B	6.1 ± 0.1 β, B	**5.7 ± 0.1 b**
	2	3.4 ± 0.0 α, A	5.3 ± 0.1 α, B	5.6 ± 0.6 α, B	5.6 ± 0.3 α, B	**5.0 ± 0.1 a**
	3	3.3 ± 0.4 α, A	5.9 ± 0.0 $\alpha\beta$, B	5.6 ± 0.3 α, B	5.4 ± 0.1 α, B	**5.0 ± 0.1 a**
	global	**3.5 ± 0.1 a**	**5.8 ± 0.1 b**	**5.8 ± 0.1 b**	**5.7 ± 0.1 b**	***p*-interaction value = 0.6579**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). Total PA, total proanthocyanidins; mDP, mean degree of polymerization; %PD, percentage of prodelphinidins; %Gal, percentage of galloylation. Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

Table 9. Proanthocyanidin Analysis by Phloroglucinolysis: Tempranillo Wines[a]

parameter	maturity level	maceration length 1 week	2 weeks	3 weeks	4 weeks	global
total PA (mg/L)	1	1049 ± 96 α, A	1238 ± 100 α, AB	1248 ± 10 α, AB	1304 ± 128 β, B	**1211 ± 34 a**
	2	1150 ± 128 α, A	1084 ± 234 α, A	1176 ± 120 α, A	1076 ± 51 α, A	**1121 ± 36 a**
	3	1061 ± 74 α, A	1266 ± 153 α, AB	1178 ± 77 α, A	1415 ± 46 β, B	**1229 ± 37 a**
	global	**1087 ± 42 a**	**1197 ± 42 ab**	**1201 ± 40 ab**	**1264 ± 40 b**	***p*-interaction value = 0.1131**
mDP	1	9.06 ± 0.15 β, C	7.27 ± 0.18 α, B	6.21 ± 0.22 α, A	6.24 ± 0.29 α, A	**7.19 ± 0.08 a**
	2	9.35 ± 0.37 β, C	7.76 ± 0.44 α, B	7.02 ± 0.17 β, A	6.56 ± 0.33 α, A	**7.67 ± 0.08 b**
	3	7.27 ± 0.35 α, B	8.9 ± 0.02 β, C	7.57 ± 0.03 γ, B	6.34 ± 0.05 α, A	**7.52 ± 0.09 b**
	global	**8.56 ± 0.10 d**	**7.97 ± 0.10 c**	**6.93 ± 0.10 b**	**6.38 ± 0.10 a**	***p*-interaction value = 0.0000**
%PD	1	18.9 ± 0.2 β, C	17.8 ± 0.1 α, B	16.6 ± 0.6 α, A	16.8 ± 0.2 α, A	**17.5 ± 0.3 a**
	2	18.3 ± 1.3 β, A	17.5 ± 1.4 α, A	18.4 ± 0.8 α, A	17.2 ± 1.1 α, A	**17.8 ± 0.3 a**
	3	15.4 ± 1.0 α, A	27.2 ± 0.7 β, C	24.6 ± 4.0 β, BC	22.0 ± 0.7 β, B	**22.3 ± 0.4 b**
	global	**17.5 ± 0.4 a**	**20.8 ± 0.4 b**	**19.9 ± 0.4 b**	**18.7 ± 0.4 a**	***p*-interaction value = 0.0000**
%Gal	1	2.8 ± 0.1 β, A	3.2 ± 0.0 β, B	4.1 ± 0.3 β, C	3.8 ± 0.1 β, C	**3.5 ± 0.1 c**
	2	2.3 ± 0.1 α, A	2.7 ± 0.2 α, B	2.9 ± 0.1 α, B	3.6 ± 0.2 β, C	**2.9 ± 0.1 b**
	3	3.0 ± 0.2 β, B	2.3 ± 0.3 α, A	2.5 ± 0.5 α, AB	2.6 ± 0.1 α, AB	**2.6 ± 0.1 a**
	global	**2.7 ± 0.1 a**	**2.8 ± 0.1 a**	**3.2 ± 0.1 b**	**3.4 ± 0.1 b**	***p*-interaction value = 0.0000**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). Total PA, total proanthocyanidins; mDP, mean degree of polymerization; %PD, percentage of prodelphinidins; %Gal, percentage of galloylation. Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

thorough extraction was achieved quickly. It seems therefore that the riper the grapes are, the faster the solubilization of proanthocyanidins.

In contrast, the total proanthocyanidin concentration in Tempranillo wines was not affected by the maturity level of the grapes. In fact, no significant differences in this parameter were found at any maturity level with the only exception being the second harvest and 4 weeks of maceration. These unexpected data seem to indicate that the Tempranillo grapes used in this study were not really well-ripened, at least as far as the skins and seeds are concerned. Nevertheless, maceration length affects Tempranillo in a similar way as Cabernet Sauvignon wines inasmuch as the global proanthocyanidin concentration was greater when the maceration was longer,[45] despite this behavior not being shown in all maturity levels.

In general terms, the mDP of proanthocyanidins of wines from both cultivars increased significantly when the grapes were riper. This increase in mDP throughout ripening has previously been described by other authors.[27,28] As is well-known, seed proanthocyanidins have a lower mDP than skin proanthocyanidins.[11,59−61] Consequently, the mDP of wines from riper grapes may be higher for two reasons. First, the mDP of grape proanthocyanidins increases with maturity or, second, riper grapes release a higher proportion of proanthocyanidins from skins than from seeds. Both alternatives are possible. In our particular case, the increase in mDP in wines from riper grapes was much clearer in Cabernet Sauvignon than in Tempranillo wines, which indicates, as has been mentioned above, that Tempranillo skins and seeds are less ripe than those of Cabernet Sauvignon.

Maceration length also had a significant effect on the proanthocyanidin mDP of wines from both cultivars. In the wines from the first and second harvests the mDP decreased continuously throughout maceration time. In contrast, the wines from the third harvest of both cultivars behaved somewhat differently because the mDP increased between the first and second weeks and then decreased significantly. These interesting data suggest that the solubilization kinetics of skin and seed proanthocyanidins are different and confirm that proanthocyanidins are released more quickly from skins than from seeds.[10,45]

Moreover, the maturity level of grapes also seems to have a different effect on proanthocyanidin extraction kinetics from skins and seeds during the wine maceration process. The observed increase in proanthocyanidin mDP between the first and second weeks of maceration in wines from both cultivars in the third harvest suggests that the riper grapes can release higher amounts of skin proanthocyanidins and for a longer time. These data also suggest that seed proanthocyanidins from riper grapes are released more slowly.

The percentage of prodelphinidins also supports these findings. Specifically, the proportion of prodelphinidins tends to increase in wines from the riper grapes and to decrease throughout maceration time in both cultivars. Because prodelphinidins are present only in skins,[9,10] these data confirm that maturation increases the amount of skin proanthocyanidins released into wine. The changes in the percentage of galloylation also support this behavior. It is well-known that seed proanthocyanidins have a higher presence of (−)-epicatechin gallate than skin proanthocyanidins.[10,11] Consequently, the observed decrease in this percentage when the grapes are riper indicates that the contribution of seeds to total wine proanthocyanidins tends to decrease with maturity.

These results also indicate that the maceration length significantly affects the percentage of prodelphinidins and galloylation. These two parameters behaved quite differently as

Table 10. Polysaccharide Analysis by HRSEC (Milligrams Polysaccharide per Liter): Cabernet Sauvignon Wines[a]

parameter	maturity level	maceration length: 1 week	2 weeks	3 weeks	4 weeks	global
total polysaccharides	1	463.0 ± 18.1 α, A	552.4 ± 28.2 αβ, B	613.2 ± 2.6 α, C	677.1 ± 26.5 αβ, D	**576.4 ± 6.8 a**
	2	488.5 ± 45.5 α, A	542.2 ± 9.6 α, A	667.9 ± 21.5 β, B	658.6 ± 16.2 α, B	**589.3 ± 6.8 a**
	3	610.9 ± 12.4 β, A	591.1 ± 23.2 β, A	653.5 ± 14.7 β, B	710.1 ± 0.7 β, C	**641.4 ± 6.8 b**
	global	**520.8 ± 8.6 a**	**561.1 ± 7.5 b**	**644.9 ± 7.5 c**	**682.0 ± 8.1 d**	***p*-interaction value = 0.0009**
HMW polysaccharides	1	119.7 ± 0.1 α, A	134.5 ± 7.4 α, B	161.8 ± 7.2 β, C	171.6 ± 7.2 β, C	**147.7 ± 3.3 b**
	2	125.5 ± 8.6 α, A	130.1 ± 9.1 α, A	155.9 ± 13.7 β, B	199.0 ± 31.9 β, C	**154.6 ± 3.3 b**
	3	115.7 ± 4.6 α, A	121.3 ± 7.2 α, A	132.8 ± 9.2 α, AB	142.4 ± 9.8 α, B	**125.5 ± 3.3 a**
	global	**120.3 ± 4.1 a**	**128.6 ± 3.6 a**	**152.0 ± 3.6 b**	**169.4 ± 3.8 c**	***p*-interaction value = 0.0083**
MMW polysaccharides	1	200.7 ± 8.4 α, A	240.1 ± 23.5 α, B	250.4 ± 19.3 α, BC	290.9 ± 18.0 β, C	**247.5 ± 4.9 a**
	2	225.0 ± 34.6 α, A	252.0 ± 4.6 αβ, A	304.7 ± 2.6 β, B	256.1 ± 4.3 α, A	**259.8 ± 4.9 a**
	3	243.9 ± 7.1 α, A	277.4 ± 9.0 β, B	303.0 ± 1.8 β, BC	318.0 ± 17.4 γ, C	**284.7 ± 4.9 b**
	global	**223.2 ± 6.2 a**	**256.5 ± 5.4 b**	**288.2 ± 5.4 c**	**288.2 ± 5.8 c**	***p*-interaction value = 0.0220**
LMW polysaccharides	1	142.5 ± 9.8 α, A	177.8 ± 12.5 αβ, A	186.6 ± 15.4 α, B	222.3 ± 21.7 α, C	**181.2 ± 4.6 a**
	2	138.0 ± 17.8 α, A	160.1 ± 13.3 α, A	194.8 ± 5.2 α, B	203.5 ± 11.4 α, B	**174.9 ± 4.6 a**
	3	251.2 ± 24.0 β, B	192.4 ± 16.7 β, A	231.4 ± 18.9 β, B	236.8 ± 23.4 β, B	**231.2 ± 4.6 b**
	global	**177.3 ± 5.8 a**	**176.8 ± 5.1 a**	**204.6 ± 5.1 b**	**224.3 ± 5.5 c**	***p*-interaction value = 0.0012**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). HMW, high molecular weight fraction (MW > 75 kDa); MMW, medium molecular weight fraction (75 kDa > MW > 15 kDa); LMW, low molecular weight fraction (MW < 15 kDa). Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

Table 11. Polysaccharide Analysis by HRSEC (Millgrams Polysaccharide per Liter): Tempranillo Wines[a]

parameter	maturity level	maceration length: 1 week	2 weeks	3 weeks	4 weeks	global
total polysaccharides	1	369.8 ± 11.0 α, A	429.1 ± 34.5 α, BC	405.0 ± 12.3 α, AB	445.5 ± 14.9 α, C	**412.4 ± 15.0 a**
	2	385.1 ± 59.6 α, A	501.9 ± 95.9 α, A	503.6 ± 96.8 α, A	505.3 ± 47.3 β, A	**473.9 ± 15.0 b**
	3	350.2 ± 50.1 α, A	468.8 ± 47.1 α, B	453.0 ± 36.0 α, B	570.2 ± 10.8 γ, C	**460.6 ± 15.9 b**
	global	**368.4 ± 17.3 a**	**466.5 ± 18.7 bc**	**453.9 ± 17.3 b**	**507.0 ± 17.3 c**	***p*-interaction value = 0.2635**
HMW polysaccharides	1	119.0 ± 7.1 α, A	132.7 ± 6.6 α, B	139.0 ± 9.8 α, C	168.6 ± 16.7 α, D	**138.8 ± 2.8 a**
	2	120.8 ± 11.3 α, A	164.8 ± 15.0 β, B	167.3 ± 20.1 β, BC	191.1 ± 10.1 β, C	**161.9 ± 2.8 b**
	3	128.2 ± 6.5 α, A	156.6 ± 17.0 αβ, B	175.7 ± 8.8 β, C	195.3 ± 4.6 β, D	**164.8 ± 3.0 b**
	global	**122.7 ± 3.3 a**	**151.4 ± 3.5 b**	**164.6 ± 3.3 c**	**181.9 ± 3.3 d**	***p*-interaction value = 0.0787**
MMW polysaccharides	1	147.5 ± 11.2 α, A	175.7 ± 14.8 α, B	143.1 ± 12.4 α, A	185.5 ± 22.7 α, B	**162.2 ± 5.6 a**
	2	146.9 ± 25.7 α, A	182.6 ± 31.0 α, A	181.5 ± 47.1 α, A	188.3 ± 20.1 αβ, A	**176.8 ± 5.6 a**
	3	122.0 ± 14.2 α, A	158.9 ± 14.1 α, B	169.5 ± 10.0 α, B	198.6 ± 4.1 β, C	**161.0 ± 5.9 a**
	global	**138.8 ± 6.5 a**	**172.4 ± 7.0 bc**	**168.1 ± 6.5 b**	**187.4 ± 6.5 c**	***p*-interaction value = 0.2120**
LMW polysaccharides	1	103.3 ± 7.9 α, A	120.7 ± 13.7 α, A	104.3 ± 6.2 α, A	119.2 ± 21.0 α, A	**111.3 ± 7.4 a**
	2	117.4 ± 23.5 α, A	154.2 ± 54.9 α, A	137.1 ± 48.0 α, A	125.9 ± 17.1 α, A	**135.2 ± 7.4 b**
	3	99.9 ± 32.2 α, A	153.2 ± 16.0 α, BC	139.8 ± 41.9 α, AB	180.3 ± 9.7 β, C	**134.8 ± 7.9 b**
	global	**106.1 ± 8.6 a**	**142.7 ± 9.3 b**	**121.1 ± 8.6 b**	**137.6 ± 8.6 b**	***p*-interaction value = 0.1799**

[a]Triplicate data are expressed as the average values of three replicates ± standard deviation ($n = 3$). Global data for maturity level and maceration length are expressed as the average values ± standard error ($n = 12$ for maturity level, $n = 9$ for maceration length). HMW, high molecular weight fraction (MW > 75 kDa); MMW, medium molecular weight fraction (75 kDa > MW > 15 kDa); LMW, low molecular weight fraction (MW < 15 kDa). Different letters indicate statistical differences ($p < 0.05$). Greek letters are used to compare the wines of the same maceration length and different maturity level by one-way ANOVA. Capital Roman letters are used to compare the wines of the same maturity level and different maceration length by one-way ANOVA. Small Roman letters are used to compare all data by two-factor ANOVA.

the percentage of prodelphinidins decreased throughout maceration, whereas the percentage of galloylation increased. Because seeds have no (−)-epigallocatechin and have a higher proportion of (−)-epicatechin gallate,[11] these results also

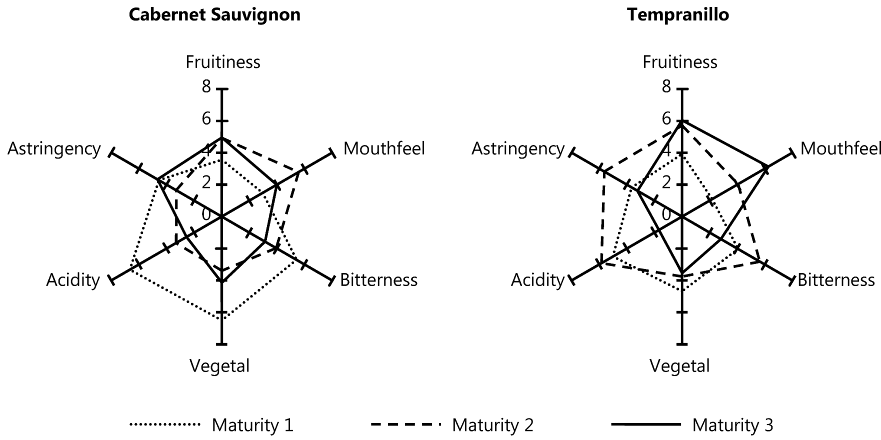

Figure 1. Cobweb diagram of six sensory attributes (fruitiness, mouthfeel, bitterness, vegetal, acidity, and astringency) obtained from sensory analysis of wines elaborated with three different maturity levels, comparing samples with different maturity levels and with the same maceration length.

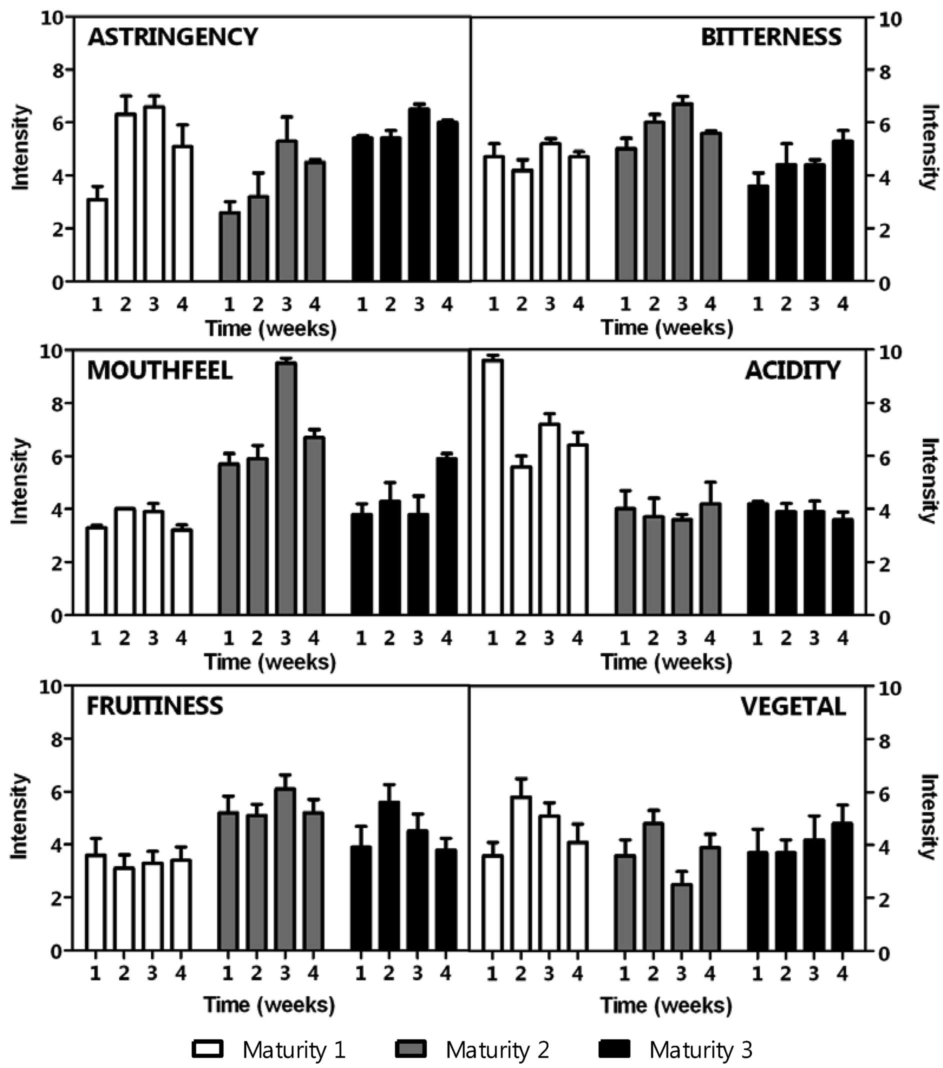

Figure 2. Sensory analysis results for the wines elaborated with cv. Cabernet Sauvignon comparing the four different macerations lengths (from 1 to 4 weeks) for each maturity level.

support that skin proanthocyanidins are released more quickly than seed proanthocyanidins.

Tables 10 and 11 show the polysaccharide concentration of Cabernet Sauvignon and Tempranillo wines, respectively. In general terms, the total wine polysaccharide concentration tended to increase with maturity in both cultivars and at any maceration time. However, the increase was slight and, sometimes, nonsignificant. The molecular weight fractions also tended to increase. The one exception was the medium molecular weight fraction of Tempranillo wines, which did not

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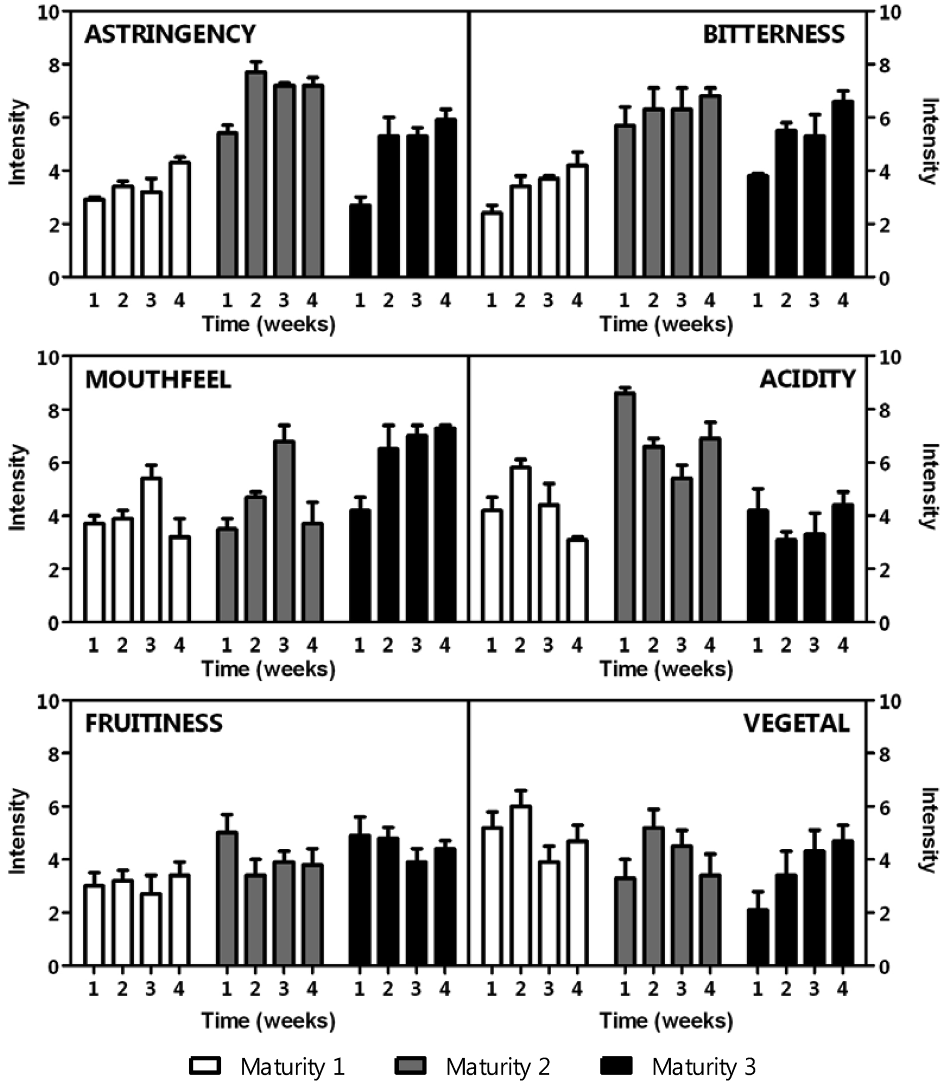

Figure 3. Sensory analysis results for the wines elaborated with cv. Tempranillo, comparing the four different maceration lengths (from 1 to 4 weeks) for each maturity level.

change, and the high molecular weight fraction of Cabernet Sauvignon wines, which decreased in wines from the ripest grapes.

Theoretically, the progressive pectin degradation that takes place throughout ripening in skin cell walls[30,31] should favor polysaccharide solubilization in the grape juice. Consequently, wines from riper grapes should have a higher polysaccharide concentration. However, the higher ethanol concentration of wines from riper grapes could also induce greater precipitation of polysaccharides, which would explain why their concentration increased only slightly with maturation.

In contrast, the polysaccharide concentration increased significantly with maceration time in both cultivars and at any ripening stage. This effect, which was much clearer than that exerted by maturity, was also observed in nearly all molecular weight fractions. Fanzone et al.[62] have reported that highly prized Argentinean wines contain significantly higher polysaccharide concentration than cheaper ones, which was related with the fact that these wines are usually elaborated with longer macerations.

It seems quite logical that this increase in polysaccharide concentration came from two possible sources. The first was direct polysaccharide solubilization from skins due to the longer contact time, and the second was the release of yeast mannoprotein and polysaccharides. However, the analytical procedure used cannot distinguish among the different types of polysaccharide, so it is not possible to establish the extent to which they all contribute.

Figure 1 shows two cobweb diagrams that compare the sensory attributes of wines from both cultivars at the three different levels of grape maturity. The diagrams were built using the average value of the second and third weeks of maceration for each maturity level. In general, astringency, acidity, bitterness, and vegetal notes tended to decrease when the grapes were riper, whereas fruitiness and mouthfeel tended to increase. There were some exceptions to this behavior. For example, in Tempranillo wines the astringency of the second harvest was greater than that of the first harvest, probably because the wines of the second harvest were more tannic. The mouthfeel of second-harvest Cabernet Sauvignon wines was higher than in the riper grapes. However, the general tendency confirms that riper grapes produce fruitier and full-bodied wines, which are less acidic, less vegetal, less astringent, and less bitter.

Figure 2 compares the sensory attributes of wines as a function of maceration time for the three maturity levels for Cabernet Sauvignon and Figure 3, for Tempranillo.

In the case of Tempranillo wines, astringency, bitterness, and mouthfeel tended to increase when macerations were longer. However, a decrease in mouthfeel was observed between the third and fourth weeks of maceration of the less ripe grapes. In contrast, no clear tendency was detected in the other sensory attributes, except in the case of the wines from the third harvest, in which vegetal notes increased with the maceration length. In the case of Cabernet Sauvignon wines, astringency and bitterness tended to increase when maceration was longer, although this was not so in all cases. The increase in astringency was particularly clear in the wines from the less ripe grapes, whereas no increase in bitterness was observed in the wines from the first harvest. Mouthfeel also tended to increase with maceration length, but this was less clear than in the case of Tempranillo. No clear trend was observed in acidity, fruitiness, or vegetal notes.

It can be concluded that grape maturity and maceration length really have a considerable influence on the color, chemical composition, and sensory quality of wines. In general, color intensity and the concentrations of anthocyanins, proanthocyanidins, and polysaccharides are higher when the grapes are riper. The changes in proanthocyanidins are also interesting inasmuch as the percentages of mDP and prodelphinidin are greater and the galloylation percentage is lower in wines from riper grapes. This suggests that grape maturity favors skin proanthocyanidin extraction. These chemical changes may explain the differences observed in some of the sensory attributes of these wines. Specifically, the decrease in astringency and bitterness may be associated with the increase in prodelphinidins and polysaccharides and with the decrease in the percentage of proanthocyanidin galloylation. On the other hand, when macerations were longer, color and anthocyanin concentration tended to decrease, whereas polysaccharide and proanthocyanidin concentration tended to increase. The mDP and prodelphinidin percentage also decreased and the galloylation percentage increased when the maceration time was longer. This suggests that the maceration length favors proanthocyanidin extraction from seeds. In this case, these chemical changes may also explain the differences observed in some of the sensory attributes. The increase in astringency and bitterness may be associated with the increase in proanthocyanidins and the percentage of galloylation, whereas the increase in mouthfeel may be related to the increase in polysaccharide concentration. Further studies are needed to better understand how maturity and maceration length influence the chemical composition of wine and, in particular, to understand the relationship between chemical composition and some of the key sensory attributes of red wines such as astringency, bitterness, and mouthfeel.

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