Comparison between the phenolic composition of Petit Verdot wines elaborated at different maceration/fermentation temperatures

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\textbf{ABSTRACT}

The objective of this work was to contribute to the knowledge of the relationship between phenolic composition and the temperature of maceration/fermentation on Petit Verdot red wines, which were made with three treatments (17°C, 21°C, and 25°C). The phenolic compounds of \textit{Vitis vinifera} cv. Petit Verdot wines elaborated at different maceration/fermentation temperatures were determined and compared by high-performance liquid chromatography coupled with diode array detector and electrospray ionization mass spectrometry. A total of 45 phenolic compounds were detected in all wines. The phenolic composition was affected by the temperature of maceration. The increment of maceration/fermentation temperature had a positive effect on the total concentration of the phenolic compounds and chromatic characteristics of these wines. The colour of Petit Verdot young red wines showed more colour intensity and their luminosity descended due to the increase in temperature. The highest total content of anthocyanins was determined in wines macerated at 21°C. However, other groups of phenolic compounds (flavonols, proanthocyanidins, and stilbenes) increased their total content by raising the maceration/fermentation temperature, reaching maximum values at 25°C.

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\textbf{Introduccion}

The phenolic content of red wine is responsible for the colour, mouthfeel, and ageing potential of the wine\textsuperscript{[1,2]}. The phenolic content of the grape is distributed throughout the various tissues of the berry, with the skin, seed, and pulp each possessing a distinct phenolic content. In the winemaking process, phenolic compounds are extracted from grapes into the wine during the maceration step\textsuperscript{[3]}. The diffusion of the different polyphenols from grape to must-wine and, consequently, their extractability and final concentration at the end of the maceration process largely depends on their location in the berry and the characteristics of the different grape varieties, especially those related to the phenolic composition content and the extractability of these polyphenols\textsuperscript{[4]}

Most phenolic compounds found in red wines may be grouped into two classes based on fundamental chemical structures: the flavonoids and the nonflavonoids\textsuperscript{[5]}. Flavonoids, located in grape skins, seeds, and stems, include anthocyanins, flavan-3-ol monomers, oligomeric and polymeric proanthocyanidins, flavonols, flavanones, and flavones. Nonflavonoids, which derive primarily from the pulp and skins of grape berries, include hydroxycinnamic and hydroxybenzoic acids, resveratrol, and its derivatives. All are important constituents of both grapes and wine due to their direct and indirect contribution to wine sensorial properties such as colour, flavour, astringency, bitterness, and structure of the wines\textsuperscript{[6]}

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The phenolic compound concentration and the distribution of the individual phenolic species in each class vary across different wines and are affected by the grape variety, growing conditions, and the winemaking practices employed.\(^7\) Although many fermentation parameters and winemaking techniques affect phenolic extraction, it is generally agreed that one of the prime factor is fermentation temperature.\(^2\) Fermentation temperature\(^8,9\) and maceration duration\(^7\) also impact significantly in red wine style and quality.

Elevating fermentation temperature has frequently been used to get a good extraction of phenolic compounds from the skins and to obtain wines with colour, body, and structure,\(^9\) increasing the total polyphenol content in wines.\(^8\) When the temperature at the end of the fermentation is increased, a promotion of colour extraction and an increase in the concentration of phenolic compounds have been observed in Pinot noir wines, but, at the same time, the wine is exposed to the risk of increasing the concentration of volatile phenols.\(^10\) Nevertheless, studies demonstrated that Cabernet Sauvignon wines elaborated at low fermentation temperature (15°C) showed higher total phenolic content and better chromatic parameters due to the maceration time increase.\(^11\) The objective of this work was to contribute to the knowledge of the relationship between phenolic composition and the temperature of maceration/fermentation. The effects of three maceration/fermentation temperatures (17°C, 21°C, and 25°C) in red wine phenolic composition have been studied.

**Materials and methods**

**Fermentation assays**

The experimental work was carried out with Petit Verdot grapes from Mancha Region (Spain), harvested during crop 2014 on the optimal timing of technological maturity: 25.80 Brix, 6.02 g/L total acidity, 3.21 pH, and 1.29 g/L L-malic acid. Just after the grapes were crushed, 50 mg/L of SO\(_2\) were added to musts.

Three maceration/fermentation temperatures were studied: 17°C, 21°C, and 25°C and all the experiments were carried out in triplicate using 100 L stainless steel tanks equipped with a wrap stainless steel cooling jacket and thermometer connected to a control temperature system. Alcoholic fermentation was induced by inoculating *S. cerevisiae* VN* (Uvaferm VN* Lallemand Inc., Zug, Switzerland), according to the manufacturer’s instructions, and daily tanks were manually punched down. The evolution of the fermentation was followed by daily measurement of density and wine was pressed when a density of 995 g/L was reached. Alcoholic fermentation was then finished at room temperature, as determined by glucose and fructose levels.

At the end of alcoholic fermentation, wines were decanted, free SO\(_2\) was adjusted to 30 mg/L to prevent the development of the malolactic fermentation, and all wines were stored at 5°C for 30 days. Subsequently, wines were decanted again and cold stabilized at −5°C for 15 days, free SO\(_2\) level was again adjusted to 30 mg/L, and the wines were filtered with 0.2 micras, bottled, and stored at 18 ± 2°C for 3 months.

**Chemical analysis**

The wines were analytically characterized. The following parameters were determined: alcohol content, total acidity (expressed as tartaric acid), pH, volatile acidity (expressed as acetic acid), L-malic acid, colour intensity, and tonality following the official analytical methods established by the International Organisation of Vine and Wine.\(^{12}\)

Colour parameters were obtained following the OIV method for the determination of chromatic characteristics according to CIELab (Resolution Oeno 1/2006) Method OIV-MA AS2-11 using an Agilent 8453 diode array spectrophotometer (Agilent Technologies, Santa Clara CA, USA), with a homemade program for spectra treatment. The measuring conditions were transmittance between 770 and 380 nm at 5-nm intervals, 1-mm cuvettes, D65 illuminant, and a 10 reference pattern observer. Results expressed were referred to 1-cm optical length.\(^{12}\)
**Sample preparation for flavonols analysis**

PCX SPE cartridges (500 mg, 6 mL; Bond Elut Plexa, Agilent, Palo Alto, CA, USA) allowed the isolation of non-anthocyanin phenolic compounds from wines, and these anthocyanin-free fractions were used to analyse flavonols. To carry out this separation, 3 mL of wine were diluted with 3 mL of HCl 0.1 N, and the prepared samples were then passed through the solid phase extraction (SPE) cartridges that had been previously conditioned with 5 mL of methanol and 5 mL of water. After the cartridges were washed with 5 mL of HCl 0.1 N and 5 mL of water, the anthocyanin-free fraction was eluted with 3 × 5 mL of methanol. This eluate was dried in a rotary evaporator (35°C) and redissolved in 1.5 mL of 20% methanol in water and directly injected into the HPLC equipment.

**Analysis of monomeric phenolic compounds in wines by HPLC**

Individual phenolic compounds were determined by high-performance liquid chromatography coupled with diode array detector and electrospray ionization mass spectrometry following the conditions of previously described methods[13] to the use of narrow-bore, smaller particle size, chromatography columns. Analysis was performed on an Agilent 1100 series system equipped with a photodiode array detector and a LC/MSD Trap VL electrospray ionization mass spectrometry (ESI-MS/MS), both coupled to an Agilent ChemStation for data processing.

For anthocyanin analysis, wines were filtered (0.2 µm CHROMAFIL PET, Macherey-Nagel, Düren, Germany), and injection volume was 10 µL. Separation was achieved on a narrow-bore column Zorbax Eclipse XDB-C18 (2.1 × 150 mm; 3.5-µm particle; Agilent), with pre-column Zorbax Eclipse XDB-C8 (2.1 × 12.5 mm; 5-µm particle; Agilent), both thermostated at 40°C. Eluents used were (a) acetonitrile/water/formic acid (3:88.5:8.5 v/v/v) and (b) acetonitrile/water/formic acid (50:41.5:8.5 v/v/v). The linear solvents’ gradient for anthocyanin analysis was as follows: 0 min, 6% B; 10 min, 30% B; 30 min, 50% B; 34 min, 100% B; 36 min, 100% B; 42 min, 4% B; and 50 min, 4% B.

For non-anthocyanin analysis, free-anthocyanin fractions (see sample preparation for flavonols analysis) were filtered (0.2 µm CHROMAFIL PET, Macherey-Nagel, Düren, Germany), and injection volume was 20 µL. The same column was used while eluents were (a) acetonitrile/water/formic acid (3:88.5:8.5 v/v/v), (b) acetonitrile/water/formic acid (50:41.5:8.5 v/v/v), and (c) methanol/water/formic acid (90:1:5:8.5 v/v/v). The linear solvents’ gradient for non-anthocyanin analysis was as follows: 0 min, 2% B and 0% C; 8 min, 4% B and 0% C; 37 min, 17% B and 13% C; 51 min, 30% B and 20% C; 51.5 min, 40% B and 30% C; 56 min, 50% B and 50% C; 57 min, 50% B and 50% C; and 64 min, 4% B and 0% C. The use of a narrow-bore column allowed establishing a slow low rate (0.19 mL/min).

For identification, Ion Trap ESI-MS/MS detector was used in both positive (anthocyanins) and negative (flavonols) ion modes, setting the following parameters: dry gas N₂, 8 L/min; drying temperature, 325°C; nebulizer, N₂, 50 psi; ionization and fragmentation parameters were optimized by direct infusion of appropriate standard solutions (malvidin-3-O-glucoside in positive ionization mode; quercetin-3-O-glucoside in negative ionization mode); scan range, 50–1,200 m/z. Identification was based on spectroscopic data (UV-Vis and MS/MS) obtained from authentic standards, when available, or previously reported data.[13,14] Quantification was made using the diode-array detector (DAD) chromatograms recorded at 520 nm (anthocyanins), 360 nm (flavonols), and the calibration graphs of the respective standards ($R^2 > 0.999$). Quantification of noncommercial compounds was made according to the calibration graphs of the most similar compounds. Hence, anthocyanins were expressed as mg/L of malvidin-3-O-glucoside and flavonols were expressed as mg/L of quercetin-3-O-glucoside.

**Sample preparation for flavan-3-ols analysis**

Flavan-3-ols (monomers, B-type dimers, and polymeric proanthocyanidins) were isolated from wines using SPE on C18 cartridges (Sep-pak Plus C18, Waters Corp., Milford, MA; cartridges filled with 820 mg of adsorbent). A mixture of 2 mL of each wine with 6 mL of water was then passed through the
C18 cartridge, which had been previously conditioned with methanol (5 mL) and water (5 mL); after the cartridge was dried under reduced pressure, methanol (15 mL) and ethyl acetate (5 mL) were added in order to recover adsorbed phenolics; after the solvent was evaporated in a rotary evaporator (35°C), the residue was dissolved in methanol (2 mL) and stored at −18°C until analysis.

**Identification and quantification flavan-3-ols and stilbenes using multiple reaction monitoring HPLC-ESI-MS/MS**

The analysis was carried out using a HPLC Agilent 1200 series system equipped with DAD (Agilent, Germany) and coupled to an AB Sciex 3200 TRAP (Applied Biosystems) with triple quadrupole, turbo spray ionization (electrospray assisted by a thermonebulization) mass spectroscopy system (ESI-MS/MS). The chromatographic system was managed and the mass spectra data was processed using the Analyst MSD software (Applied Biosystems, version 1.5).

Structural information concerning the proanthocyanidins was obtained using the pyrogallol-induced acid-catalyzed depolymerization method.[15] The reaction consisted of adding 0.50 mL of the pyrogallol solution (100 g/L pyrogallol plus 20 g/L of ascorbic acid in 0.3 N HCl) to 0.25 mL of the sample in methanol and incubating 40 min at 30°C. The hydrolysis reaction was stopped by adding 2.25 mL of sodium acetate (67 mM). An aliquot of 2 mL of the reacted sample was placed in a vial and injected directly into the equipment for analysis.

The samples, before and after the acid-catalyzed depolymerization reaction, were injected (20 μL) into an Ascentis C18 reversed-phase column (150 mm × 4.6 mm with 2.7 μm of particle size) (Supelco, Bellefonte, USA), with the temperature controlled at 16°C. The solvents and gradients used for this analysis and the multiple reaction monitoring settings as well as all the mass transitions (m/z) for identification and quantitation were according to the methodology reported by Lago-vanzela.[14]

**Statistical analysis**

Data were subjected to the Student–Newman–Keuls test to identify any statistically significant differences between different maceration temperatures. The SPSS 12.0 software statistical package for Windows (Chicago, USA) was used for this analysis. Multivariate data analysis (principal component analysis (PCA)) was used to obtain an overview of the chemical and phenolic compounds analysed and to investigate possible correlations between the samples. SPSS 12.0 software (IBM, USA) was used for both analyses.

**Results and discussion**

**Oenological parameters and colour**

The oenological parameters are shown in Table 1. All wines presented glucose + fructose concentrations bellow 0.5 g/L (data not shown). Wines produced at 25°C showed significantly lower ethanol contents than the corresponding at 17°C and 21°C. Higher ethanol concentration at lower maceration/fermentation temperature has been previously documented.[16,17] In contrast, an increase in the content of glycerine is observed with increase in maceration/fermentation temperature. Several parameters have been shown to influence the final glycerol levels in wine, among them is the maceration/fermentation temperature.[18]

Oenological parameters of these wines were similar to those obtained in Petit Verdot wines elaborated in other regions.[19] Total and volatile acidity were lower in the wine obtained by the maceration at lower temperature (17°C) compared to wines made at higher temperature (21 and 25°C). Several studies have reported increased total and volatile acidity with increasing fermentation temperature.[9,17] Winemaking variables and techniques are known to affect the colour of red wines.
Less colour intensity was obtained in the wines made at 17°C. In this way, higher maceration/fermentation temperatures have been reported to increase phenolic extraction. In the Cielab parameters, although having not found statistically significant differences, it is observed that when the maceration/fermentation temperature decreased, the wines showed lower colour intensity and tonality described the change of red colour toward orange tones (higher values of a* and b* coordinates).

**Phenolic composition of wines**

**Anthocyanins**

Table 2 shows the concentrations (mg/L as malvidin-3-O-glucoside) of the anthocyanin monomers identified in the wines at different maceration/fermentation temperature. The completed series of nonacylated, acetylated, and p-coumaroylated anthocyanins of the five anthocyanidins usually found in *V. vinifera* grape varieties (delphinidin, cyanidin, petunidin, peonidin, and malvidin) were detected in these wines, as well as the 3-O-caffeoyl glucosides of malvidin and peonidin. Trisubstituted anthocyanins (delphinidin, petunidin, and malvidin) predominated over disubstituted ones (cyanidin and peonidin), being more abundant malvidin derivatives as expected in these wines. With regard to pyranoanthocyanins, vitisins A and B were detected in low concentration, two pyranoanthocyanins derived from the reaction of malvidin-3-glucoside with two yeast metabolites (pyruvic acid and acetaldehyde, respectively). All wines, regardless of maceration/fermentation temperature used for their elaboration, showed a greater amount of non-acylated anthocyanins than acylated ones. Anthocyanin profile and concentration of wines are largely dependent on grape variety and climatic and agronomic conditions, but also on the technology applied during winemaking and the reactions that take place during maturation and ageing in wood. The results showed a lower concentration of total anthocyanins, acylated anthocyanins, and pyranoanthocyanins in wines produced at 17°C, which seems to indicate lower extraction of anthocyanins at lower temperatures. Total anthocyanins content of wines under the three treatments were lower (380–419 mg/L) than those reported for Petit Verdot wines made at 24°C (542 mg/L). 

Maceration and fermentation at 21°C produced wines with higher total anthocyanin concentration (non-acylated and acylated). On the other hand, wines produced at 25°C showed similar concentration of non-acylated anthocyanins than those made at 17°C. With respect to the anthocyanidins, except cyanidin and delphinidin types whose concentrations are not modified by maceration/fermentation temperature, the rest of the anthocyanins were higher in wines elaborated at 21°C. With respect to pyranoanthocyanins, the contents of vitisin A were higher in wines macerated at 21°C and 25°C. These

| Table 1. Oenological and colour parameters at the end of alcoholic fermentation of Petit Verdot wines elaborated at different temperatures of maceration and fermentation. |
|---------------------------------|------------------|------------------|------------------|
|                                | 17°C             | 21°C             | 25°C             |
| Alcoholic content (% v/v)      | 15.36 ± 0.00     | 15.10 ± 0.18     | 14.68 ± 0.02     |
| Glycerine (g/L)                | 4.08 ± 0.10      | 4.89 ± 0.70      | 5.63 ± 0.14      |
| Total acidity (g/L)            | 4.61 ± 0.01      | 4.70 ± 0.02      | 4.85 ± 0.03      |
| pH                             | 3.78 ± 0.00      | 3.78 ± 0.03      | 3.73 ± 0.00      |
| Volatile acidity (g/L)         | 0.12 ± 0.01      | 0.13 ± 0.01      | 0.16 ± 0.01      |
| L-malic acid (g/L)             | 1.43 ± 0.05      | 1.42 ± 0.02      | 1.38 ± 0.01      |
| L*                             | 18.20 ± 1.85     | 16.01 ± 0.78     | 15.40 ± 0.01     |
| a*                             | 50.42 ± 1.83     | 48.22 ± 0.96     | 47.66 ± 0.05     |
| b*                             | 28.58 ± 1.75     | 26.30 ± 1.12     | 25.60 ± 0.04     |
| C*                             | 57.96 ± 2.46     | 54.93 ± 1.38     | 54.10 ± 0.03     |
| H*                             | 29.52 ± 0.62     | 28.60 ± 0.54     | 28.25 ± 0.06     |
| Colour intensity               | 8.14 ± 0.76      | 8.97 ± 0.22      | 9.47 ± 0.09      |
| Tonality                       | 0.60 ± 0.01      | 0.59 ± 0.00      | 0.57 ± 0.02      |

Values are the mean of triplicates. L*: luminosity; a*: redness; b*: yellow; C*: chroma; H*: hue angle. Different superscripts (a, b, c) indicate significant differences between maceration temperature for α = 0.05 according to the Student–Newman–Keuls test.
results showed that the wines obtained at higher maceration/fermentation temperature did not contain higher concentrations of anthocyanins, at least with statistically significant differences, except those obtained at an intermediate temperature of 21°C.

**Flavonols**

Flavonols contribute to bitterness and colour,[23] and they originate from the berry skins of grapes and are transferred to wine during the process of winemaking.[24] They vary in colour from white to yellow, closely related in structure to the flavones.[25] They also contribute to the colour stabilization of red wines by reinforcing the pigmentation due to anthocyanins, a phenomenon known as copigmentation.[26] In this study, there were 14 flavonols detected in wines in the three treatments.

Table 3 shows the flavonol content (mg/L) in wines elaborated at 17°C, 21°C, and 25°C, displaying all wine values in the range of other red wines.[27] Regarding the flavonol profile, the 3-glucosides of the six aglycones (quercetin, myricetin, laricitrin, syringetin, isorhamnetin, and kaempferol) were detected and quantified in the studied wines. In addition, myricetin and quercetin 3-glucuronides, and the galactoside forms of myricetin, quercetin, and laricitrin were also detected in these wines. Trisubstituted flavonols (myricetin, laricitrin, and syringetin) predominated over disubstituted (quercetin and isorhamnetin) and monosubstituted ones (kaempferol), being more abundant myricetin derivatives. Myricetin-3-glucoside was the main flavonol found in Petit Verdot wines, being in good agreement with other studies.[13,28] Syringetin-3-glucoside was the second mayor flavonol in these wines, followed by quercetin-3-glucuronide and laricitrin-3-glucoside. Flavonols are present in the grape exclusively in the form of glycosides, and the presence of

| Table 2. Concentration of anthocyanins (mg/L as malvidin-3-O-glucoside) at the end of alcoholic fermentation in Petit Verdot wines elaborated at different temperatures of maceration and fermentation. |
|---------------------------------|-----------------|-----------------|-----------------|
|                                 | 17°C            | 21°C            | 25°C            |
| Cyanidin 3-O-glucoside          | 0.49 ± 0.02     | 0.61 ± 0.11     | 0.66 ± 0.03     |
| Cyanidin 3-O-acetylglucoside    | 0.18 ± 0.01     | 0.17 ± 0.03     | 0.15 ± 0.00     |
| Cyanidin 3-O-coumaroylglucoside| 0.07 ± 0.00     | 0.07 ± 0.01     | 0.11 ± 0.04     |
| Peonidin 3-O-glucoside          | 1.73 ± 0.01     | 2.20 ± 0.28     | 2.11 ± 0.08     |
| Peonidin 3-O-acetylglucoside    | 3.34 ± 0.02     | 3.73 ± 0.14     | 3.34 ± 0.03     |
| Peonidin 3-O-coumaroylglucoside| 0.77 ± 0.03     | 0.91 ± 0.12     | 0.82 ± 0.05     |
| ∑ Disubstituted                | 6.56 ± 0.09     | 7.70 ± 0.68     | 7.20 ± 0.24     |
| Delphinidin 3-O-glucoside      | 6.78 ± 0.10     | 7.48 ± 0.88     | 6.44 ± 0.09     |
| Delphinidin 3-O-acetylglucoside| 3.71 ± 0.10     | 4.00 ± 0.47     | 3.18 ± 0.01     |
| Delphinidin 3-O-coumaroylglucoside| 0.84 ± 0.05  | 1.05 ± 0.10     | 0.89 ± 0.00     |
| Petunidin 3-O-glucoside        | 14.97 ± 0.44    | 16.57 ± 1.41    | 14.01 ± 0.22    |
| Petunidin 3-O-acetylglucoside  | 7.17 ± 0.09     | 7.73 ± 0.55     | 6.60 ± 0.16     |
| Petunidin 3-O-coumaroylglucoside| 1.05 ± 0.06  | 1.39 ± 0.16     | 1.25 ± 0.01     |
| Malvidin 3-O-glucoside         | 191.14 ± 7.53   | 210.11 ± 7.51   | 196.26 ± 4.89   |
| Malvidin 3-O-acetylglucoside   | 113.89 ± 4.36   | 122.65 ± 2.10   | 118.86 ± 4.02   |
| Malvidin 3-O-coumaroylglucoside| 24.83 ± 1.52    | 30.03 ± 2.02    | 29.58 ± 0.89    |
| Malvidin 3-O-cafeoylglucoside  | 4.08 ± 0.06     | 4.53 ± 0.12     | 4.05 ± 0.29     |
| ∑ Trisubstituted              | 368.46 ± 13.90  | 405.54 ± 15.32  | 381.13 ± 10.28  |
| Vitisin A                     | 2.78 ± 0.12     | 3.41 ± 0.15     | 3.53 ± 0.17     |
| Vitisin B                     | 1.87 ± 0.08     | 2.01 ± 0.12     | 1.89 ± 0.09     |
| ∑ Pyranoanthocyanins          | 4.65 ± 0.20     | 5.42 ± 0.27     | 5.42 ± 0.26     |
| ∑ Cyanidin type               | 0.73 ± 0.03     | 0.86 ± 0.14     | 0.92 ± 0.07     |
| ∑ Peonidin type               | 5.83 ± 0.06     | 6.84 ± 0.54     | 6.28 ± 0.17     |
| ∑ Delphinidin type            | 11.33 ± 0.15    | 12.53 ± 1.44    | 10.51 ± 0.08    |
| ∑ Petunidin type              | 23.19 ± 0.58    | 25.69 ± 2.13    | 21.86 ± 0.37    |
| ∑ Malvidin type               | 333.94 ± 13.41  | 367.32 ± 11.63  | 348.75 ± 9.80   |
| ∑ Non-acylated                | 215.10 ± 7.91   | 236.97 ± 10.18  | 219.47 ± 5.12   |
| ∑ Acylated                    | 159.92 ± 6.09   | 176.26 ± 5.81   | 168.85 ± 5.40   |
| ∑ Total anthocyanins          | 379.66 ± 14.20  | 418.66 ± 16.26  | 393.74 ± 10.78  |

Values are the mean of triplicates. Different superscripts (a, b, c) indicate significant differences between maceration temperature for α = 0.05 according to the Student–Newman–Keuls test.
flavonols aglycones in wine is attributed to hydrolysis processes, being unclear if they are chemical or enzymatic and lactic acid bacteria glycosidase enzymes could have some impact on wine flavonols.\[29\]

Maceration and fermentation at 17°C produced wines with lower concentration of total flavonols. Higher concentration of disubstituted flavonols was observed in wines macerated at 25°C. On the other hand, wines made at 21°C and 25°C showed similar total flavonol concentration (non-methoxylated and trisubstituted). The monosubstituted and methoxylated flavonols were found in similar concentration in all samples. In this study, the maceration/fermentation temperature caused no important changes, neither the profile nor concentration of flavonols, although some slight statistically significant differences, with lower concentration of the derivatives of quercetin, myricetin, and laricitrin were observed in wines macerated and fermented at 17°C.

Flavan-3-ols and stilbenes
Flavan-3-ols are the main phenolic compounds related to the astringency, bitterness, and structure of wines, and also an important factor in stabilizing the colour of ageing wines as anthocyanin copigments.\[23,30,31\] Proanthocyanidins are polymers of flavan-3-ols and the primary source of astringency and bitterness.\[2,32\] Studies showed that overall intensity and persistence are positively correlated with astringency, and therefore to proanthocyanidin content.\[4,33\]

The results obtained are shown in Table 4. Maceration and fermentation at 25°C provided wines with higher concentration of proanthocyanidins, according to increase of these compounds by raising the fermentation temperature,\[34,35\] although no differences were statistically significant. Wines elaborated at 21°C and 25°C showed lower concentration of flavan-3-ols monomers and dimers than wines macerated at 17°C. However, the mean degree of polymerization (mDP) was similar in all three treatments although there were decreases in macerated and fermented wines at 25°C. For galloylation and prodelphinidin percentages, our results seem to indicate that Petit Verdot wines macerated a higher temperature...
showing lower percentage of galloylation and higher percentage of prodelphinidins, showing that the wines macerated at 25°C had higher proportion of proanthocyanidins from the grape skins, because of the fact that there are a higher proportion of prodelphinidins in grape skins\[36,37\] and lower proportion of galloylated procyanidins than seeds.\[36,38\] Moreover, it has been accepted that the extraction of proanthocyanidins needs less maceration time than seeds.\[39,40\] The increase of proanthocyanidin content at 25°C and lower mDP may indicate that proanthocyanidin skin with low mDP were extracted more easily at higher temperature than those in skins with high mDP.

Regarding stilbenes, Petit Verdot wines elaborated for this study did not contain resveratrol in detectable levels, and only its glycoside forms (cis and trans isomers of piceid) were quantified, being in good agreement with other studies.\[5\] The results indicated that the macerated and fermented wines at 21°C and 25°C showed higher concentration of these compounds, wherein differences are statistically significant. Stilbenes are important due to their putative protective effects against cardiovascular diseases and a remarkable inhibitory potential of various stages of tumour development.\[41\] It was suggested that the concentrations of these compounds in wines vary, depending on many factors such as grape variety, fungal infections, winemaking procedures, and weather conditions.\[42\]

### Multivariate data analysis

PCA was applied to the results obtained from phenolic compound studied in the wines. Table 5 shows the variables with the highest correlation with principal component 1 (PC1) and principal component 2 (PC2). A total of 86.00% of the variance was explained by the first two principal components.

Figure 1 shows the distribution of the wines on the plane formed by the two principal components PC1 and PC2. For PC1, two different groups were evident; in positive value, the wines elaborated at 21°C had higher concentration of total anthocyanins, specifically derivatives of malvidin, petunidin, and peonidin. On the other hand, CP2 separates the samples again in two other groups, differentiating the wines obtained at 25°C by its composition in galloycatequin and flavonols (mainly quercetin-3-glucoside).

### Table 4. Concentration of flavan-3-ol monomers and dimers, proanthocyanidins, and stilbenes (mg/L) at the end of alcoholic fermentation in Petit Verdot wines elaborated at different temperatures of maceration and fermentation.

|             | 17°C         | 21°C         | 25°C         |
|-------------|--------------|--------------|--------------|
| Catechin    | 21.79 ± 1.99 | 19.68 ± 0.24 | 19.08 ± 1.61 |
| Epicatechin | 10.80 ± 0.73 | 9.30 ± 0.65  | 8.15 ± 0.56  |
| Gallocatechin| 3.12 ± 0.26  | 3.83 ± 0.38  | 4.66 ± 0.06  |
| Epigallocatechin| 1.16 ± 0.10 | 1.71 ± 0.28  | 1.50 ± 0.30  |
| Catechin gallate| 0.11 ± 0.03 | 0.09 ± 0.02  | 0.10 ± 0.02  |
| Epicatechin gallate| 0.41 ± 0.15 | 0.32 ± 0.15  | 0.46 ± 0.01  |
| **Σ Monomers** | **37.40 ± 2.39** | **34.93 ± 1.37** | **33.95 ± 2.50** |
| Procyanidin B1 | 22.29 ± 0.29 | 22.59 ± 1.86 | 24.11 ± 1.72 |
| Procyanidin B4 | 2.86 ± 0.53  | 2.64 ± 0.02  | 2.47 ± 0.25  |
| Procyanidin B2 | 10.10 ± 1.06 | 9.00 ± 0.63  | 8.42 ± 0.33  |
| Dimer 1 (Unknown dimer 1) | 3.97 ± 0.00 | 3.03 ± 0.56  | 3.26 ± 0.14  |
| Dimer 2 (Unknown dimer 2) | 1.16 ± 0.04 | 0.97 ± 0.03  | 1.06 ± 0.01  |
| **Σ Dimers** | **40.37 ± 1.35** | **38.23 ± 3.03** | **39.33 ± 2.43** |
| mDP\(^1\) | 3.95 ± 0.22  | 3.93 ± 0.15  | 3.63 ± 0.18  |
| Galloylation (%) | 5.75 ± 0.37 | 5.20 ± 0.67  | 5.00 ± 0.17  |
| Prodelphinidins (%) | 27.2 ± 0.55 | 28.4 ± 0.05  | 29.2 ± 0.37  |
| **Σ Proanthocyanidins** | **305.39 ± 6.66** | **371.71 ± 12.2** | **519.38 ± 55.45** |
| trans-piceid | 0.05 ± 0.00  | 0.06 ± 0.01  | 0.06 ± 0.00  |
| cis-piceid | 0.08 ± 0.00  | 0.12 ± 0.01  | 0.12 ± 0.01  |
| **Σ Stilbenes** | **0.13 ± 0.01** | **0.18 ± 0.02** | **0.18 ± 0.02** |

Values are the mean of triplicates. mDP\(^1\): mean degree of polymerisation. ΣFlavan-3-ol monomers and dimers, proanthocyanidins and stilbenes represents different superscripts (\(^a, b, c\)) indicate significant differences between maceration temperature for \(\alpha = 0.05\) according to the Student–Newman–Keuls test.
Conclusion

Phenolic characterization of Petit Verdot wines elaborated at different temperatures of maceration and fermentation (17°C, 21°C and 25°C) was carried out and discussed. It is clear that maceration/fermentation temperature plays a very important role in the phenolic composition of wine. However, according to the results obtained, wines elaborated at the highest temperature (25 °C) did not show the greatest concentration of all phenols families. In the case of anthocyanins, they were found in greater quantity in wines made at 21°C but proanthocyanidins in wines elaborated at 25°C. Therefore, with regard to the phenolic content and composition of the resulting wines, it is important to control maceration/fermentation temperature. This will enable winemakers to choose the most

Table 5. Principal component analysis (PCA) applied to the data from phenolic compounds.

| Principal component | Variance explained (%) | Total variance (%) | Variables highly correlated with the axis and their loadings |
|---------------------|------------------------|--------------------|-------------------------------------------------------------|
| 1                   | 46.103                 | 46.103             | Peonidin 3-acetylglucoside (0.993)                           |
|                     |                        |                    | Delphinidin 3-coumaroylglucoside (0.976)                     |
|                     |                        |                    | Malvidin 3-glucoside (0.941)                                |
|                     |                        |                    | Malvidin 3-cafeoylglucoside (0.929)                         |
|                     |                        |                    | Σ Peonidin type (0.927)                                     |
|                     |                        |                    | Σ Total anthocyanins (0.925)                                |
|                     |                        |                    | Σ Petunidin type (0.922)                                    |
|                     |                        |                    | Petunidin 3-glucoside (0.912)                               |
|                     |                        |                    | Σ Malvidin type (0.883)                                     |
|                     |                        |                    | ∑ Peonidin type (0.927)                                     |
|                     |                        |                    | ∑ Total anthocyanins (0.925)                                |
|                     |                        |                    | ∑ Petunidin type (0.922)                                    |
|                     |                        |                    | ∑ Malvidin type (0.883)                                     |
|                     |                        |                    | ∑ Peonidin type (0.927)                                     |
|                     |                        |                    | ∑ Total anthocyanins (0.925)                                |
|                     |                        |                    | ∑ Petunidin type (0.922)                                    |
|                     |                        |                    | ∑ Malvidin type (0.883)                                     |
| 2                   | 40.078                 | 86.181             | Quercetin-3-glucoside (0.981)                               |
|                     |                        |                    | Gallocaatechin (0.973)                                      |
|                     |                        |                    | Gallocatechin (0.981)                                       |
|                     |                        |                    | Epigallocatechin (0.962)                                    |
|                     |                        |                    | Σ Disubstituted flavonols (0.911)                           |
|                     |                        |                    | Σ Non-methoxylated flavonols (0.861)                        |
|                     |                        |                    | Procyanidin B2 (−0.860)                                    |
|                     |                        |                    | Vitisin A (0.859)                                           |
|                     |                        |                    | Σ Quercetin type (0.849)                                    |

Figure 1. Plotting of the samples on the plane defined by the two principal components obtained by principal component analysis (PCA) of the data from phenolic compounds.
suitable maceration and alcoholic fermentation temperature for Petit Verdot grapes according to the type of wine desired, since phenolic composition will have a great influence on the final organoleptic characteristics of the wine.

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