Effects of malolactic fermentation on colour stability and phenolic composition of Petit Verdot red wines

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Abstract

In the present work we evaluate the effects of malolactic fermentation (MLF) on the colour parameters and polyphenolic composition of Petit Verdot red wines. MLF caused a significant decrease in colour intensity in wines, approximately 9%. In line with that, the results showed a decrease in the concentration of anthocyanins (acylated and non-acylated), but an increase of the pyranothocyannins concentration. MLF did not produce important variations on the content of hydroxycinnamic acids derivatives, stilbenes and flavonols. Regarding flavan-3-ols, MLF caused a decrease in monomers, total flavan-3-ols and percentage of galloylation, and an increase on the percentage of prodelphinidins. However, no effect over mean degree of polymerisation was observed. Thus, it is unlikely that these changes may affect the acceptability of wines by consumers.

Introduction

Red winemaking includes several phases besides alcoholic fermentation (AF), being malolactic fermentation (MLF) and ageing of wine in barrels and/or in bottles between the main ones. The central purpose of MLF is to reduce wine acidity, transforming malic acid, a dicarboxylic acid, into lactic acid, a monocarboxylic acid.[1] Moreover, during this process volatile compounds that enrich the wine's aromatic quality, together with those formed during AF, are also formed.[2,3]

The influence of monomeric and polymeric phenolic compounds in the colour parameters and sensory quality of wine is obvious. Grape and wine phenolics belong to two main groups: flavonoid and nonflavonoid compounds. Flavonoids, located in grape skins, seeds and stems, include anthocyanins, flavan-3-ol monomers, oligomeric and polymeric proanthocyanidins, flavonols, flavanonols and flavones. Nonflavonoids, which derive primarily from the pulp and skins of grape berries, include hydroxycinnamic and hydroxybenzoic acid derivatives and stilbenes. All of them 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.[4]

On this context, the studies regarding the effects of MLF on wine aroma, biogenic amines content and other microbiological parameters are common.[3,5-7] However, literature and studies about the consequences of MLF on red wine colour parameters and phenolic composition are scarce, although the empirical accumulated knowledge.[1,8,9] Moreover, the effects of MLF on wine composition is usually measured by analysing the samples of wine taken immediately after the AF and then just finished the MLF and by hence there are no available information on the effect of the FML on the stability of phenolic compounds and wine color during wine aging.

Given the importance of phenolic compounds to the final quality, and therefore, to the consumer acceptance of red wines, in the present work we evaluate the differences in the colour parameters and phenolic composition of two sets of Petit Verdot red wines which have been identically elaborated and stored for a period of nine months before analysed, being the only difference between them that one of the sets has undergone the MLF.

Materials and Methods

Fermentation assays

Vinifications were carried out in the wine cellar of the Vine and Wine Institute of Castilla-La Mancha (Tomelloso, Castilla-La Mancha, Spain) using red grapes *Vitis vinifera cv.* Petit Verdot. They were harvested on the vintage 2013 at commercial maturity: 23.9° Brix. For the winemaking, grapes were destemmed and distributed into three 100 L tanks, sulphited with 5 g/HL SO_2 and inoculated with 25 g/HL Uvaferm VN® yeast strain (Lallemand Inc., Montreal, Canada). AF was carried out at 25±2°C. The fermentation was monitored daily by measuring the density and grape must was pressed upon reaching a density of 995 g/L. The completion of AF was carrying out at room temperature. After AF, the wine in each tank was divided into two batches in 50 L tanks maintained at 22°C. The first batch was sulphited until a final free SO_2 concentration of 30.0 mg/L to avoid the development of MLF (batch without MLF). The second one was inoculated with a commercial lactic acid bacteria Alpha strain (MBR®), according to the manufacturer's instructions (Lallemand Inc.). MLF development was controlled by monitoring the L-malic acid concentration of the wines. When malic acid content reached values ≤0.2 g/L, the wines were sulphited until a final free SO_2 concentration of 30.0 mg/L. Finally all the wines (with and without MLF) were then cold-stabilised, filtered through 0.2 m filters and bottled and stored for nine months at controlled temperature at 16°C until analysis. Vinifications were carried out in triplicate and average values of the three tanks are presented.

Wine analysis

Physicochemical analysis

The wines were analytically characterised. The following parameters were determined: alcohol content, total acidity (expressed as tartaric acid), pH, volatile acidity (expressed as acetic acid), L-malic acid, L-lactic acid, citric acid, colour intensity, tonality and shade following the official analytical methods established by the International Organisation of Vine and Wine.[10]

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

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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.[10]

Sample preparation for flavonols and hydroxycinnamic acid derivatives analysis

PCX solid phase extraction (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 and hydroxycinnamic acid derivatives. 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 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 re-dissolved in 1.5 mL of 20% methanol in water and directly injected into the high-performance liquid chromatography (HPLC) equipment.

Analysis of monomeric phenolic compounds in wines by high-performance liquid chromatography

Individual phenolic compounds were determined by HPLC-coupled with diode array detection-electrospray ionisation mass spectrometry (-DAD-ESI-MS/MS) following the conditions of previously described methods[11,12] 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 (PDA) and a LC/MSD Trap VL 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 x 150 mm; 3.5 µm particle; Agilent), with pre-column Zorbax Eclipse XDB-C8 (2.1 x 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: zero min, 6% B; 10 min, 30% B; 30 min, 50% B; 34 min, 100% B; 36 min, 100% B; 42 min, 4% B; 50 min, 4% B. For non-anthocyanin analysis, free-anthocyanin fractions (see sample preparation for flavonols and hydroxycinnamic acid derivatives analysis) were filtered (0.2 µm Chromafil Pet; Macherey-Nagel) 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: zero 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; 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, hydroxycinnamic acids) ion modes, setting the following parameters: dry gas N_2, 8 L/min; drying temperature, 325°C; nebulizer, N_2, 50 psi; ionisation and fragmentation parameters were optimised by direct infusion of appropriate standard solutions (malvidin-3-O-glucoside in positive ionisation mode; quercetin-3-O-glucoside and caftaric acid in negative ionisation mode); scan range, 50-1200 m/z. Identification was based on spectroscopic data (UV-Vis and MS/MS) obtained from authentic standards, when available, or previously reported data.[12,13] Quantification was made using the DAD chromatograms recorded at 520 nm (anthocyanins), 360 nm (flavonols), 320 nm (hydroxycinnamic acid derivatives), and the calibration graphs of the respective standards (R2>0.999). Quantification of non-commercial 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, flavonols were expressed as mg/L of quercetin-3-O-glucoside, and hydroxycinnamic acid derivatives were expressed as mg/L of trans-caftaric acid.

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., Millipore, MA, USA; 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 high-performance liquid chromatography-electrospray ionisation mass spectrometry

The analysis was carried out using a HPLC Agilent 1200 series system equipped with DAD (Agilent Technologies) and coupled to an AB Sciex 3200 TRAP (Applied Biosystems, Carlsbad, CA, USA) with triple quadrupole, turbo ESI-MS/MS system. The chromatographic system was managed and the mass spectra data was processed using the Analyst MSD software (Applied Biosystems).

Structural information concerning the proanthocyanidins was obtained using the pyrogallol-induced acid-catalyzed depolymerisation method.[14] 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 depolymerisation reaction, were injected (20 L) into an Ascentis C18 reversed-phase column (150 mm×4.6 mm with 2.7 m of particle size) (Supelco, Bellefonte, PA, 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.[13]

Statistical analysis

The paired Student *t*-test was used to identify any significant differences between chemical analysis results and volatile compounds. IBM SPSS Statistics 22.0 software was used for both analyses.

Results and Discussion

Oenological parameters and colour

The oenological and colour parameters are shown in Table 1. The values of total acidity were in agreement with the normal content found in Spanish wines.[15] As expected, MLF produced an increase of wine pH, a decrease of total acidity and a slight increase in volatile acidity. Wines without MLF, showed high values of colour intensity, indicating a good potential for ageing. MLF caused significant decreases in colour intensity in the wines, about 9%. These results are similar to those observed by Martínez-Pinilla[8] in Tempranillo and other varieties wines when studying the effects of MLF.[16]

Statistical significative differences were observed in the CIELAB parameters a* (redness), b* (yellow) and L* (lightness) among wines without and with MLF. Although these differences were quite small, wines which

undergone MLF showed higher lightness and also higher contributions of components a* and b* to their colour.

All these changes have been attributed to three different phenomena: firstly, the increase of pH that occurs in wine during MLF produces a reduction of the proportion of anthocyanins in form of flavilium cation (the red colored form of the anthocyanins) and therefore reduces the color intensity and increases L*. Secondly, the dissociation of the copigmentation complexes, probably due to the ionic shifts occurring during the MLF.[17] And finally, the formation of new and stable pigments resistant not only to the bisulfite addition but also to pH changes and oxidation[18,8] but showing lower molar extinction coefficient (increase of wine lightness) and orangish colour (higher contribution to b* component).

Anthocyanins

Table 2 shows the concentrations (mg/L) of the different anthocyanins identified in the wines without and with MLF. The anthocyanin profile and concentration of wines is 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.[1] The main anthocyanins identified in *Vitis vinifera* spp. grapes and wines are the 3-O-glucosides, 3-O-acetyl glucosides and 3-O-p-coumaroyl glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, as well as the 3-O-caffeoyl glucosides of malvidin and peonidin.[19,20]

The results show a lower concentration of anthocyanins (acylated and non-acylated) and a higher content of pyranoanthocyanins in wines with MLF, which seems to indicate that anthocyanins are less stable in the wine subjected to MLF. The differences in the content of anthocyanins does not depend on the type of aglycone since for both disubstituted and trisubstituted anthocyanins it was around 10%, but the difference in acylated anthocyanins is slightly superior to that in the non acylated anthocyanins (14 *vs* 9% respectively). These results are similar with those observed by Martínez-Pinilla.[8] These authors observed a decrease of 30% in the total anthocyanins concentration and attributed this diminution to the participation of monomeric anthocyanins in the formation of polymeric structures which tend to precipitate, and also to degradation and oxidation of the anthocyanins. Similar results were also observed by Burns.[21]

With regard to the pyranoanthocyanins, the contents of vitisin and acetyl vitisin A were higher in wines with MLF. Burns and colleagues [21] found lower concentrations of vitisin B in wines with FML.

Hydroxycinnamic acid derivatives and stilbenes

The only hydroxycinnamic acids (Table 3) present in the wines were those corresponding to tartaric acid esters forms, while the free forms (caffeic, coumaric and ferulic acids) were below the quantification limits. In all the

Table 1. Oenological and colour parameters of Petit Verdot wines without and with malolactic fermentation.

	Without MLF	With MLF
Alcohol content (% v/v)	14.65±0.01	14.61±0.08
Total acidity (g/L)	4.94±0.09	4.16±0.07°
pH	3.84±0.01	3.92±0.04°
Volatile acidity (g/L)	0.28±0.01	0.31±0.03°
L-malic acid (g/L)	1.37±0.01	0.00±0.00°
L-lactic acid (g/L)	0.05±0.06	0.98±0.04°
Citric acid (g/L)	0.29±0.00	0.26±0.02°
L*	12.15±0.16	12.99±0.44°
a*	43.37±0.18	44.47±0.72°
b*	20.58±0.27	21.67±0.70°
Colour intensity	10.51±0.02	9.55±0.11°
Tonality	0.58±0.00	0.60±0.00°

MLF, malolactic fermentation; L*, lightness; a*, redness; b*, yellow. Values are the mean of triplicate assays. °Statistically significant differences (P≤0.05).

Table 2. Concentration of anthocyanins (mg/L) in Petit Verdot wines without and with malolactic fermentation.

	Whitout MLF	With FML
Delphinidin-3-glc	4.54±0.25	4.07±0.07*
Cyanidin-3-glc	0.23±0.01	0.21±0.01*
Petunidin-3-glc	9.81±0.19	8.80±0.05*
Peonidin-3-glc	2.55±0.08	2.39±0.01*
Malvidin-3-glc	144.33±2.77	132.19±4.80*
Delphinidin-3-acglc	3.22±0.05	2.71±0.11*
Petunidin-3- acglc	6.35±0.11	5.60±0.06*
Peonidin-3-acglc	2.14±0.02	1.91±0.05*
Malvidin-3-acglc	81.11±2.25	70.80±3.01*
trans-delphinidin-3-cmglc	0.88±0.04	0.74±0.04*
trans-petunidin-3-cmglc	0.90±0.02	0.87±0.02*
trans-peonidin-3-cmglc	0.47±0.01	0.39±0.00*
cis-malvidin-3-cmglc	0.81±0.12	0.53±0.04*
trans-malvidin-3-cmglc	17.91±0.37	15.65±0.63*
Malvidin-3-cfgcl	2.25±0.17	2.20±0.15
Vitisin A	16.76±0.77	21.78±0.60*
Vitisin B	9.68±0.66	9.59±0.14
Acetyl vitisin A	12.57±0.45	15.38±0.43*
Acetyl vitisin B	3.51±0.30	3.81±0.01
Coumaroyl vitisin A	5.04±0.23	6.19±0.09*
∑ Trisubstituted anthocyanins	5.39±0.05	4.90±0.03*
∑ Disubstituted anthocyanins	272.09±5.06	244.17±8.42*
∑ Piranoanthocyanins	47.56±0.95	56.75±1.80*
∑ Non-acylated anthocyanins	161.46±2.25	147.66±4.78*
∑ Acylated anthocyanins	116.03±2.72	101.40±3.70*
∑ Total anthocyanins	325.04±7.38	305.80±9.74*

MLF, malolactic fermentation. Values are the mean of triplicates. *Statistically significant differences (P≤0.05).

analysed wines, the trans-form of the acids presented higher concentrations than its *cis* isomer and, as reported in other red wine varieties,[22] the *trans*-caftaric acid was by far the major compound.

MLF did not produce important effects on the total content of hydroxycinnamic acid derivatives, however slight differences in the concentration of all of them were observed, although for *trans*-caftaric and *trans*-coutaric acids the changes were not statistically significant. The *cis-coutaric* acid was that experimenting a higher proportional difference, but his absolute concentración was always lower than 1 mg/L. These results are similar to that found by[23,24] showed losses of hydroxycinnamic acid derivates that tartaric esters were hydrolysed to their corresponding free forms during MLF. Chescheir and colleagues[25] who demonstrated cinnamic esterase activity in *O. oeni*. The different esterasic activity of the lactic acid bacteria strains responsible for the MLF could expalin the results found in these studies.[26,27] Regarding to stilbenes, Petit Verdot wines elaborated for this study did not contained resveratrol in detectable levels, and only *cis* and *trans* isomers of piceid were quantified. The results presented in Table 3 indicate that the MLF had no appreciable effect on the concentration of these compounds. Similar results have been obtained by other authors.[8,23]

Flavonols

Table 4 shows the flavonols content in wines without and with MLF, displaying all wines values in the range of other red wines.[28]

Regarding the flavonol profile, myricetin-3-glucoside was the main flavonol found in Petit Verdot both without and with MLF, being in good agreement with other studies.[29,30] Syringetin-3-glucoside was the second mayor flavonol in these vines, followed of quercetin-3-glucuronide and laricitrin-3-glucoside.

Flavonols are present in the grape exclusively in the form of glycosides and the presence of flavonols aglicones 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.[30] In this study, the MLF did not cause important changes neither the profile nor concentration of flavonols, although some slight statistically significant differences, with lower concentration of the derivatives of kaempferol, myricetin and laricitrin were observed in wines subjected to MLF. These results are similar with those obtained when MLF was carried out in oak barrels[8] and those obtained when stainless steel tanks were employed.[23]

Flavan-3-ols

As in the case of flavonols, hydroxycinnamic acid and stilbenes, there are very few studies that have focused on study of wine flavan-3-ols comparing their stability in wines with have undergone the MLF and wines with only AF. The results obtained are shown in Table 5. Wine subjected to MLF showed lower concentrations for almost all the analysed flavan-3-ols, although for a high proportion of the compounds the changes were not statistically significant. Total flavan-3-ols content was 22.6% lower in the wines with MLF, being flavan-3-ols the group of phenolic compounds showing the highest differences between the two sets of wines. Previous works analysing flavan-3-ols of different varietal red wines before and after

Table 3. Concentration of hydroxycinnamic acid derivatives and stilbenes (mg/L) in Petit Verdot wines without and with malolactic fermentation.

	Without MLF	With MLF
trans-caftaric acid	52.01±2.09	48.64±2.33
trans-coutaric acid	5.15±0.16	4.83±0.26
cis-coutaric acid	0.98±0.01	0.52±0.02*
trans-fertaric acid	5.34±0.09	5.23±0.03*
∑ Hydroxycinnamic acid derivatives	63.48±2.16	59.22±2.64*
trans-piceid	4.18±0.15	3.65±1.05
cis-piceid	4.59±1.02	4.04±0.13
∑ Stilbenes	8.77±1.17	7.69±1.18

MLF, malolactic fermentation. Values are the mean of triplicates. *Statistically significant differences (P≤0.05).

Table 4. Concentration of flavonols (mg/L) in Petit Verdot wines without and with malolactic fermentation.

	Without MLF	With MLF
Kaempferol-3-glucoside	0.37±0.06	0.33±0.06
Kaempferol	1.05±0.00	0.95±0.03*
∑ Monosustituted	1.42±0.06	1.28±0.03*
Querecetin-3-glucoside	4.43±0.36	4.39±0.44
Querecetin-3-galactoside	1.50±0.05	1.53±0.01
Querecetin-3-glucuronide	8.65±0.09	8.75±0.09
Querecetin-3-rutinoside	0.35±0.05	0.33±0.03
Querecetin	4.80±0.29	4.73±0.27
Isorhamnetin-3-glucoside	1.39±0.07	1.39±0.06
Isorhamnetin-3-galactoside	0.17±0.01	0.16±0.00
Isorhamnetin	0.34±0.01	0.34±0.02
∑ Disustituted	21.63±0.22	21.62±0.28
Myricetin-3-glucoside	12.33±0.11	11.65±0.03*
Myricetin-3-galactoside	1.78±0.02	1.67±0.00*
Myricetin-3-glucuronide	2.19±0.03	2.15±0.00*
Myricetin	1.62±0.12	1.44±0.12
Laricitrin-3-glucoside	6.78±0.24	6.57±0.15
Laricitrin	0.17±0.00	0.21±0.00*
Syringetin-3-glucoside	9.87±0.49	9.85±0.27
Syringetin-3-galactoside	0.32±0.01	0.32±0.01
Syringetin	0.23±0.01	0.23±0.01
∑ Trisustituted	35.29±0.77	34.07±0.36*
∑ Total flavonols	58.34±1.05	56.96±0.67
∑ Kaempferol-type	1.42±0.06	1.28±0.03*
∑ Quericitin-type	19.73±0.16	19.73±0.23
∑ Isorhamnetin-type	1.90±0.06	1.89±0.05
∑ Myricetin-type	17.92±0.04	16.90±0.08*
∑ Laricitrin-type	6.96±0.24	6.77±0.15
∑ Syringetin-type	10.41±0.49	10.39±0.29

MLF, malolactic fermentation. Values are the mean of triplicates. *Statistically significant differences (P≤0.05).

Table 5. Concentration of flavan-3-ols (mg/L) in Petit Verdot wines without and with malolactic fermentation.

	Without MLF	With MLF
Catechin	26.57±2.67	22.98±0.91*
Epicatechin	12.28±2.83	9.55±0.84
Galocatechin	5.39±0.16	4.32±0.11*
Epigalocatechin	2.13±0.06	2.10±0.01
Epicatechin gallate	0.26±0.03	0.19±0.03*
∑ Monomers	46.64±5.76	39.15±1.90*
Procyanidin B1	11.36±0.79	10.95±0.52
Procyanidin B2	4.36±0.63	3.68±0.71
Procyanidin B4	1.16±0.31	1.03±0.17
Unknown procyanidin dimer	0.88±0.04	0.88±0.29
∑ Dimers	17.78±1.77	16.55±1.69
mDP	2.42±0.18	2.44±0.15
Galloilation (%)	2.72±0.11	2.16±0.45*
Prodelphinidins (%)	26.36±1.14	29.65±1.26*
∑ Flavan-3-ols	359.99±25.78	278.63±46.84*

MLF, malolactic fermentation; mDP, mean degree of polymerisation. Values are the mean of triplicates. *Statistically significant differences (P≤0.05).

MLF also found important decreases in flavan-3-ols content during MLF.[8] These changes could be explained considering the participation of flavan-3-ols in condensation reactions and a later precipitation of the polymeric compounds formed. However, the mean degree of polymerisation (mDP) remains almost constant, and condensation reactions with anthocyanins seems hardly probable as cause of this decrease, since free anthocyanin content of wines which undergo MLF was higher than that of the wines not subjected to MLF, in which total flavan-3-ols content was higher. Regarding to the proanthocyanidins percentages of galloylation and prodelphinidins, our results seems to indicate that Petit Verdot wines subjected to MLF contains a higher proportion of flavan-3-ols coming from grape skins, as in this wine flavan-3-ols showed a slight, but statistically significant, higher percentage, of prodelphinidis and a lower percentage of galloylation.

Conclusions

The usual way of studying the effect of MLF on wine phenolic composition is analysing the wine just after the fermentation analysis, wait until the wine complete the later MLF and then analyse again the phenolic composition of the wine. In these studies the differences observed in the colour parameters and phenolic composition of the wines before and after MLF could be due to lactic acid bacteria enzymes and metabolites, and also to different chemical reactions, precipitation, *etc*. However, the effect of MLF is not limited to the time while LABs are growing in wine, changes occurred in wine during MLF (pH and acidity changes, accumulation of dicacetyl, *etc*.) affect also the stability of phenolic compounds and wine colour during wine storage.

To overcome these limitations, when designing this study we decided to let both kinds of wine, those subjected only to AF and those which have undergone both AF and MLF, to stabilise for 9 months after bottling.

Wines not submitted to MLF maintained, after 9 months of storage, greater colour intensity, higher anthocyanin content and lower pyranoanthocyanins content than those, which underwent MLF. Among the other phenolic compounds, also related to wine colour and stability, flavan-3-ols were those most affected, with a concentration decrease higher than 20% in wines with underwent FML.

Although the changes observed in colour intensity and chromatic characteristics were statistically significant, they were not very important from a quantitatively point of view. Hence, it is unlikely that these changes may affect the acceptability of wines by consumers.

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