SENSORY PROFILING AND CHANGES IN COLOUR AND PHENOLIC COMPOSITION PRODUCED BY MALOLACTIC FERMENTATION IN RED MINORITY VARIETIES

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ABSTRACT

This research studies the sensory properties and chemical composition of varietal wines made with minority varieties from La Rioja (Spain) and it analyses the transformations in phenolic compounds and colour parameters occurring during the malolactic fermentation. In this sense, all the analysed parameters underwent changes of the same magnitude during this stage and both anthocyanin and hydroxycinnamic acid distribution was found to be dependent on the grape variety and not on the winemaking process. Wines made with these varieties showed high values of resveratrol that could lead to healthier wines, and the variety Maturana Tinta de Navarrete was found to share quite similarities in the chemical parameters with Tempranillo, studied as a reference variety. In the sensory analysis, and although all the wines obtained good punctuations, Monastel and Maturana Tinta de Navarrete were the best valued. Monastel had the highest color intensity and both varieties showed high aromatic intensity and great complexity. In mouth, Monastel showed the greater persistence, mount length and structure and Maturana Tinta de Navarrete was described as fresh and pleasant. In conclusion, this research shows the first characterization of these wines and provides data to be used as a chemotaxonomic tool to fingerprint them. Moreover, it opens the door to the use of minority varieties providing a viable alternative to traditional grape varieties cultivated in La Rioja and favouring consumer offer and wine differentiation.

Keywords: red minority varieties, polyphenols, colour, sensory analysis, alcoholic fermentation, malolactic fermentation.

1. INTRODUCTION

Over the last few decades the introduction and spread of world recognized varieties has caused a massive loss of indigenous grapevine varieties traditionally grown in various grape-growing regions. Initiatives has been ongoing in recent years to safeguard biodiversity in the oenological sector via a process of enhancement of ancient varieties, under a pressure of a market strongly oriented towards production deriving from native vines of specific geographical zones. In that sense, La Rioja (Spain), an autonomous community with a large vitiviniculture tradition, has raised the need to preserve and characterize its minority vine varieties in order to maintain the authenticity and quality of its wines. This community has different vine-growing zones with an important number of minority grape varieties, which are perfectly adapted to these zones.

Previous studies of local and minority varieties from La Rioja were carried out in experimental plots in order to know their possibilities of production and winemaking (Martínez de Toda, Martínez, Sancha, Blanco & Martínez, 2004a; Martínez de Toda, Martínez, Sancha, Blanco & Martínez, 2004b). The results of these works highlighted for their oenological interest red Maturana Tinta, Monastel and red Maturana Tinta de Navarrete. These minority varieties could be able to provide differentiation of red wines from La Rioja and be a good complement to the widespread and most representative variety of the area, Tempranillo, which implies 85% of the surface of red grapes cultivated in La Rioja.

Although there are a few studies on the growing potential of these minority varieties (Martínez de Toda et al., 2004a), studies on the oenological potential are limited to measures of general oenological parameters and sensory analysis in experimental microvinifications after alcoholic fermentation (Martínez de Toda et al. 2004b). No scientific researches have been carried out to study the composition, behaviour and evolution of these wines during the winemaking. Therefore, we aimed to evaluate the oenological characteristics of these minority varieties during the winemaking by analyzing their behaviour both after the alcoholic fermentation and malolactic fermentation, necessary to elaborate high quality wines that could be aged.

It is obvious the influence of monomeric and polymeric phenolic compounds in the colour parameters and sensory quality of the wines. 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 acids and 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 (Garrido & Borges, 2011). The content and profile of these polyphenols have not yet been studied in these minority varieties, hence the importance of its knowing, that could provide information about their oenological potential. Moreover, researches on the effect of malolactic fermentation on phenolics or colour parameters are limited and they are frequently aimed at the study of aromas, biogenic amines or microbiological researches (Pramateftaki, Metafa, Karapetrou & Marmaras, 2012; Bartowsky & Borneman, 2011; López et al., 2011; Miller, Franz, Cho & du Toit, 2011; Pan, Jussier, Terrade, Yada & Mira de Orduña 2011; Mendoza, Manca de Nadra & Farías, 2010; Hernádez-Orte et al., 2009; Terrade, Noël, Couillaud & Mira de Orduña, 2009).

Therefore, the aim of this work was to evaluate the oenological characteristics of the selected varieties by analyzing the sensory profiling of the wines obtained as well as the changes occurring in the colour parameters and polyphenolic composition during the malolactic fermentation. Wines were elaborated in an industrial wine cellar Tempranillo was also studied as a reference variety.

2. EXPERIMENTAL

2.1. Vinifications and samples

Vinifications were carried out in the wine cellar of Juan Carlos Sancha S.L. (Baños de Río Tobia, La Rioja, Spain) using the red grapes *Vitis vinífera cv.* Tempranillo (TE), Maturana Tinta (MA), Monastel (MO) and Maturana Tinta de Navarrete (MNAV). They were harvested on the vintage 2009 at commercial maturity: 24.7 ºBrix for TE, 24.2 ºBrix for MA, 25.3 ºBrix for MO and 23.6 ºBrix for MNAV.

For the winemaking, grapes were destemmed and distributed into 500 L French oak barrels, sulphited with 3 g/HL SO_2 and inoculated with 25 g/HL *S. cerevisiae* yeast strain**.** The prefermentation process went on for 72 h at 12 ± 1ºC, the fermentation-maceration process was carried out at a maximum temperature of 28 ± 2ºC and lasted 10 days. Wines were then run off and introduced in eight 500 L French oak barrels, two for each variety. Barrels were maintained at controlled wine cellar temperature and after malolactic fermentation, wines were racked. Malolactic fermentation lasted around 2 months in all wines. Samples were taken at the end of alcoholic fermentation (OH) and at the end of malolactic fermentation (ML). All vinifications were carried out in duplicate and average values of the two barrels are presented.

2.2. Determination of usual oenological parameters

All wines were analyzed for pH, ethanol concentration, titratable and volatile acidity according to the OIV official practices (1990). Malic acid was analyzed by the autoanalyzer LISA 200 (Biocode Hyad, Le Rhem, France).

2.3. Analysis of colour parameters and total polyphenol index

Wine red colour (WC), monomeric anthocyanin colour (MAC), copigmentation colour (CC), and bisulphite-stable colour (BSC) were determined by the method proposed by Levengood & Boulton (2004) in a Cary 300 Scan UV-vis spectrophotometer (Varian Inc., Madrid, Spain). Wine stable colour (SC) was calculated as the sum of CC and BSC. Colour intensity (CI) was calculated as the sum of absorbances at 420, 520, and 620 nm, and Hue as A_{420}/A_{520}, at wine pH. The CIELAB rectangular parameters (L*, a* and b*, illuminant D65 and 10º observer conditions) were determined according to Ayala, Echávarri & Negueruela (1997). Total polyphenol index (TPI) was determined by absorbance at 280 nm of diluted wine with synthetic wine. All measurements were performed in triplicate and referred to 10 mm path length quartz cells.

2.4. Analysis of monomeric phenolics

Anthocyanins, hydroxycinnamic and hydroxybenzoic acids, flavonols, flavan-3-ols and resveratrol derivatives were analyzed by high performance liquid chromatography in a modular 1100 Agilent liquid chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with one G1311A quaternary pump, an on-line G1379A degasser, a G1316A column oven, a G1313A automatic injector, and a G1315B photodiode-array detector (DAD) controlled by the Chemstation Agilent software.. Separation was achieved in an ACE HPLC (5 C18-HL) particle size 5 μm (250 mm x 4.6 mm) column according to the methodology described in Gómez-Alonso, García-Romero &

Hermosín-Gutiérrez (2007). Quantification of non-commercial compounds was made using the calibration curves belonging to the most similar compound: malvidin-3-glucoside for the anthocyanins; quercetin-3-glucoside for myricetin-3-glucoside and quercetin-3-glucuronide; caffeic acid for *cis*- and *trans*-caftaric acids (*cis*- and *trans*-caffeoyl-tartaric acid); *p*-coumaric acid for *cis*- and *trans*-coutaric acids (*cis*- and *trans*-*p*-coumaryl-tartaric acid); ferulic acid for *cis*- and *trans*-fertaric acids (*cis*- and *trans*-ferulic-tartaric acid); and *trans*-resveratrol for its glucoside. The content of non-acylated anthocyanins (A) was calculated as the sum of delphinidin, cyanidin, petunidin, peonidin and malvidin-3-glucosides; the content of acetyl-glucoside anthocyanins (A-Ac) as the sum of delphinidin, cyanidin, petunidin and malvidin-3-(6-acetyl)-glucosides; the content of coumaryl-glucoside anthocyanins (A-Cm) included delphinidin, petunidin, and malvidin-3-(6-*p*-coumaryl)-glucosides. The sum of A, A-Ac and A-Cm was referred to as total monomeric anthocyanins (T-A). Total hydroxycinnamic acids (T-HA) were calculated as the sum free acids, i. e., caffeic, ferulic and coumaric acid, and hydroxycinnamates, i. e., *trans*-caftaric, *cis*-caftaric, *trans*-coutaric, *cis*-coutaric and *trans*-fertaric. Total flavonol content (T-Flavo) was calculated as the sum of myricetin-3-glucoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-glucuronide, myricetin, quercetin, kaempferol and isorhamnetin. Total Resveratrol (T-resveratrol) was calculated as the sum of *cis*-resveratrol, *trans*-resveratrol and *trans*-resveratrol glucoside. All analyses were performed in duplicate.

2.5. Analysis of proanthocyanidins

Wine samples were directly fractionated by gel permeation chromatography (GPC) on a Toyopearl gel HP-50F column as described by Fernández, Martínez, Hernández, Guadalupe and Ayestarán (2011) and Guadalupe, Soldevilla, Sáenz-Navajas &

Ayestarán (2006). Fractionation was performed in triplicate and phloroglucinol adducts were analyzed in F2 fractions by reversed-phase HPLC as described by Kennedy & Jones (2001). Proanthocyanidins cleavage products were estimated using their response factors relative to (+)-catechin which was used as the quantitative standard. Total proanthocyanidin content (PA) was calculated as the sum of all the subunits: extension subunits (phloroglucinol adducts) and terminal subunits (catechin, epicatechin and epicatechin-gallate). To calculate the apparent mean degree of polymerization (mDP), the sum of all subunits was divided by the sum of the terminal subunits. All analyses were performed in duplicate.

2.6. Sensory Analysis

Wines after malolactic fermentation were analyzed for sensory profiling and they were judged for visual (colour), olfactory (volatile fraction), and gustatory (taste and mouth-feel sensations) quality conformance to wine typology.

A panel of twelve tasters, wine professionals from the D. O. Ca. Rioja, was convened. All wine tasters had participated on previous aroma and mouth-feel sensory descriptive panels and had regularly participated in quality scoring varietal wine sensory panels. Tasters rated 9 attributes for the olfactory phase and 6 for the gustative, scoring the intensity of each attribute on an interval scale with 5 levels of intensity (0 = no aroma or no taste; 1 =weak aroma or weak taste; 5 = strong aroma or strong taste; intermediate values did not bear description). The colour was also judged and blue-red colour was rated according to its intensity on an anchored scale with five levels of intensity (0 = no blue-red colour; 5 = extremely strong blue-red colour). After tasting, panellist were also asked to score the wines according to their global perception on a structured scale from 1 to 5, with 5 being the highest and 1 the lowest; and they were allowed to make any additional comment about the sensory properties of the wines. Wines were presented at 18ºC in coded standard wine-tasting glasses according to standard 3591 (ISO 3591, 1997). Assessment took place in a standard sensory analysis chamber (ISO 8589, 1998) equipped with separate booths. One wine was replicated in order to ascertain judges' consistency.

2.7. Statistical procedures

Significant differences between analytical determinations were analyzed by an analysis of variance (ANOVA) if the data adhered to assumptions of normality. If these assumptions were not adhered to, a Kruskal−Wallis test was used. Separate principal component analysis (PCA) was carried out on full data for colour parameters and phenolic compounds, and it was conducted using the correlation matrix with no rotation. Sensory data were subjected to ANOVA analysis to determine the within judges reproducibility in rating two replicated wines. Generalized Procrusters Analysis (GPA) was applied on the mean ratings for olfactory and gustatory attributes, and a permutation test was also made to explain that the results obtained were significant (85.12%). ANOVA evaluations were performed using the Statistica 8.0 program for Microsoft Windows (Statsoft Inc., Tulsa, Oklahoma) and PCA and GPA analyses by using the Senstools Version 3.3.2. Program (Utrecht, the Netherlands).

3. RESULTS AND DISCUSSION

3.1. Oenological parameters

Alcohol content, pH, titratable acidity, volatile acidity and malic acid content in wines after alcoholic and malolactic fermentation are shown in Table 1. The values obtained after alcoholic fermentation for volatile acidity confirmed a suitable winemaking with absence of microbial alterations. In general, all the wines reached high levels in the ethanol content after the alcoholic fermentation and Monastel showed the highest value. The highest pH corresponded to Tempranillo, which was attributed to a varietal factor due to the high potassium content usually observed in this variety (Aleixandre, Lizama, Álvarez & García, 2002). The values of titratable acidity were in agreement with the normal content found in Spanish wines after alcoholic fermentation (Escudero-Gilete, González-Miret & Heredia, 2010). The content of malic acid differed significantly among varieties, with Tempranillo showing the highest value.

As expected, malolactic fermentation produced an increase of wine pH, a decrease of titratable acidity and an increase in volatile acidity. No important differences were found among varieties after the malolactic fermentation except for the highest alcohol content in Monastel and the lowest titratable acidity in the MNAV wine.

3.2. Colour parameters and total polyphenol index

Colour characteristics and total polyphenol index (TPI) in wines after alcoholic and malolactic fermentation are shown in Table 2. After alcoholic fermentation, all wines showed high values of colour intensity (CI) and TPI, indicating a good potential for aging, but it was Monastel the wine that showed the highest values in both parameters. No noteworthy differences were observed in the CIELAB parameters a^* (redness) and L^* (lightness) among wines although Maturana Tinta wine showed the lowest value in the yellow-blue component b^*, indicating bluish tonalities. Regarding the colour components, and in good agreement with the CI values, Monastel showed the highest wine colour (WC) and it was due to the monomeric anthocyanin colour (MAC) and bisulfite-stable colour (BSC). On the other hand, Maturana Tinta de Navarrete showed the lowest value of WC and MAC and Tempranillo showed the lowest copigmentation colour (CC). In all the wines the contribution of copigmentation colour to WC was around 38% while bisulfite stable colour, attributed in bibliography to polymeric pigments, accounted between 13 and 19% of WC, and monomeric anthocyanin colour represented between 44 and 47%. All these data were found to be in agreement with others studies (Guadalupe & Ayestarán, 2008; Boulton, 2001) except for anthocyanin color which was found to be slightly lower than data described in literature for young wines (Han, Zhang, Pan Zheng, Chen & Duan, 2008; Boulton, 2001).

Malolactic fermentation caused significant decreases in colour intensity in all the wines, ranging between 5-8% in Tempranillo and Monastel to 10-14% in Maturana Tinta and Maturana Tinta de Navarrete, due to changes in absorbance at 520 nm (data not shown). The CIELAB parameters a^* and L^* were maintained while the b^* value decreased by more than 50%, indicating a shift to bluish tonalities. Hue showed a slight increase in all the wines, as well as the TPI, probably due to the contribution of the wood of the oak barrel. With regard to the colour components, malolactic fermentation resulted in a significant decrease in WC (~ 20% in all the wines) due to a decrease of 58 to 65% in the copigmentation colour. On the contrary, MAC was maintained and BSC increased from 17% in Tempranillo to 40% in Maturana Tinta. All these changes were attributed to two different phenomena: firstly, to the dissociation of the copigmentation complexes, probably due to the ionic shift occurring during the malolactic fermentation, and secondly, to the formation of new and stable pigments resistant not only to the bisulfite addition but also to pH changes and oxidation (Asenstorfer, Hayasaka & Jones, 2001). In this point it is important to highlight that malolactic fermentation produced similar changes in the colour parameters and total polyphenol index in all the wines and they were independent on the variety used. Therefore, the differences observed among varieties after alcoholic fermentation were maintained after malolactic fermentation.

3.3. Monomeric anthocyanins

All wines showed significant differences in the content of anthocyanins after the alcoholic fermentation (Figure 1). Monastel reached the highest value of total-anthocyanins (T-A), in good agreement with its highest values in colour intensity and red wine colour. Non-acylated anthocyanins (A) were the major anthocyanins in all the wines, varying from 67% in Maturana Tinta de Navarrete to 88% in Tempranillo. Acetylated anthocyanins (A-Ac) were found to be higher than coumarylated anthocyanins (A-Cm) in Monastel (19% vs. 8%), Maturana Tinta (19 vs. 5%) and Maturana Tinta de Navarrete (28% vs. 5%) while Tempranillo showed more coumaryl than acetylated derivatives (7% vs. 5%). These differences were attributed to varietal differences and, although there is no bibliography in this respect for the minority varieties, studies on Tempranillo variety (Gómez-Alonso et al., 2007; Monagas, Gómez-Cordovés, Bartolomé, Laureano & da Silva, 2003) show that the content of coumaryl derivatives is always higher than the concentration of acetylated anthocyanins while other varieties such as Cabernet Sauvignon or Graciano show the opposite (Monagas et al., 2003). The profile of major non-acylated anthocyanins was also studied in detail (Figure 2) and, as expected, malvidin-3-glucoside was the major anthocyanin in all the varietal wines, representing from 60% to 76%, and its derivatives were also the main anthocyanins in the acetylated and coumarylated forms (data not shown). Some authors consider that anthocyanin profile of varietal wines can be used as an analytical tool to certify their authenticity (Pérez-Trujillo, Hernández, López-Bellido & Hermosín-Gutiérrez, 2011; Ferrandino & Guidoni, 2010; Castillo-Muñoz et al., 2009). In this sense, it must be noticed that the percentages of each non-acylated anthocyanin varied between wines. Therefore, Tempranillo and Monastel showed quite similar proportions in the major anthocyanins while Maturana Tinta and Maturana Tinta de Navarrete showed more similarities between them (Figure 2). Moreover, this anthocyanin profile together with the anthocyanin profile of the rest coumaryl and acetylated forms (data not shown) was exactly maintained in the wines after the malolactic fermentation, indicating that the anthocyanin distribution in the wines was dependent on the variety and not on the winemaking process. It should be pointed out that the acylated forms of the non-malvidin pigments seem to be involved in strong copigmentation (Boulton, 2001), which would explain that wines with predominantly malvidin in the anthocyanin profile, i. e., Maturana Tinta and Maturana Tinta de Navarrete, showed the lowest value of red wine colour (Table 2).

Malolactic fermentation produced a decrease of around 30% in the content of total monomeric anthocyanins (T-A) in all the wines, which coincided with the significant loss observed in CI and WC. This reduction, which affected similarly to A, A-Ac and A-Cm, was attributed to their conversion into to polymeric anthocyanins, also in agreement with the BSC increase (Table 2), and to degradation, oxidation, complexation and precipitation reactions of the monomeric anthocyanins during the malolactic fermentation. It is important to remark again that the changes occurring in the anthocyanin compounds during this winemaking stage were the same, both in quantity and profile, in all the studied varieties. All final wines showed anthocyanin concentrations in the range usually described in bibliography for red wines (Ginjom, D´Arcy, Caffin & Gidley, 2011; Guadalupe & Ayestarán, 2008).

3.4. Hydroxycinnamic acid derivatives, gallic acid and total resveratrol

All wines after alcoholic fermentation showed similar values of total hydroxycinnamic acids (T-HA) except for Monastel, which showed significantly lower quantity (Table 3).

The only cinnamic acids present in all wines were the sterified forms while the free forms were below the quantification limits. In all the analysed wines, the *trans*-form of the acids presented higher concentrations than its *cis* isomer and, as reported in other red wine varieties (Ginjom et al., 2011), the *trans*-caftaric acid was by far the major acid (> 50%) followed by the *trans*-coutaric acid (20-30%). Taking into account the ratio *trans*-coutaric/*trans*-caftaric, considered by some authors as a varietal factor and proposed as a possible chemotaxonomic tool (Ferrandino & Guidoni, 2010), a clear difference was observed between Tempranillo, with a ratio of 0.65, and the rest of the wines, with a ratio between 0.34 and 0.39. Curiously, the rest *cis*-caftaric, *cis*-coutaric and *trans*-fertaric acids showed the same proportions in all the wines, i.e., 8-9%, 5-6% and 2-3%, respectively. Regarding the effect of the malolactic fermentation on the content and profile of the hydroxycinnamic acids, different aspects should be highlighted. Firstly, the malolactic fermentation did not produce important effects on the content of total hydroxycinnamic acids. Secondly, and as in the case of anthocyanins, malolactic fermentation did not affect the distribution of hydroxycinnamic acids and without exception the wines showed the same profile as observed after the alcoholic fermentation. This fact confirmed the varietal origin of the acids and indicated that the *trans*-coutaric acid/*trans*-caftaric acid ratio may characterize the wines according to their grape origin. Finally, and opposed to other authors that observe that tartaric esters are hydrolysed to their corresponding free forms during malolactic fermentation (Cabrita et al., 2008; Hernández et al., 2007), in the present study the concentration of free acids after malolactic fermentation was below the limit of quantification while the tartaric esters were in the range described for other red varieties (Ginjom et al., 2011).

With regards to benzoic acids, gallic acid, considered one of the most potent antioxidants in wines (Ginjom et al. 2011), was the most abundant both before and after alcoholic fermentation and it was Monastel the wine that showed the highest content (Table 3). Malolactic fermentation prompted an increase in gallic acid in all the wines and it was attributed to the fact that malolactic fermentation was carried out in oak barrels and thus it was released from their tannin galloylated precursors (Ginjom et al., 2011). Therefore, and taking into account its antioxidant activity, conducting the malolactic fermentation in oak barrels would increase the antioxidant capacity of the wines.

Finally, and relative to the content of resveratrol (Table 3), Monastel, Maturana Tinta and Maturana Tinta de Navarrete showed values of total resveratrol above the mean of Spanish wines (Abril, Negueruela, Pérez, Juan & Estopañán, 2005). In this sense they could be considered as *healthier wines* as it is widely known the positive biological effect of this compound in human health. Although malolactic fermentation produced a decrease in the content of total resveratrol in Tempranillo and Monastel, the latter still showed higher values than those described in bibliography for Spanish wines (Abril et al., 2005).

3.5. Flavonols

Table 4 shows the flavonol content in wines after alcoholic and malolactic fermentation. Although all wines showed values in the range of other wines (Hermosín, Sánchez-Palomo & Vicario, 2005), Maturana Tinta showed the highest content in total flavonols (T-Flavo) at the end of both fermentations followed by far by Tempranillo and finally Monastel and Maturana Tinta de Navarrete. Regarding the flavonol profile, myricetin-3-glucoside was the main flavonol found in Tempranillo both before and after the malolactic fermentation, being in good agreement with other studies (Gómez-Alonso et al., 2007; Hermosín et al., 2005). Myricetin-3-glucoside was also the main flavonol in Maturana Tinta and Maturana Tinta de Navarrete after the alcoholic fermentation whereas the main flavonols in Monastel corresponded to the aglycones of quercetin and myricetin. Flavonols are present in the grape exclusively in the form of glycosides and the fact the free flavonols were detected in wines could be due to the hydrolysis of their glycosides during the alcoholic fermentation. With the exception of quercetin-3-galactoside and some flavonols present in very low concentrations, all these compounds, considered as the best kind of copigmentation cofactors (Hermosín et al., 2005; Boulton, 2001), decreased significantly during malolactic fermentation in agreement with a decrease in the copigmentation colour (Table 2). On the other hand, and contrary to what was observed with anthocyanins or hydroxycinnamic acids, the distribution of individual flavonols changed in all the wines during the malolactic fermentation and it was not possible to establish a concrete pattern of changes. Decreases in flavonol-glycosides could not be explained by the hydrolysis of glycoside linkages because increases in their correspondent free aglycones were not observed and thus other kind of reactions such as condensation, oxidation and copigmentation with anthocyanins (Hermosín et al., 2005) may have occurred. Querecetin-3-glucuronide became the major flavonol in Monastel and Maturana Tinta de Navarrete after the malolactic fermentation and, although myricetin-3-glucoside was again the major flavonol in Tempranillo and Maturana Tinta, the proportion of the rest flavonols was not maintained. To sum up, the results of the present study indicated that the flavonol profile was not a good indicator to differentiate grape varieties.

3.6. Catechin and proanthocyanidins

The concentration of (+)-catechin and total proanthocyanidins (PA) as well as the proanthocyanidin mean Degree of Polymerization (mDP) in wines after alcoholic and malolactic fermentation is shown in Table 5. The only flavan-3-ol detected in the wines within the quantification limits was (+)-catechin while epicatechin, epicatechin-gallate and catechin gallate were below the limit of quantification (0.90 mg/L). After alcoholic fermentation, Maturana Tinta de Navarrete reached by far the highest value in catechin, which is involved in the formation of stable colour through copigmentation and formation of polymeric pigments (González-Manzano, Dueñas, Rivas-Gonzalo, Escribano-Bailón & Santos-Buelga, 2009) and in condensation reactions with other flavanols affecting the astringency and bitterness of the final wines (Fortes et al., 2011; Chira, Schmauch, Saucier, Fabre & Teissedre, 2009). Although the content of PA was significantly higher in Monastel than in the rest of wines, the mDP, which can influence the flavan-3-ol bioavailability and bioactivity and it is related with the astringent and bitter properties of proanthocyanidins (Vidal et al., 2003), was quite high in all the wines.

Malolactic fermentation prompted a significant decrease in the concentration of catechin, PA and mDP in all the wines and, as it was observed with other polyphenols, both PA and mDP experienced changes in the same magnitude in all the wines. Hence, PA content decreased from 37 to 42% in all wines, while mDP decreased from 35 to 45%. Maturana Tinta de Navarrete continued showing the highest value in catechin and Monastel in proanthocyanidins. Looses in catechin were attributed to their conversion into more stable polymers by reacting with other flavanols, anthocyanins, and small molecules such as pyruvic acid and vinylphenol; decreases in PA were due to tannin-anthocyanin combination and precipitation of unstable colloids tannin-tannin, tannin-polysaccharides and tannin-proteins.

PA values after malolactic fermentation were in the range described in bibliography for other red wines (Fortes et al., 2011; Cosme, Ricardo-Da-Silva & Laureano, 2009). The results of mDP, also in agreement with other authors (Fortes et al., 2011; Cosme et al., 2009; Monagas et al., 2003), indicated that the proanthocyanidins present in wines after malolactic fermentation were mainly oligomers or short-chain polymers (Fortes et al., 2011). This fact, which could be attributed to a higher precipitation of larger PA and/or to the hydrolysis of higher PA forming lower ones may be related with a change in the astringent sensation (Chira et al, 2009; Vidal et al., 2003).

3.7. Differentiation of wines in the PCA space

Principal component analysis (PCA) was applied to the chemical data to clarify the interpretation of the data and highlight those variables that best explain the differences between wines. In Figure 3, a PCA of the varietal wines on colour parameters and phenolic compounds explained the 91% of the accumulative variance. PC1 (69% of variance) was mainly associated with copigmentation colour (CC), stable colour (SC), wine colour (WC), total proanthocyanidin content (PA) and total anthocyanins (T-A) whereas colour intensity (CI), monomeric anthocyanin colour (MAC), bisulphite-stable colour (BSC), total hydroxycinnamic acids (T-HA) and total flavonols (T-Flavo) were associated with both PC1 and PC2 (22% of the variance). It was observed a highly positive correlation between CC, T-A, PA, SC and WC and a highly negative correlation between CI and T-HA (r^2= -0.70), and between BSC and T-Flavo (r^2= -0.74). The distribution of the wines in the PCA space showed quite interesting aspects to be highlighted. On the one hand, wines were clearly differentiated according to their winemaking stage. Thus, wines after the alcoholic fermentation were located in the right of the PCA space and they were characterised by higher values of CC, T-A, PA, SC, WC, CI and MAC than wines after the malolactic fermentation, which were located in the left of the PCA space. This shift indicated that malolactic fermentation produced a decrease of the values of these parameters and an increase in T-HA and BSC. On the other hand, and taking into account the position of each wine, a similar distribution of the individual wines was observed both before and after the malolactic fermentation. Therefore, Tempranillo and Maturana Tinta de Navarrete were quite close in the PCA space both before and after the malolactic fermentation, indicating that they shared quite similarities in the analytical parameters. However, Monastel and Maturana Tinta were located separately from the rest and just in opposite positions both before and after the alcoholic fermentation, showing that Maturana Tinta was clearly characterised by higher values of T-HA and T-Flavo, whereas Monastel showed higher correlation with BSC, MAC, CI and WC. The results observed in the PCA space showed again that the malolactic fermentation produced the same changes in all the analysed parameters in all the wines, and that the differences observed among wines after the alcoholic fermentation were maintained after the malolactic fermentation.

3.8. Sensory analysis

Sensory evaluations of wines after malolactic fermentation were performed to verify the differences observed between wines on the organoleptic perception. On the visual phase, and in good agreement with what was observed in the analysis of colour, Monastel and Tempranillo wines showed the highest scores in color intensity although all the wines obtained high punctuations, 4.56 for Monastel, 4.38 for Tempranillo, 3.73 for Maturana Tinta and 3.62 for Maturana Tinta de Navarrete. With regards to judge’s comments on color it is noteworthy that Monastel was described as presenting the highest red tonalities while Maturana Tinta de Navarrete colour intensity was coupled to orange tonalities. Figure 4 provides a GPA consensus configuration of the relationship of the wines as determined for their olfactory and gustatory perceptions. Generalised Procrustes Analysis (GPA) was applied to sensory data to ascertain consistency among the 12 tasters and provide information on relationship between wines and attributes. Before that, the within judges reproducibility was evaluated by mean of two replicated wines in the tasting session and replications were demonstrated not to be a source of variation.

In the olfactory GPA space (Figure 4a), wines were properly located in the vectorial dimension defined by the two factors, which accounted for 82% of the total variance. The consensus plot showed the wines quite spread, thus indicating a marked difference among wines. Tempranillo showed a higher correlation with herbaceous and liquorice aromas, the last being a characteristic varietal descriptor of the Tempranillo wines. Monastel was more correlated with fruity, coffee and toasted aromas while Maturana Tinta was described to be related with dairy and also liquorice aromas and Maturana Tinta de Navarrete with pepper odours. Relative to aromatic intensity, Monastel wine was the best valued as it obtained an average score of 3.91 while Tempranillo obtained the lowest score (2.51) and the other wines had values around 3.0. Figure 4b shows the wine and attribute average space obtained from the gustatory space where PC1 explained 45% of the total variance and PC2 accounted for 33.5%. The first aspect to be highlighted is that Maturana and Maturana Tinta de Navarrete were perceived by the tasters as being very similar in their gustative descriptors as they were located very close in the consensus space. Tempranillo wine showed a higher correlation with bitterness and mouth length and obtained low punctuations in relation with fatty, sweet and acid sensations. On the contrary, Monastel was more correlated with mouth length and astringency while Maturana Tinta and Maturana Tinta de Navarrete were related with acidity, sweetness and fatty sensations.

Finally, and although all the wines obtained good punctuations in the global perception, Monastel and Maturana Tinta de Navarrete were best valued as they obtained the highest punctuations. Monastel, with a global score of 4.45, was described by tasters as a wine of high intensity and great aromatic complexity with very pleasant dairy and coffee aromas. In mouth, it resulted the most valuable due to its great persistence, mount length and structure. Maturana Tinta de Navarrete (4.36 in global perception) was also described as highly aromatic and it was fresh and pleasant in mouth. Lastly, Tempranillo and Maturana Tinta obtained lower punctuations in the global perception (3.55 and 3.75 respectively) because they showed lower aromatic complexity although both were described as very pleasant and balanced in the mouth.

4. CONCLUSIONS

This work evaluates the sensory profiling of wines Maturana Tinta, Monastel and Maturana Tinta de Navarrete and monitors the chemical changes occurring in phenolic compounds and colour parameters during the malolactic fermentation process. In this sense, it was observed that malolactic fermentation produced changes of the same magnitude in all the analysed compounds in all the wines and both anthocyanin and hydroxycinnamic acid distribution was found to be dependent on the variety and not on the winemaking process. Therefore, this study permitted to characterize for the first time wines manufactured with these minority varieties and provided data that could be used as a chemotaxonomic tool to fingerprint them. Data also revealed that all the varieties produced wines with high values of resveratrol which could lead to healthier wines. Moreover, in sensory analysis all wines were found to present a great potential to produce high quality wines, which would provide a viable alternative to grape varieties cultivated in La Rioja and would favour the differentiation of the Rioja wines on the national and international markets. However, and in order to complement these findings, further studies would be needed on the biogenic ammine, amino acid and volatile composition of these wines and on consumer preferences.

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REFERENCES

Abril, M., Negueruela, A. I., Pérez, C., Juan, T., & Estopañán, G. (2005). Preliminary study of resveratrol content in Aragón red and rosé wines. *Food Chemistry, 92,* 729-736.

Aleixandre, J. L., Lizama, V., Álvarez, I., & García, M. J. (2002). Varietal differentiation of red wines in the Valencian region (Spain). *Journal of Agricultural and Food Chemistry, 50,* 751-755.

Asenstorfer, R. E., Hayasaka, Y., & Jones, G. P. (2001). Isolation and structures of oligomeric wine pigments by bisulphite mediated ion-exchange chromatography. *Journal of Agricultural and Food Chemistry, 49,* 5957-5963.

Ayala, F., Echávarri, J. F., & Negueruela, A. I. (1997). A new simplified method for measuring the colour of wines. I. Red and rosé wines. *American Journal of Enology and Viticulture, 48,* 357-363.

Bartowsky, E., & Borneman, A. R. (2011). Genomic variations of *Oenococcus oeni* strains and the potential to impact on malolactic fermentation and aroma compounds in wines. *Applied Microbiology and Biotechnology, 92,* 441-447.

Boulton, R. B. (2001). The copigmentation of anthocyanins and its role in the colour of red wine: a critical review. *American Journal of Enology and Viticulture, 52,* 67-87.

Cabrita, M. J., Torres, M., Palma, V., Alves, E., Patão, R., & Costa Freitas, A. M. (2008). Impact of malolactic fermentation on low molecular weight phenolic compounds. *Talanta, 74,* 1281-1286.

Castillo-Muñoz, N., Gómez-Alonso, S., García-Romero, E., Gómez, M. V., Velders, A. H., & Hermosín-Gutiérrez, I. (2009). Flavonol 3-O-glycosides series of *Vitis vinífera* cv. Petit Verdot red wine grapes. *Journal of Agricultural and Food Chemistry, 57,* 209-219.

Chira, K., Schmauch, G., Saucier, C., Fabre, S., & Teissedre, P. L. (2009). Grape variety effect on proanthocyanidin composition and sensory perception of skin and seed tannin extracts from Bordeaux wine grapes (Cabernet Sauvignon and Merlot) for two consecutive vintages (2006 and 2007). *Journal of Agricultural and Food Chemistry, 57,* 545-553.

Cosme, F., Ricardo-Da-Silva, J. M., & Laureano, O. (2009). Tannic profiles of *Vitis vinífera* L. grapes growing in Lisbon and from their monovarietal wines. *Food Chemistry, 112,* 197-204.

Escudero-Gilete, M. L., González-Miret, M. L., & Heredia, F. J. (2010). Implications of blending wines on the relationships between the colour and the anthocyanic composition. *Food Research International, 43, 745-752*.

Fernández, O., Martínez, O., Hernández, Z., Guadalupe, A., & Ayestarán, B. (2011) Effect of the presence of lysated lees on polysaccharides, color and main phenolic compounds of red wine during barrel ageing. *Food Research International*, *44*, 84-91.

Ferrandino, A., & Guidoni, S. (2010). Anthocyanins, flavonols and hydroxycinnamates: an attempt to use them to discriminate *Vitis vinífera* L. cv "Barbera" clones. *European Food Research and Technology, 230,* 417-427.

Fortes Gris, E., Mattivi, F., Ferreira, E., Vrhovsek, U., Pedrosa, R. C., & Bordignon-Luiz, M. T. (2011). Proanthocyanidin profile and antioxidant capacity of Brazilian *Vitis vinífera* red wines. *Food Chemistry, 126,* 213-220.

Garrido, J., & Borges, F. (2011). Wine and grape polyphenols-A chemical perspective. *Food Research International*, *44*, 3134-3148.

Ginjom, I., D´Arcy, B., Caffin, N., & Gidley, M. (2011). Phenolic compound profiles in selected Queensland red wines at all stages of the wine-making process. *Food Chemistry, 125,* 823-834.

Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). HPLC analysis of diverse grape and wine phenolics using direct injection and multidetection by DAD and fluorescence. *Journal of Food Composition and Analysis, 20,* 618-626.

González-Manzano, S., Dueñas, M., Rivas-Gonzalo, J. C., Escribano-Bailón, M. T., & Santos-Buelga, C. (2009). Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine. *Food Chemistry, 114,* 649-656.

Guadalupe, Z., & Ayestarán, B. (2008). Changes in the colour components and phenolic content of red wines from *Vitis vinífera* L. Cv. “Tempranillo” during vinification and aging. *European Food Research and Technology, 228,* 29-38.

Guadalupe, Z., Soldevilla, A., Sáenz-Navajas, M. P., & Ayestarán, B. (2006). Analysis of polymeric phenolics in red wines using different techniques combined with gel permeation chromatography fractionation. *Journal of Chromatography A, 1112,* 112-120.

Han, F. L., Zhang, W. N., Pan, Q. H., Zheng, C. R., Chen, H. Y., & Duan, C. Q. (2008). Principal component regression analysis of the relation between CIELAB color and monomeric anthocyanins in young Cabernet Sauvignon wines. *Molecules, 13,* 2859-2870.

Hermosín Gutiérrez, I., Sánchez-Palomo Lorenzo, E., & Vicario Espinosa, A. (2005). Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars Cabernet Sauvignon, Cencibel and Syrah. *Food Chemistry, 92*, 269-283.

Hernández, T., Estrella, I., Pérez-Gordo, M., Alegría, E. G., Tenorio, C., Ruiz-Larrea, F., & Moreno-Arribas, M. V. (2007). Contribution of malolactic fermentation by *Oenococcus oeni* and *Lactobacillus plantarum* to the changes in the nonanthocyanin phenolic composition of red wine. *Journal of Agricultural and Food Chemistry, 55,* 5260-5266.

Hernádez-Orte, P., Cersosimo, M., Loscos, N., Cacho, J., García-Moruno, E., & Ferreira, V. (2009). Aroma development from non-floral grape precursors by wine lactic acid bacteria. *Food Research International, 42,* 773-781.

Kennedy, J. A., & Jones, G. P. (2001). Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of phloroglucinol. *Journal of Agricultural and Food Chemistry, 49,* 1740-1746.

Levengood, J., & Boulton, R. (2004). The variation in the colour due to copigmentation in young Cabernet Sauvignon wines. In: Red wine colour. Revealing the mysteries. A. Waterhouse and J. Kennedy (Eds.), pp. 35-52. American Chemical Society, Washington.

López, R., López-Alfaro, I., Gutiérrez, A. R., Tenorio, C., Garijo, P., Gónzalez-Arenzana, L., & Santamaría, P. (2011). Malolactic fermentation of Tempranillo wine: contribution of the lactic acid bacteria inoculation to sensory quality and chemical composition. *International Journal of Food Science and Technology, 46,* 2373-2381.

Martínez de Toda, F., Martínez, M. T., Sancha, J. C., Blanco, C., & Martínez, J. (2004a). Variedades minoritarias de vid en la D.O.Ca. Rioja. Ed. Gobierno de La Rioja, Logroño.

Martínez de Toda, F., Martínez, M. T., Sancha, J. C., Blanco, C., & Martínez, J. (2004b). Interés de las variedades locales y minoritarias de vid. Ed. Gobierno de La Rioja, Logroño.

Mendoza, L. M., Manca de Nadra, M. C., & Farías, M. E. (2010). Antagonistic interaction between yeast and lactic acid bacteria of oenological relevance. Partial characterization of inhibitory compounds produced by yeasts. *Food Research International, 43,* 1990-1998.

Miller, B. J., Franz, C. M. A. P., Cho, G.-S., & du Toit, M. (2011). Expression of the malolactic enzyme gene (*mle*) from *Lactobacillus plantarum* under winemaking conditions. *Current Microbiology, 62,* 1682-1688.

Monagas, M., Gómez-Cordovés, C., Bartolomé, B., Laureano, O., & Ricardo da Silva, J. M. (2003). Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinífera* L. cv. Graciano, Tempranillo and Cabernet Sauvignon. *Journal of Agricultural and Food Chemistry, 51,* 6475-6481.

O. I. V. Compendium of International Methods of Analysis of Wine and Must (1990) París.

Pan, W., Jussier, D., Terrade, N., Yada, R. Y., & Mira de Orduña, R. (2011). Kinetics of sugars, organic acids and acetaldehyde during simultaneous yeast-bacterial fermentations of white wine at different pH values. *Food Research International, 44,* 660-666.

Pérez-Trujillo, J. P., Hernández, Z., López-Bellido, F. J., & Hermosín-Gutiérrez, I. (2011). Characteristic phenolic composition of single cultivar red wines of the Canary Islands (Spain). *Journal of Agricultural and Food Chemistry, 59,* 6150-6164.

Pramateftaki, P. V., Metafa, M., Karapetrou, G., & Marmaras, G. (2012). Assessment of the genetic polymorphism and biogenic amine production of indigenous *Oenococcus oeni* strains isolated from Greek red wines. *Food Microbiology, 29,* 113-120.

Terrade, N., Noël, R., Couillaud, R., & Mira de Orduña, R. (2009). A new chemically defined medium for wine lactic acid bacteria. *Food Research International, 42,* 363-367.

Vidal, S., Francis, L., Williams, P., Kwitkowski, M., Gawel, R., Cheynier, V., & Waters, E. (2004). The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chemistry, 85,* 519-525.

Yada & Mira de Orduña 2011

FIGURE CAPTIONS

Figure 1. Concentration (mg/L) of total anthocyanins (T-A), non-acetylated anthocyanins (A), acetyl-glucoside anthocyanins (A-Ac) and coumaryl-glucoside anthocyanins (A-Cm) in wines after alcoholic and malolactic fermentation.

Figure 2. Relative proportions of non-acylated anthocyanins (A) in wines after alcoholic and malolactic fermentation. Df-3-G: delphinidin-3-glucoside; Ci-3-G: cyaniding-3-glucoside; Pt-3-G: petunidin-3-glucoside; Pn-3-G: peonidin-3-glucoside; Mv-3-G: malvidin-3-glucoside.

Figure 3. PCA on the mean ratings for colour parameters and phenolic compounds in wines after alcoholic and malolactic fermentation.

Figure 4. GPA on the mean ratings for a) olfactory and b) gustatory attributes in wines after malolactic fermentation.

Table 1**.** Oenological parameters in wines after alcoholic and malolactic fermentation

Wine	Alcohol content[1]	pH	TA[2]	VA[3]	H_2M[4]
TEOH	14.58±0.2[ab]	3.64±0.02[d]	6.01±0.1[c]	0.28±0.05[a]	2.64±0.02[e]
MOOH	15.02±0.3[a]	3.46±0.01[ab]	6.75±0.05[d]	0.29±0.03[a]	1.64±0.03[c]
MAOH	14.28±0.2[bc]	3.41±0.03[a]	6.96±0.2[d]	0.38±0.07[a]	2.22±0.05[d]
MNAVOH	13.95±0.2[c]	3.51±0.02[bc]	5.63±0.05[b]	0.30±0.03[a]	1.25±0.02[b]
TEML	14.55±0.2[abc]	3.81±0.02[f]	5.40±0.03[b]	0.38±0.05[a]	0±0.01[a]
MOML	15.00±0.2[a]	3.65±0.02[de]	5.52±0.1[b]	0.40±0.07[a]	0±0.00[a]
MAML	14.30±0.2[bc]	3.54±0.03[c]	5.54±0.03[b]	0.39±0.03[a]	0±0.00[a]
MNAVML	14.00±0.2[bc]	3.71±0.01[e]	4.99±0.1[a]	0.32±0.05[a]	0±0.01[a]

[1] Alcohol content: mL ethanol for 100 mL of wine at 20ºC; [2] TA: titratable acidity as g of tartaric acid per litre; [3] VA: volatile acidity as g of acetic acid per litre; [4] H_2M: malic acid as g of malic acid per litre. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p < 0.05$.

Table 2. Colour parameters and total polyphenol index (absorbance units) in wines after alcoholic and malolactic fermentation

Wine	CI[1]	Hue[2]	a^{*}[3]	b^{*}[3]	L^{*}[3]	WC[4]	MAC[5]	BSC[6]	CC[7]	TPI[8]
TEOH	19.41±1.1^{b}	0.55±0.01^{d}	45.93±1.8^{ab}	4.02±0.09^{d}	55.30±3.3^{ab}	11.02±0.04^{e}	5.23±0.08^{bcd}	1.99±0.01^{c}	3.79±0.04^{b}	75.3±2.2^{bc}
MOOH	21.48±0.82^{c}	0.52±0.01^{c}	48.04±0.2^{bc}	5.83±0.76^{e}	53.94±2.06^{a}	11.96±0.11^{g}	5.37±0.08^{cd}	2.24±0.01^{d}	4.36±0.11^{d}	80.0±2de
MAOH	19.83±1.30^{b}	0.47±0.01^{a}	49.62±3.58^{c}	-1.51±0.32^{b}	54.72±3.56^{ab}	11.53±0.05^{f}	5.11±0.10^{b}	1.49±0.01^{a}	4.94±0.06^{e}	71.8±4.2^{ab}
MNAVOH	19.81±0.75^{b}	0.5±0.01^{b}	44.75±1.79^{ab}	3.27±1.5^{d}	57.55±2.3^{ab}	10.46±0.09^{d}	4.62±0.06^{a}	1.81±0.01^{b}	4.03±0.09^{c}	71.0±1^{a}
TEML	18.34±0.84^{ab}	0.61±0.01^{f}	45.19±1.4^{ab}	1.73±0.05^{c}	55.78±1.54^{ab}	9.09±0.43^{b}	5.19±0.24^{bc}	2.32±0.11^{d}	1.58±0.08^{a}	78.0±2.6^{cd}
MOML	19.64±0.24^{b}	0.59±0.00^{e}	47.83±0.4^{bc}	3.33±0.14^{d}	54.08±0.19^{a}	10.04±0.2^{c}	5.47±0.07^{d}	2.88±0.01^{e}	1.70±0.08^{a}	84.0±1^{e}
MAML	17.67±0.64^{a}	0.52±0.02^{c}	47.84±1.8^{bc}	-3.46±0.18^{a}	55.94±1.88^{ab}	8.95±0.41^{ab}	5.21±0.25^{bc}	2.09±0.01^{c}	1.65±0.05^{a}	75.5±1.8^{bc}
MNAVML	17.01±0.2^{a}	0.6±0.01^{f}	43.77±1.05^{a}	0.66±0.03^{c}	58.19±0.42^{b}	8.56±0.04^{a}	4.63±0.06^{a}	2.32±0.09^{d}	1.62±0.05^{a}	75.2±1.9^{abc}

[1] CI: colour intensity as sum of absorbances at 420, 520 and 620 nm; [2] Hue: A_{420}/A_{520}; [3] a^{*}: from green to red; b^{*}: from blue to yellow; L^{*}: lightness; [4] WC: red wine colour; [5] MAC: monomeric anthocyanin colour; [6] BSC: bisulfite stable colour; [7] CC: copigmentation colour; [8] TPI: total polyphenol index. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p < 0.05$.

Table 3. Concentration of hydroxycinnamic acid derivatives, gallic acid and total resveratrol (mg/L) in wines after alcoholic and malolactic fermentation

Wine	*cis*-caftaric	*trans*-caftaric	*cis*-coutaric	*trans*-coutaric	*trans*-fertaric	T-HA[1]	t-cout/ t-caft[2]	Gallic acid	T-resveratrol[3]
TEOH	5.42±0.05[bc]	34.3±0.2[a]	3.82±0.01[f]	22.23±0.9[e]	1.86±0.01[c]	67.6±0.5[d]	0.65	45.6±0.5[b]	4.57±0.05[b]
MOOH	4.72±0.03[a]	33.93±0.2[a]	2.57±0.02[b]	12.72±0.6[a]	1.82±0.02[c]	55.76±0.9[a]	0.37	56.4±0.54[d]	10.2±0.05[g]
MAOH	5.60±0.06[c]	38.97±0.15[c]	2.96±0.05[c]	15.95±0.4[c]	1.14±0.00[a]	64.62±0.25[c]	0.39	39.5±0.36[a]	8.87±0.05[f]
MNAVOH	5.64±0.14[c]	40.36±0.32[c]	3.10±0.09[d]	13.93±0.45[b]	1.57±0.01[b]	64.6±0.07[c]	0.34	46.2±0.45[b]	6.03±0.02[d]
TEML	5.71±0.24[d]	34.54±1.23[a]	3.53±0.03[e]	21.06±0.5[d]	3.61±0.13[g]	68.46±1.69[de]	0.61	50.20±0.96[c]	3.16±0.13[a]
MOML	4.84±0.21[a]	36.39±0.79[b]	2.86±0.14[c]	12.79±0.71[a]	3.40±0.10[f]	60.28±1.10[b]	0.35	59.13±2.08[e]	4.43±0.14[b]
MAML	5.28±0.21[b]	42.76±1.75[d]	3.76±0.05[f]	15.48±0.43[c]	2.84±0.08[d]	70.11±1.95[e]	0.36	46.04±0.47[b]	8.39±0.38[e]
MNAVML	5.69±0.13[c]	42.96±0.11[d]	2.03±0.03[a]	13.87±0.33[b]	3.12±0.08[e]	67.64±0.41[d]	0.33	47.07±1.50[b]	5.55±0.08[c]

[1] T-HA: total hydroxycinnamic acids; [2] *t*-cout /*t*-caft: ratio *trans*-coutaric/*trans*-caftaric; [3] T-resveratrol: total resveratrol. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p < 0.05$.

Table 4. Concentration of flavonols (mg/L) in wines after alcoholic and malolactic fermentation

Wine	Myricetin-3-G[1]	Quercetin-3-Gal[2]	Quercetin-3-G[3]	Quercetin-3-Glc[4]	Myricetin	Quercetin	Kaempferol	Isorhamnetin	T-Flavo[5]
TEOH	21.5±0.2[h]	2.42±0.03[b]	3±0.03[e]	6.86±0.07[b]	6.91±0.07[f]	1.83±0.02[a]	0.05±0.01[a]	0.15±0.01[a]	42.72±0.4[e]
MOOH	6.20±0.06[c]	2.55±0.03[b]	1.06±0.01[a]	7.83±0.08[d]	10.1±0.1[g]	10.3±0.1[g]	0.12±0.005[b]	0.79±0.01[f]	38.95±0.4[d]
MAOH	18.9±0.2[g]	3.61±0.04[c]	10.73±0.1[g]	15.06±0.2[f]	5.33±0.05[d]	13.06±0.1[h]	0.57±0.01[g]	2.05±0.01[h]	69.31±0.7[g]
MNAVOH	10.07±0.1[d]	1.6±0.02[a]	1.46±0.01[b]	8.01±0.08[d]	6.62±0.07[e]	5.02±0.05[d]	0.18±0.005[c]	0.63±0.01[e]	33.59±0.3[c]
TEML	12.25±0.08[e]	4.50±0.09[d]	2.69±0.09[d]	6.29±0.20[a]	3.42±0.06[a]	2.28±0.07[b]	0.21±0.01[d]	0.19±0.00[b]	31.82±0.2[b]
MOML	2.01±0.07[a]	4.95±0.25[e]	1.66±0.01[b]	6.55±0.29[ab]	4.84±0.07[c]	6.08±0.24[e]	0.27±0.01[e]	0.50±0.01[c]	26.84±0.7[a]
MAML	14.09±0.65[f]	5.40±0.25[f]	8.99±0.41[f]	13.65±0.2[e]	3.33±0.14[a]	7.1±0.13[f]	0.35±0.02[f]	1.27±0.04[g]	54.16±1.3[f]
MNAVML	5.31±0.13[b]	3.4±0.12[c]	2.13±0.08[c]	7.43±0.24[c]	4.00±0.15[b]	4.54±0.2[c]	0.19±0.01[c]	0.58±0.02[d]	27.57±0.07[a]

[1] Myricetin-3-G: myricetin-3-glucoside; [2] Quercetin-3-Gal: quercetin-3-galactoside; [3] Quercetin-3-G: quercetin-3-glucoside; [4] Quercetin-3-Glc: quercetin-3-glucuronide; [5] T-Flavo: total flavonols. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p < 0.05$.

Table 5. Concentration of catechin and proanthocyanidins (mg/L) and mean degree of polymerization in wines after alcoholic and malolactic fermentation

Wine	(+)-catechin	PA[1]	mDP[2]
TEOH	78.5±0.75[d]	828±46[e]	13.57±0.01[g]
MOOH	75.9±0.72[d]	1013±19[g]	10.53±0.00[d]
MAOH	66.4±0.63[c]	903±27[f]	13.12±0.00[f]
MNAVOH	90.8±0.68[f]	715±36[d]	11.57±0.00[e]
TEML	55.99±2.91[b]	483±24[a]	7.34±0.32[c]
MOML	69.95±2.7[c]	584±16[c]	6.81±0.33[b]
MAML	44.15±2[a]	535±16[b]	7.19±0.36[bc]
MNAVML	86.15±4.66[e]	448±8[a]	6.36±0.22[a]

[1] PA: total proanthocyanidins; [2] mDP: mean degree of polymerization. Values are means ± standard deviations. Different letters in the same column indicate that means significantly differ at $p < 0.05$.

Figure 1

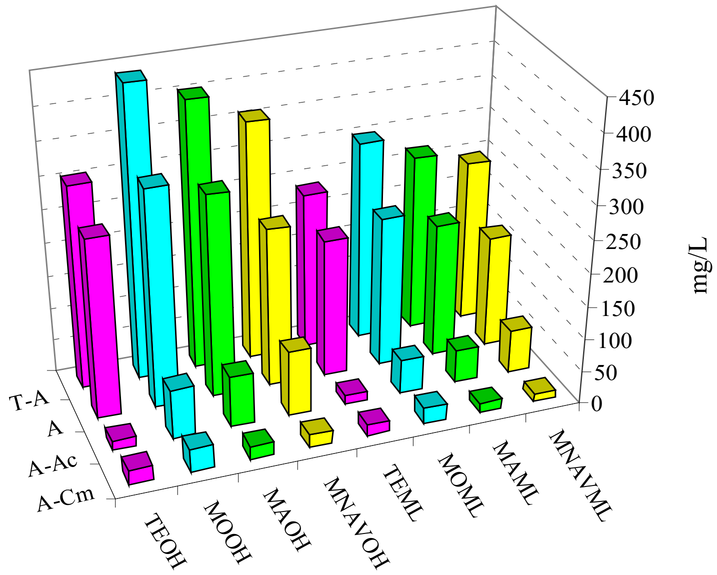
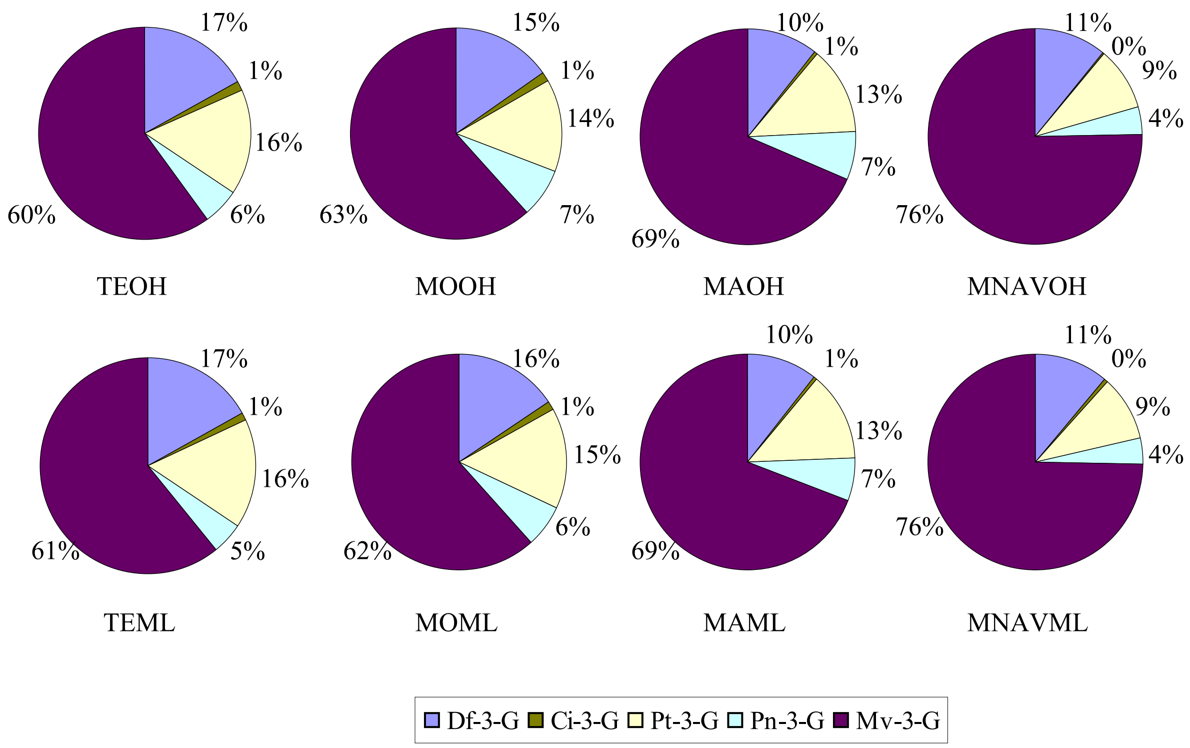

450
400
350
300
250
200
150
100
50
0
mg/L
T-A
A
A-Ac
A-Cm
TEOH
MOOH
MAOH
MNAVOH
TEML
MOML
MAML
MNAVML

Figure 2



17%
1%
16%
6%
60%
TEOH
15%
1%
14%
7%
63%
MOOH
10%
1%
13%
7%
69%
MAOH
11%
0%
9%
4%
76%
MNAVOH
17%
1%
16%
5%
61%
TEML
16%
1%
15%
6%
62%
MOML
10%
1%
13%
7%
69%
MAML
11%
0%
9%
4%
76%
MNAVML
Df-3-G
Ci-3-G
Pt-3-G
Pn-3-G
Mv-3-G

Figure 3

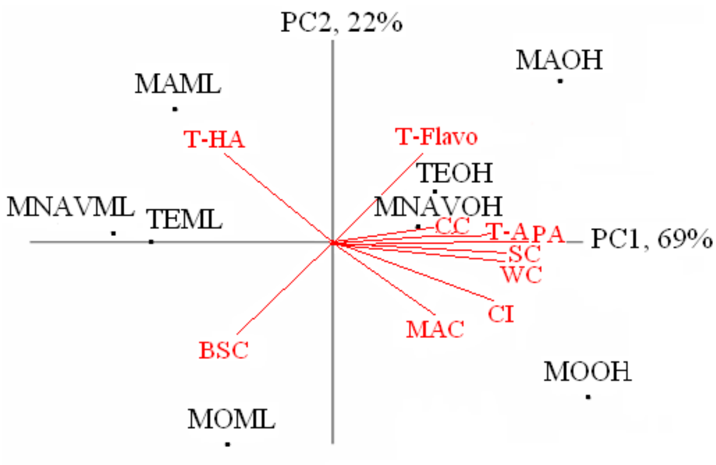
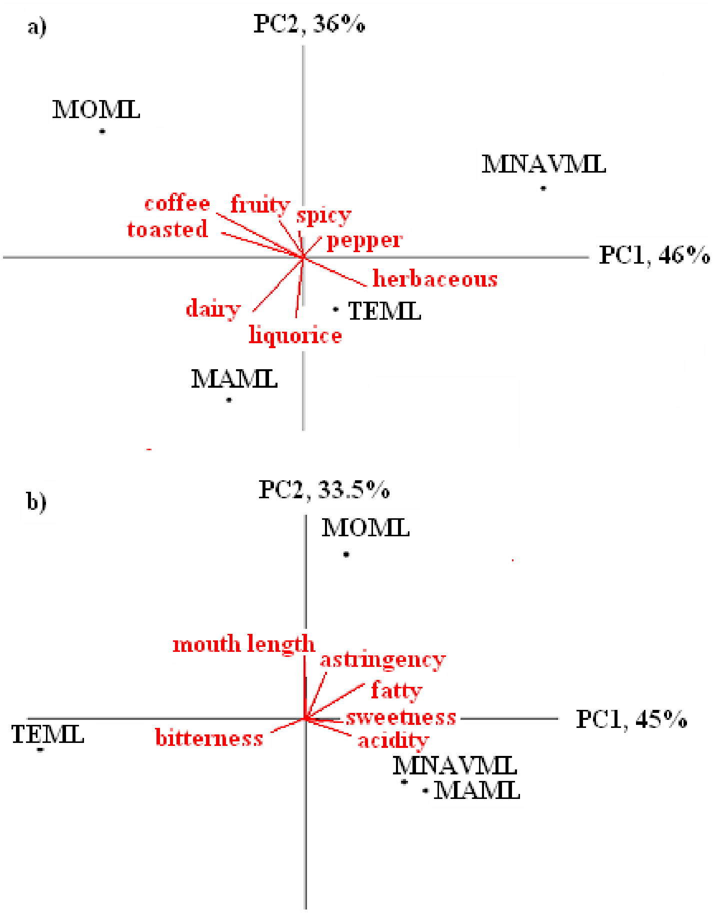

PC2, 22%
MAOH
MAML
T-HA
T-Flavo
TEOH
MNAVML
TEML
MNAVOH
CC
T-APA
PC1, 69%
SC
WC
CI
MAC
BSC
MOOH
MOML

Figure 4


a)
PC2, 36%
MOML
MNAVML
coffee
fruity
spicy
toasted
pepper
PC1, 46%
herbaceous
dairy
TEML
liquorice
MAML
b)
PC2, 33.5%
MOML
mouth length
astringency
fatty
TEML
bitterness
sweetness
PC1, 45%
acidity
MNAVML
MAML