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Colour stabilization of red wines by microoxygenation treatment before malolactic fermentation

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Abstract

Microoxygenation allows the addition of small, continuous and controlled amounts of oxygen, in order to improve wine quality. Then, the effect of this treatment on colour stabilization and phenolic composition was studied. Four single varietal red wines were elaborated during three consecutive vintages. One part of them was microoxygenated before malolactic fermentation, the other part being maintained in stainless steel tanks without microoxygenation.

The results showed that the microoxygenation treatment slightly decreased the global phenolic composition of wines but allowed the stabilization of wine colour, without showing an appreciable increase in tonality values. Thus, the slow and controlled addition of small amounts of oxygen could partially avoid the colour drop habitually occurring in red wines after malolactic fermentation.

Keywords: Microoxygenation; Red wines; Colour stabilization; Phenols

1. Introduction

Oxygen plays an important role in the different physicochemical and microbiological processes that take place during fermentation and aging of wines. Besides, oxygen has an influence on the phenolic composition and indirectly has an effect on some sensorial characteristics, such as colour, astringency and aroma which determine wine quality. This is due to the important role that oxygen plays in oxidation, condensation and polymerization reactions in which different compounds (mainly phenolic compounds) are involved. These reactions lead to the formation of new pigments and polymeric compounds which can stabilize wine colour (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Bakker & Timberlake, 1997; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & Freitas, 2003; Revilla, Pérez-Magariño, González-Sanjosé, & Beltrán, 1999; Vivar-Quintana, Santos-Buelga, & Rivas-Gonzalo, 2002). Among other kinds of reactions, those involving acetaldehyde, such as the formation of pyranoanthocyanins and of ethyl-bridged adducts, are expected to be favoured by the presence of oxygen (Atanasova et al., 2002). This is because oxygen catalyzes oxidation of ethanol to acetaldehyde, which then serves as a bridging agent, linking together phenols and/or phenol-anthocyanins.

Traditionally, oxygen supply takes place indirectly, through different processes, such as pump-over and racking. However, the amount of oxygen provided by these treatments is difficult to control, so the controlling of added oxygen should improve quality.

Microoxygenation is a technique developed in France (Madeiran) by Patrick Ducournau and Michael Moutounet in the earliest nineties (Roig & Yerle, 2003). This technique allows the addition of small, continuous and controlled amounts of pure oxygen or air into wines over

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time. Parish, Wollan, and Paul (2000) pointed out that this application can be carried out at any stage of the winemaking process, from primary fermentation to bottling. However, different experimental studies seem to confirm that the addition of oxygen during the post-fermentative phase gave better results for wine quality, especially for colour stabilization and palate structure (Amati, Arfelli, Castellari, & Simoni, 2000; Bosso, Guaita, Vaudano, & Di-Stefano, 2000; Castellari, Matricardi, Arfelli, Gallassi, & Amati, 2000; Ferrarini, Girardi, De-Conti, & Castellari, 2001; Pérez-Magariño & Gonzále, 2002; Pour-Nikfardjam & Dykes, 2003).

Winemakers recognize the beneficial aspects of limited oxygen exposure of young red wines immediately after alcoholic fermentation and during the early stages of aging. However, few scientific studies supported by analytical data have been published.

Microoxygenation allows control over the amount of oxygen, which can be regulated over time, taking into account the necessities of each wine. Therefore, some of the main purposes of the microoxygenation are to enhance colour stability, to improve mouth feel (body and texture), to decrease astringency, to reduce the undesirable vegetal or herbaceous aromas, and to avoid the presence of reductive characters (Moutounet, 2003; Roig & Yerle, 2003). Besides, the slow and constant rate of oxygen diffusion actually permits wine phenols to consume oxygen without acquiring oxidative characteristics.

Taking into account the results found, microoxygenation was proposed to improve wine quality, but the addition of oxygen must be controlled since an excess of oxygen can give rise to negative effects, such as higher astringency, appearance of anomalous characters, phenol oxidation and negative microbial activities (Parish et al., 2000).

Furthermore, the rate of oxygenation and total oxygen added depend on volatile sulfide, anthocyanin and tannin concentrations, and also on the ability of wine to consume this oxygen. Therefore, rate cannot be determined "a priori", and in general, is indirectly related to the relative concentration of polyphenols, and determined by tasting. Therefore, this treatment requires continuous control of wine evolution in order to avoid negative results on wine quality.

Among all the possible useful effects of the microoxygenation technique on red wine quality parameters, the aim of this work was to evaluate its effects on colour stabilization and phenolic composition of different single-varietal red wines when microoxygenation treatment was applied before malolactic fermentation.

2. Materials and methods

2.1. Wine elaboration and microoxygenation treatment

Four single varietal red wines were elaborated in stainless steel tanks, following the traditional red winemaking process. Wines were made from red grape of the following varieties and growing zones: Mencía from Bierzo (wines labelled as B), Tinta de Toro from Toro (wines labelled as T), Tinta del País from Ribera del Duero (wines labelled as RD) and Tempranillo from Rueda (wines labelled as RT). These vineyards were placed in "Castilla y León" an Autonomous Community of Spain. After manual harvest and selection of about 5000 kg per grape variety to remove the damaged clusters, these were destemmed with minimum physical damage, and the mass obtained was slightly sulphited (0.04 g/l); it was transferred to undergo alcoholic fermentation at controlled temperature (25–28 °C). The wines were racked off new tanks when the maximum total polyphenol index, TPI (measurement of wine absorbance at 280 nm) was raised. This moment was determined by considering the flat phases after the continuous increase of TPI value; that means that the wines were racked off when this index showed the same value during two or three consecutive days, which coincided, in most of the cases, with nearly complete consumption of reducing sugars (<3 g/l). Once alcoholic fermentation was over and before the malolactic fermentation started, 2000 l of each

Table 1
Total amounts of oxygen in ml per litre of wine added in the different wines and vintages

Variety grapes	Total amount of O_2 added/day		
	2002 vintage	2003 vintage	2004 vintage
Mencía	28.0/18	30.5/23	43.3/19
Tinta de Toro	23.3/20	26.7/20	42.0/19
Tinta del País	30.0/20	30.5/23	43.3/19
Tempranillo	30.0/20	30.0/20	42.0/19

Table 2
Data used to the identification of the anthocyanin peaks

[a]H_{313}/H_{546}	t_r (min)	[c]M^+ (m/z)	[b]A_{313}/A_{530}	Anthocyanins
	0.57	465 (303)	0.100	Dp-3-Gls
	0.60	533 (465, 371)	–	Dp-Pyr
	0.70	449 (287)	0.253	Cy-3-Gls
	0.80	479 (317)	0.128	Pt-3-Gls
	0.85	547 (479, 385)	–	Pt-Pyr
	0.93	463 (301)	0.106	Pn-3-Gls
	1.00	493 (331)	0.333	Mv-3-Gls
	1.05	531 (463, 301)	–	Pn-Pyr
	1.08	561 (493, 331)	0.592	MvPyr
0.114	1.22	507 (465, 303)	0.100	Dp-3-(6 Ac)-Gls
	1.25	517	0.433	Vitisin B
0.124	1.39	491 (449, 287)	0.141	Cy-3-(6 Ac)-Gls
0.107	1.46	521 (479, 317)	0.122	Pt-3-(6 Ac)-Gls
0.117	1.62	505 (463, 301)	0.105	Pn-3-(6 Ac)-Gls
0.120	1.67	535 (331)	0.121	Mv-3-(6 Ac)-Gls
0.745	1.73	611 (303)	0.715	Dp-3-(6 Cm)-Gls
0.530	1.83	655 (493, 331)	0.483	Mv-3-(6 Cf)-Gls
0.698	1.85	595 (287)	0.706	Cy-3-(6 Cm)-Gls
0.742	1.89	625 (317)	0.731	Pt-3-(6 Cm)-Gls
0.889	1.96	609 (301)	0.783	Pn-3-(6 Cm)-Gls
0.787	2.15	639 (331)	0.893	Mv-3-(6 Cm)-Gls

[a] Relationship between height of peaks at 313 nm and 546 nm (González San José, 1989; González San José et al., 1988).
[b] Relationships between peak areas at 313 nm and 530 nm (Pérez-Magariño, 1999).
[c] Data according to Revilla et al. (1999).

wine were separated, transferred to four different stainless steel tanks, and then these tanks were microoxygenated. Microoxygenation equipment provided by AZ3 (Oenodev, France) was used. Pure oxygen was slowly diffused through a ceramic membrane, placed close to the bottom of the stainless steel tank. This diffuser allowed a slow and constant flow rate of a few millilitres of oxygen per litre of wine per month, permitting the wine phenols to consume the oxygen without acquiring oxidative characteristics. The total amount of oxygen added in each wine and vintage is showed in Table 1. As mentioned in Section 1, the rate of oxygenation depends on the presence of off-flavours, including both reductive and vegetal aromas, and the phenolic composition, especially green tannins. After alcoholic fermentation, all wines were tasted by professional tasters, in order to establish their sensorial characteristics, and then

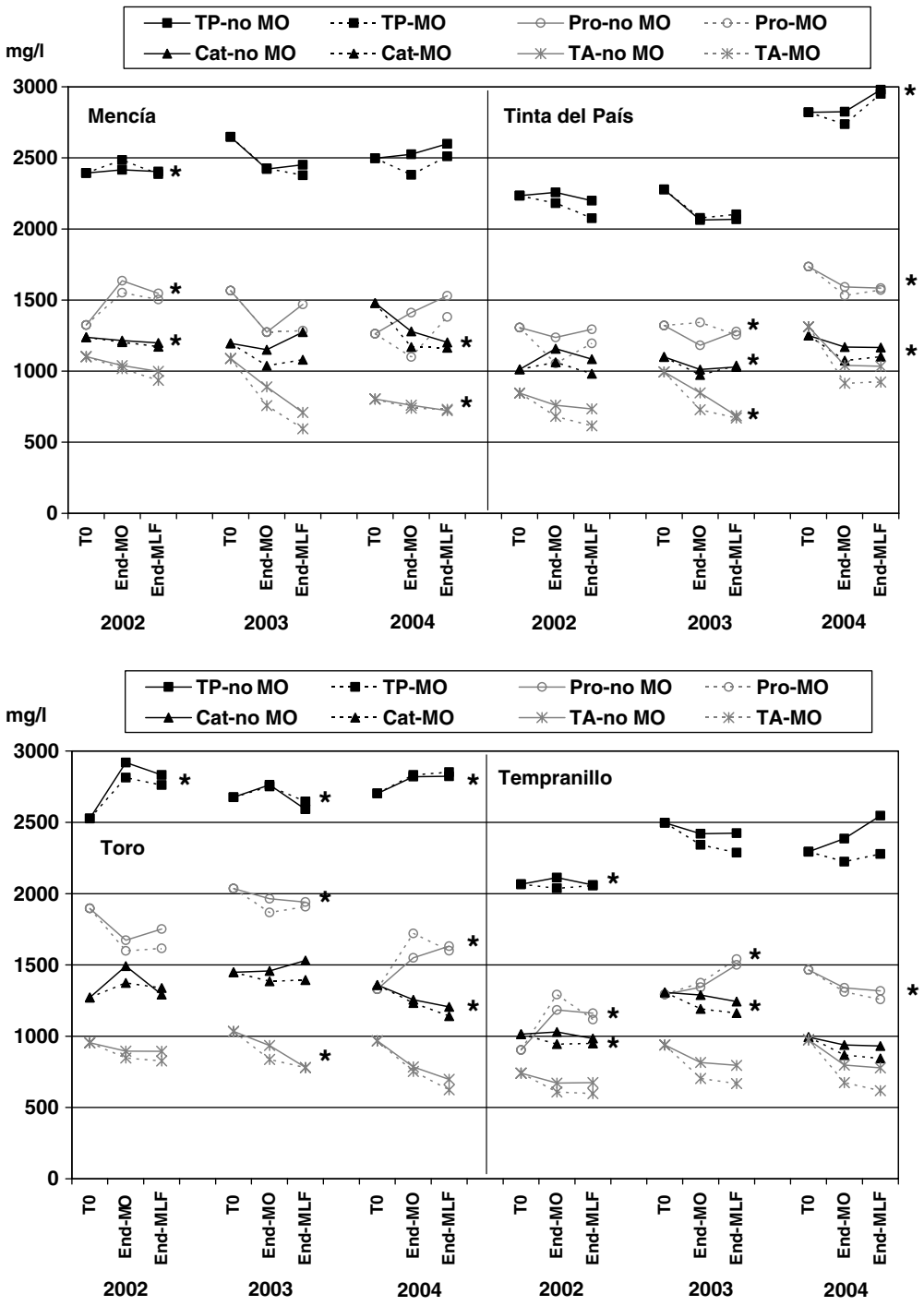

Fig. 1. Phenolic families of microoxygenated (MO) and non-microoxygenated (no MO) wines from each variety and vintage. TP: total polyphenols, Pro: proanthocyanidins, Cat: catechins, TA: total anthocyanins. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

the appropriate microoxygenation doses were determined for each single-red-varietal wine. Initially, the doses of oxygen applied were higher for a brief period of time to eliminate some reductive compounds that sometimes appear just after alcoholic fermentation, and the vegetal characters of some wines, allowing a better fruity expression. After that, the flows were reduced to provide colour and tannin stabilization and to complete the structuring phase before malolactic fermentation started. The microoxygenation treatments were stopped when the tasters considered that the vegetal characters of wines were lost and the tannins evolved from green tannins to hard ones. The temperature was controlled, and maintained around 16 °C while microoxygenation treatment was applied.

The rest of each wine, about 1500 l, was maintained in closed stainless steel tanks, one for each single-variety wine, where the malolactic fermentation was carried out spontaneously. These wines were considered as the control ones and were labelled as no MO.

After the microoxygenation treatment, the microoxygenated wines (MO) underwent the malolactic fermentation (MLF) spontaneously, as did the no MO wines. At the end of the MLF samples of both microoxygenated (MO) and control (no MO) wines were taken.

This study was carried out during three consecutive vintages, 2002, 2003 and 2004.

2.2. Analytical methods

2.2.1. Chemicals

Gallic acid, catechin, caffeic acid and quercetin were purchased from Sigma–Aldrich (St. Louis, MO, USA), and the malvidin-3-glucoside and cyanidin chloride were from Extrasynthèse (Lyon, France).

Milli-Q water, formic acid (Merck, Darmstadt, Germany), and methanol (Lab-Scan, Dublin, Ireland) were used in high-performance liquid chromatography (HPLC) analyses.

2.2.2. Analyses of SO_2 and volatile acidity

The SO_2 and volatile acidity were analyzed following the Official Methods of Analysis (OIV, 1990).

2.2.3. Analyses of levels of phenolic compounds

Several phenolic families were analyzed: otal polyphenols (TP) were determined by reaction with Folin–Ciocalteu reagent, and were expressed as mg/l of gallic acid (Paronetto, 1977); Total anthocyanins (TA) were quantified according to the variation of colour in function of pH and were expressed as mg/l of malvidin-3-glucoside (Paronetto, 1977); proanthocyanidins (Pro) were measured after their acid hydrolysis with heat, and were expressed as mg/l of cyanidin chloride (Ribéreau-Gayón & Stonestreet, 1966); catechins (Cat) were determined by their reaction with vanillin, and were expressed as mg/l of D-catechin (Swain & Hillis, 1959).

Polymeric anthocyanins were determined using Boulton's method, modified by Mazza, Fukumoto, Delaquis, Girard, and Ewert (1999), and they were expressed as mg/l of malvidin-3-glucoside.

The absorbances at 320 nm and 360 nm were used to estimate total levels of tartaric esters and flavonols (Mazza et al., 1999). These compounds were expressed as mg/l of caffeic acid and quercetin, respectively. Polymeric anthocyanins,

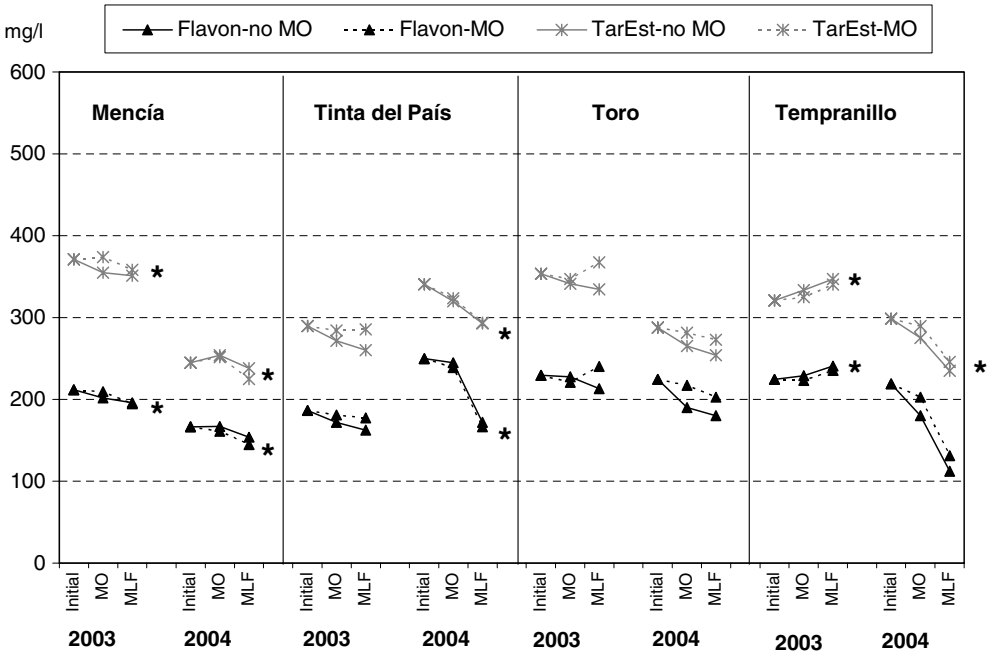

Fig. 2. Levels of total flavonols (Flavon) and total tartaric esters (TarEst) of microoxygenated (MO) and no microoxygenated (no MO) wines from 2003 and 2004 vintages. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

tartaric esters and flavonols were only evaluated in the 2003 and 2004 vintages.

2.2.4. HPLC diodo-array detection and mass spectrometry analyses of anthocyanins

The contents of the individual anthocyanins were determined by direct injection of wine, previously filtered through filters with a pore size of 0.45 μm (Millipore, Bedford, MA), into an Agilent Technologies LC–MS Series 1100, with a diode-array detection system and a mass detector, applying the chromatographic conditions established by Pérez-Magariño and González-Sanjosé (2004). Peak identification was carried out by using the absorbance ratio 530/313 (Table 2), and the relative retention times and the MS detection according to Revilla et al. (1999). The compounds considered in this study were grouped together as anthocyanin glucosides (AcyGls), acetylated anthocyanins (AcyAc), cinnamylated anthocyanins (AcyCin), the

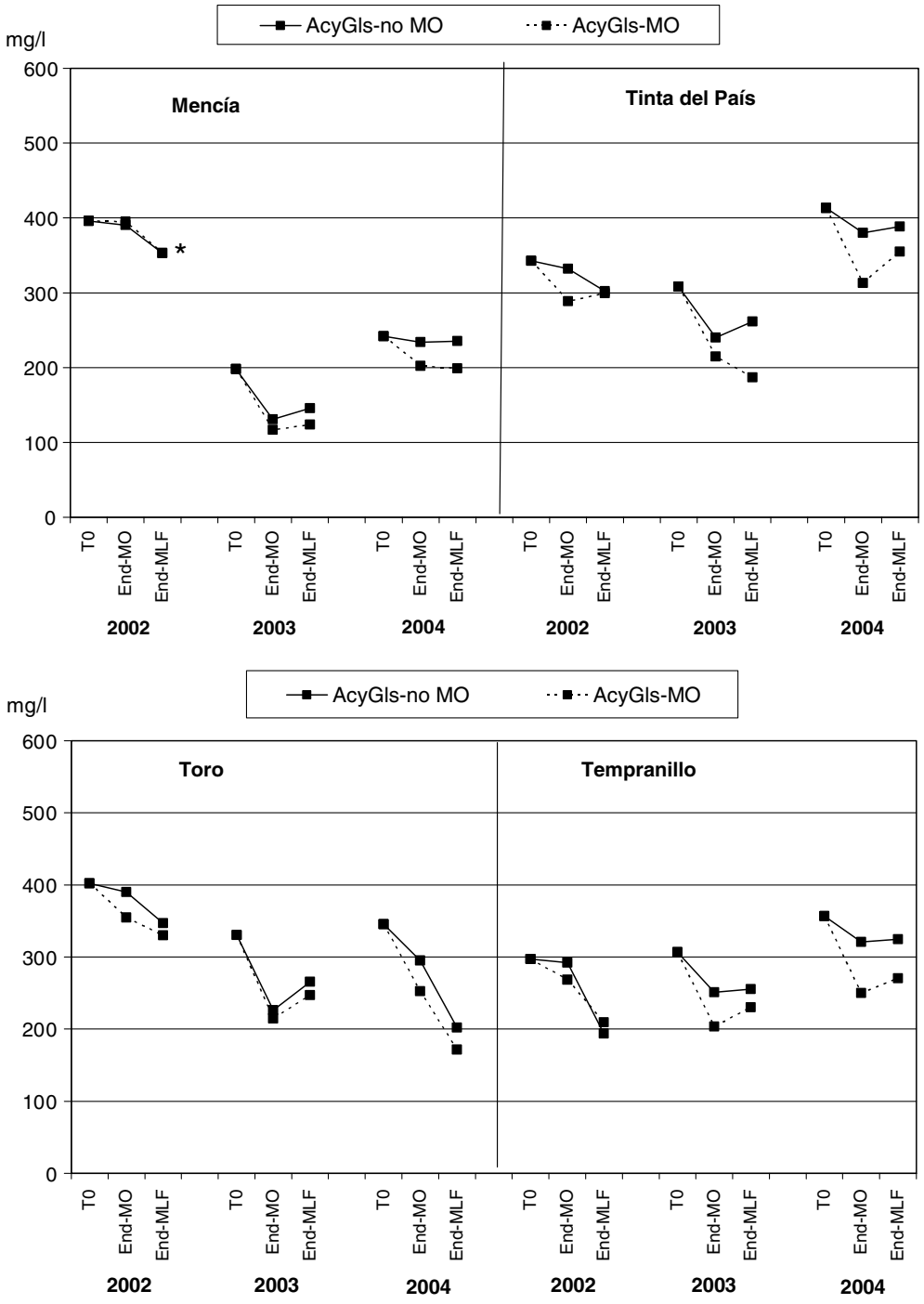

Fig. 3. Anthocyanin glucosides (AcyGls) by HPLC of microoxygenated (MO) and non-microoxygenated (no MO) wines from each variety and vintage. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

pyruvic acid derivatives of the anthocyanins (AcyPyr), and vitisin B.

All anthocyanin compounds were expressed as mg/l of malvidin 3-glucoside, the most abundant pigment in the grape varieties studied.

2.2.5. Colour evaluation of wines

The measurement of wine colour was carried out using the Glories' method (Glories, 1984), evaluating the following chromatic parameters: colour intensity (CI), tonality (To), percentage of yellow (%Ye), percentage of red (%Red) and percentage of blue (%Blue). They are widely known and the most frequently used by wine-makers.

The spectrophotometric measurements were carried out using quartz cells of 1 mm path length.

2.3. Statistical analyses

All the analyses described above were carried out in duplicate, since two samples, which were taken from in

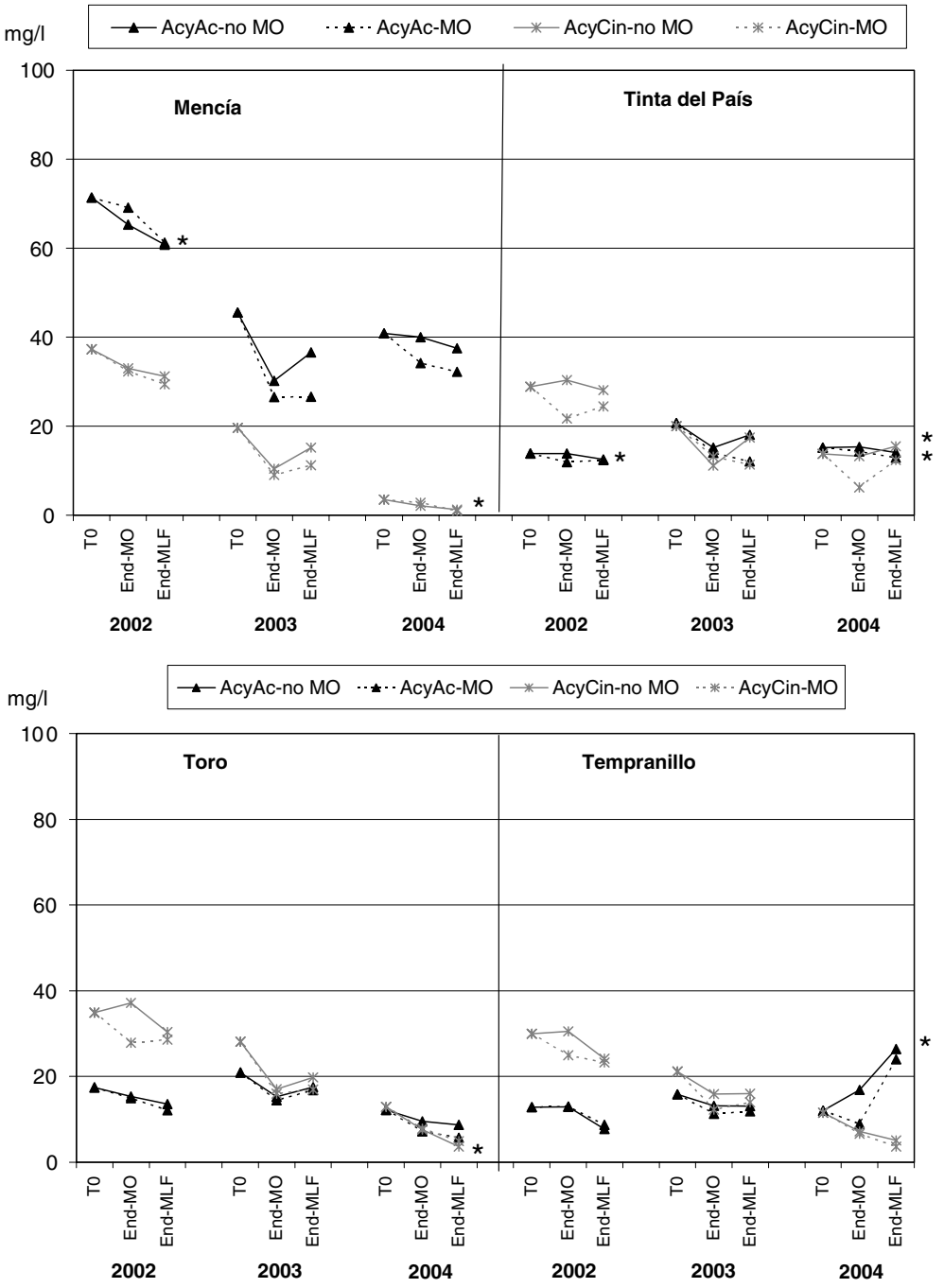

Fig. 4. Anthocyanin acetylated (AcyAc) and anthocyanin cinnamylated (AcyCin) values by HPLC of microoxygenated (MO) and non-microoxygenated (no MO) wines from each variety and vintage. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

two different points of the tanks (at the top and at the bottom), were analyzed for each kind of wine. The analysis of variance (ANOVA) and the least significant difference test (LSD) were used to detect differences and to establish which measurements could be considered statistically different. A significance level of $\alpha = 0.05$ was used.

All statistical analyses were carried out using the statistics package Statgraphics Plus 4.0 (1999).

3. Results and discussion

In order to carry out an adequate microoxygenation treatment, it was necessary to control some factors, such as temperature, volatile acidity and SO_2 levels.

The temperature has a direct influence on the speed of the reactions, leading to the structuring effect, but it also has an influence on the oxygen solubility, which increases

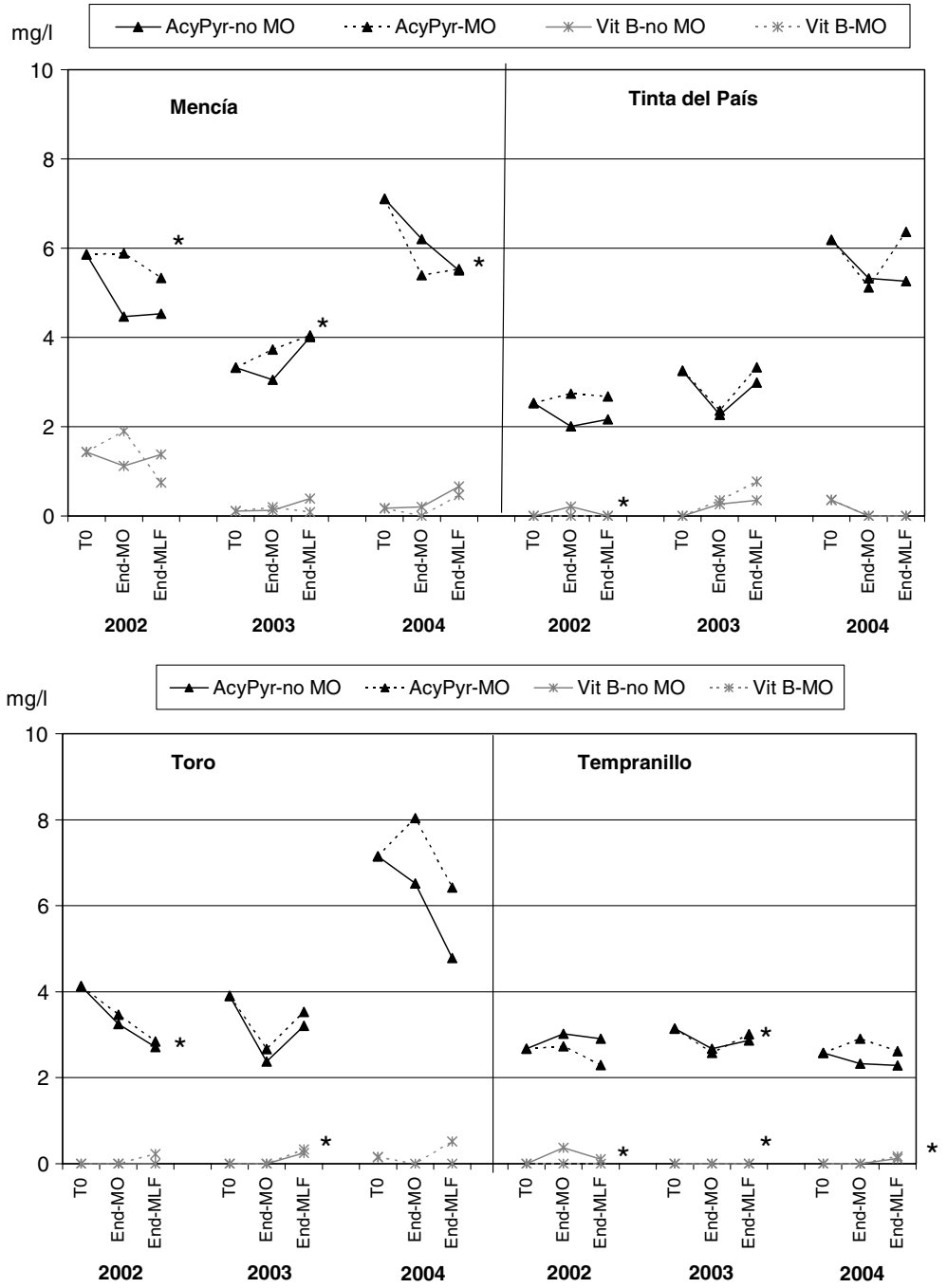

Fig. 5. Anthocyanin derivatives of microoxygenated (MO) and non-microoxygenated (no MO) wines from each variety and vintage. AcyPyr: pyruvic acid derivatives of anthocyanins, Vit B: vitisin B. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

as the temperature decreases. So this factor should be continuously controlled. In this study, temperature was maintained around 16 °C (±1 °C) to avoid the accumulation of dissolved oxygen in the wine.

The SO_2 was measured since it allows the control of the wine flora. Microoxygenation seems not to have a direct effect on SO_2, and vice versa, but if an accumulation of dissolved oxygen occurs, the free-SO_2 drops. Volatile acidity was also evaluated because is a quality parameter and provides information about a possible growth of undesirable microorganisms. Therefore, both parameters (SO_2 and volatile acidity) were checked every two days.

Table 1 shows the total amount of oxygen applied to each varietal wine from each vintage. It must be pointed out that the total amount of oxygen did not exceed of 50 ml/l, which is considered the oxygen limit that a wine can adequately consume, without acquiring undesirable oxidations (Moutounet, 2003). The rate of oxygenation was slightly different for each wine, since each one had a different need of oxygen, although these differences among wines were not very important. Nowadays, tasting is the only way to evaluate this need, and some correlations with analytical parameters such as total polyphenols, tannins and astringency are being studied. Tasting of wines was also carried out every two days in order to change (or not) the oxygen flow.

All colorimetric parameters and phenolic compounds were analyzed at three stages: just at the end of the alcoholic fermentation, initial sampling (T0), the same day as on which application of the microoxygenation treatment was finished (End-MO) and at the end of the malolactic fermentation (End-MLF).

Fig. 1 shows the results of global phenolic composition, total polyphenols, proanthocyanidins, catechins and anthocyanins of the four varietal wines and in the three vintages studied.

The ANOVA results showed some statistically significant differences. In general, the microoxygenated wines (MO) showed slightly lower phenolic compositions than did their non-microoxygenated counterparts (no MO), although in some cases no statistically significant differences were detected. These differences were observed, especially, in the total anthocyanins, the MO wines showing lower concentrations than their no MO counterparts. These results could be due to the fact that phenols took part in condensation and polymerization reactions. This fact could be corroborated by the values found for colour and polymeric anthocyanins that will be discusssed later. The losses of phenols are in agreement with experimental studies carried out by Amati et al. (2000) and Ferrarini et al. (2001), who found lower concentrations of anthocyanins, catechins and proanthocyanidins in MO wines than in no MO ones.

The levels of total tartaric esters and total flavonols, which were only evaluated in wines from 2003 and 2004 vintages, are shown in Fig. 2. No statistically significant differences were found in Mencía wines, either between vintages or compounds but, between Tempranillo and Tinta del País wines, statistically significant differences were found only in some compounds and vintages. Only wines from the Toro variety showed clear differences caused by microoxygenation treatment in the two vintages studied, the MO wines showing the highest concentrations of flavonols and tartaric esters. Therefore, these results indicated

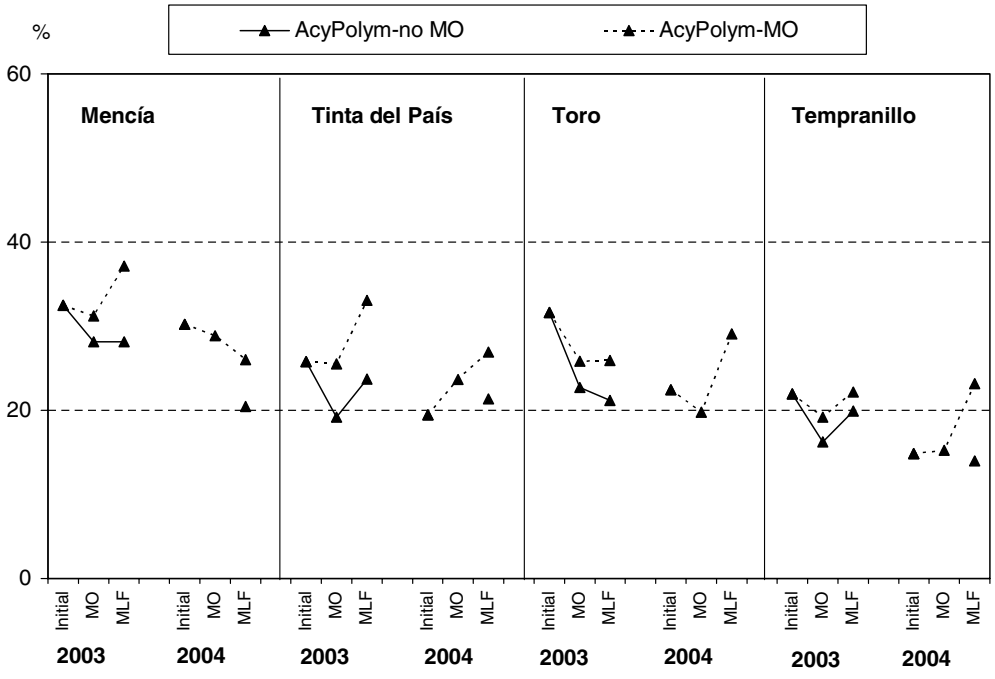

Fig. 6. Percentage of polymeric anthocyanins (AcyPolym) of microoxygenated (MO) and non-microoxygenated (no MO) wines from each variety and from 2003 and 2004 vintages. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

that the microoxygenation treatment did not produce a reduction in the content of this kind of phenol and in some wines even seemed to stabilize their levels. The highest levels of tartaric esters, in the MO wines, was a surprising result, because these phenols are very susceptible to oxidation, as has been widely studied by other authors (Singleton, 1987), and thus a decrease of these kinds of compounds in the MO wines could be expected.

Statistically significant differences were observed between MO and no MO wines in the individual anthocyanins evaluated by HPLC (Figs. 3 and 4). In general, the no MO wines showed the highest values in the monomeric anthocyanin glucosides, acetylated and cinnamylated. These results are in agreement with the lower values of total anthocyanins found in MO wines previously discussed, and with the higher percentage of polymeric anthocyanins found in the MO wines than in the no MO ones (Fig. 6). The highest values of polymeric anthocyanins in MO wines are statistically significantly different for all wines. These results made clear the influence of the microoxygenation treatment on the polymerization reactions, showing a more important formation in the MO wines.

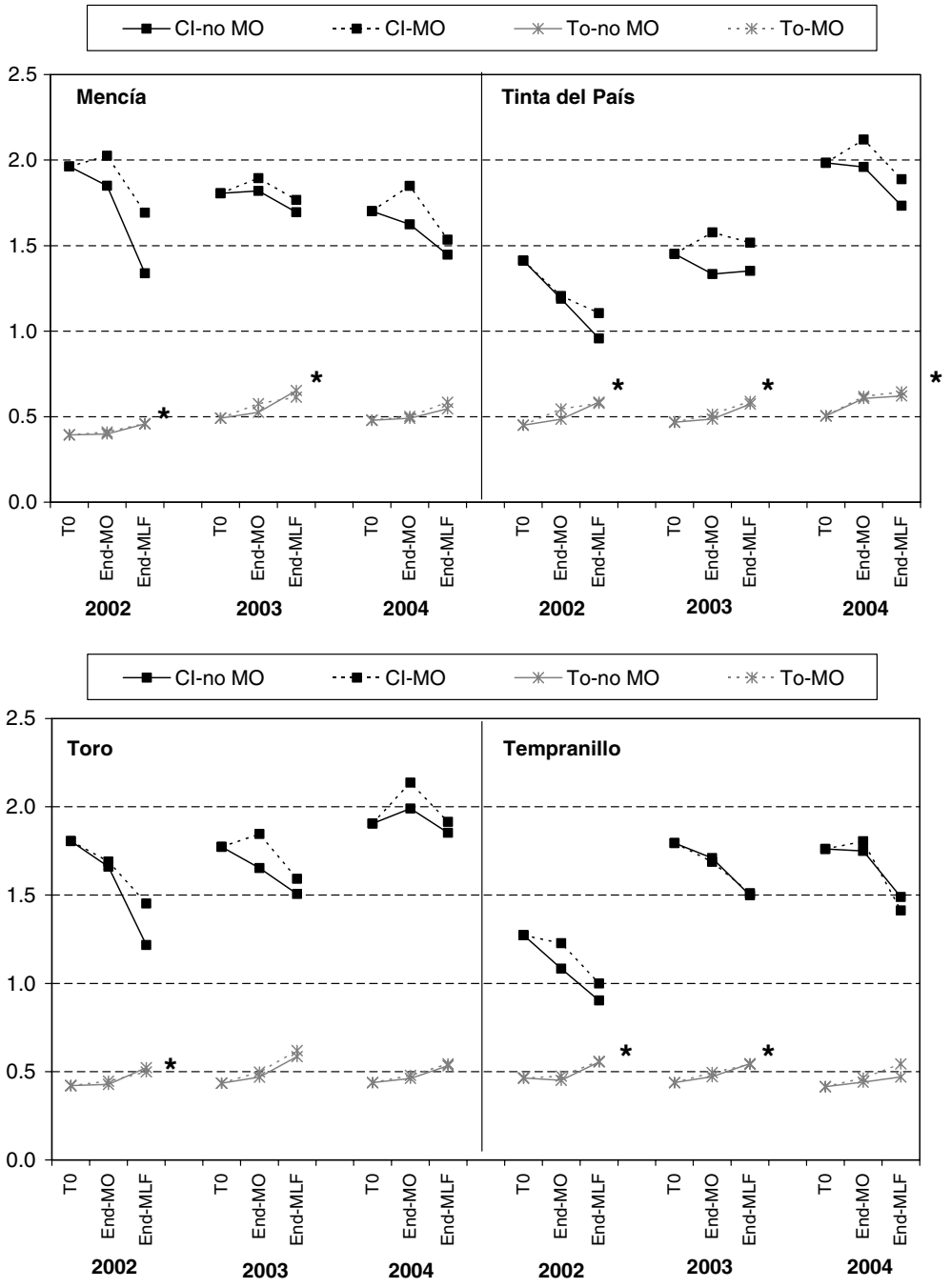

Fig. 7. Colour intensity (CI) and tonality (To) of microoxygenated (MO) and non-microoxygenated (no MO) wines from each variety and vintage. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

Bosso et al. (2000) and Castel et al. (2001) also found an increase in polymeric anthocyanins, concluding that the addition of oxygen activated the reactions among free anthocyanins and flavanols, forming new coloured compounds stable to changes of SO_2 and pH. Castel, Morand, Pujol, and Naudin (2001) also reported an increase of tannin condensation and of combined anthocyanins.

The levels of pyruvic acid derivatives of anthocyanins and vitisin B showed different tendencies depending on the anthocyanin group and the varietal wine (Fig. 5). In general, higher values of AcyPyr were found in MO wines, although some wines did not show statistically significant differences from their counterpart no MO wines. These compounds are pigments that are formed during wine-making and aging and which stabilize wine colour. Then, the losses of monomer anthocyanins could be partially due to the formation of these pigments. Vitisin B did not show statistically significant differences between MO and no MO wines, with the exception of Mencía wines.

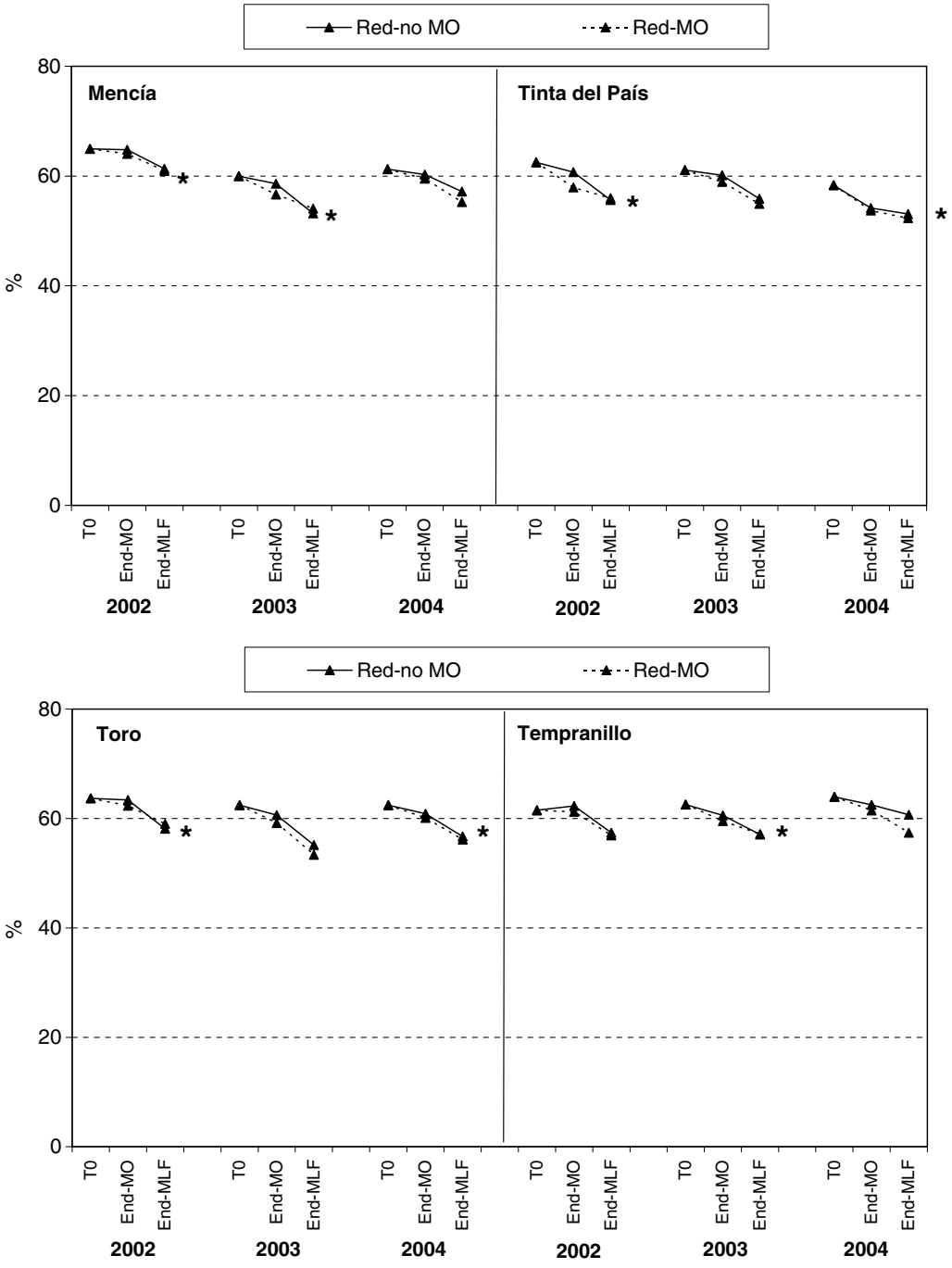

Fig. 8. Percentages of red of microoxygenated (MO) and non-microoxygenated (no MO) wines from each variety and vintage. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

These results are in accordance with the colour data obtained (Figs. 7–9). Glories colour parameters showed marked differences between microoxygenation wines and their control ones. The MO wines had higher values of colour intensity (CI) than the no MO ones (Fig. 7), which indicated that, despite the losses of total phenol levels, the MO wines better stabilized their colour, in agreement with the results of the AcyPyr and percentage of polymeric anthocyanins found.

It must be pointed out that the tonality (Fig. 7), which is an indicator of possible oxidation processes, did not show statistically significant differences between MO and no MO wines in most cases.

As was expected, the red percentage found in the MO wines was lower than that found in their no MO counterparts, although these differences were not very high (Fig. 8). So, it can be said that the microoxygenation treatment had a slight effect on the red component of

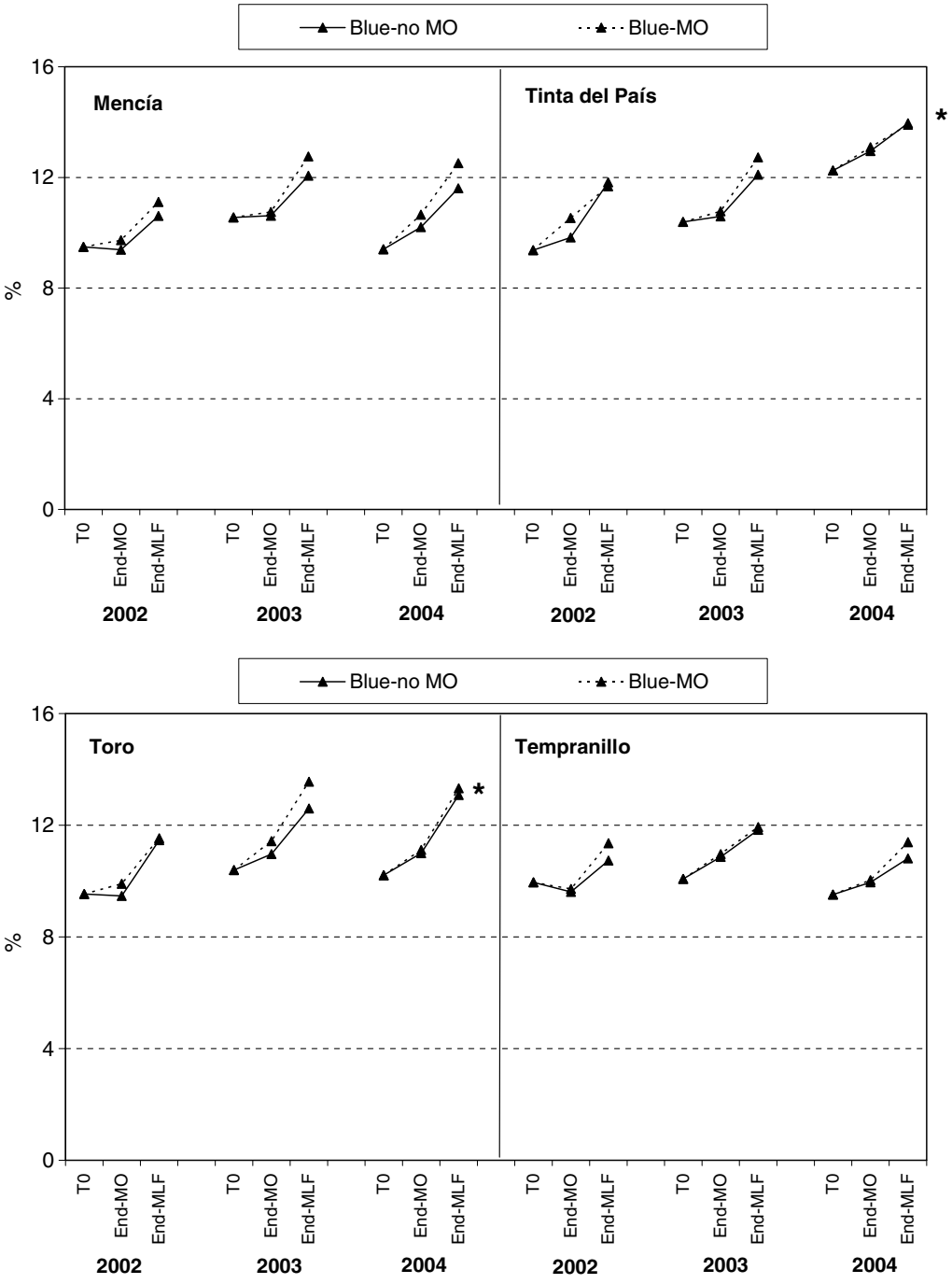

Fig. 9. Percentage of blue of microoxygenated (MO) and non-microoxygenated (no MO) wines from each variety and vintage. Values with an asterisk (*) mean no statistically significant differences between no MO and MO wines in End-MLF point.

Table 3
Difference of values of colour intensity (CI) and blue tones (%Blue) of the studied wines before and after malolactic fermentation

Wines	[a]ΔCI	Ranges of relative losses of CI[b]	[a]Δ% Blue	Ranges of relative increment of %Blue[c]
B-no MO	−17.6	1.5–2.8	16.5	
B-MO	−8.6		23.7	1.3–1.5
RD-no MO	−17.2	1.5–2.6	18.8	
RD-MO	−7.3		20.2	1.1–1.4
T-no MO	−16.8	1.5–2.4	23.1	
T-MO	−9.8		27.3	1.1–1.5
RT-no MO	−20.3	1.2–2.6	13.0	
RT-MO	−15.8		17.4	1.1–1.9

[a] Mean values of the three consecutive vintages.
[b] Relative losses of CI: comparing losses of CI in the no MO wines and in the MO ones by years.
[c] Relative increment of %Blue: comparing increment of %Blue in the MO wines and in the no MO ones by years.

the wine colour, and these differences were mainly correlated with the losses of monomer anthocyanins, previously reported.

In addition, the microoxygenation treatment increased the blue tonalities of wines (Fig. 9), which indicated that the new pigments and polymeric compounds formed contributed, not only to the maintenance of colour intensity, but also to the increase of the violet tones of wines. These data are in agreement with the results found by other authors (Amati et al., 2000; Bosso et al., 2000; Moutounet, Mazauric, Ducournau, & Lemaire, 2001; Otto, 2003; Pérez-Magariño & González-Sanjosé, 2004).

In summary, these results indicate that the microoxygenation technique slightly decreased the global phenolic composition of wines but allowed the stabilization of wine color, without showing an appreciable increase in tonality values, reducing the losses of the CI, or inducing significant increment in the blue tones (Table 3). Thus, the slow and controlled addition of small amounts of oxygen could partially avoid the colour drop which habitually occurred in red wines during the malolactic fermentation.

In general, the relative losses of CI ranged from 1.5 to 2.6 times, which means that the no MO wines showed higher losses of CI than the MO wines. Furthermore, the relative increment of blue notes ranged from 1.1 to 1.5, which indicates that the MO wines showed higher values of blue tones than did their respective no MO counterparts.

Although important quantitative differences were detected among wines, there were neither variety nor vintage factors influencing the results, Therefore, the microoxygenation effect on colour stability was independent of these two factors.

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References

Amati, A., Arfelli, G., Castellari, M., & Simoni, M. (2000). Effetto dell'ossigenazione controllata sulla frazione fenolica dei vini rossi. *Industrie delle Bevande, XXIX*, 606–612.

Atanasova, V., Fulcrand, H., Cheynier, V., & Moutounet, M. (2002). Effect of oxygenation on polyphenol changes occurring in the course of wine-making. *Analytica Chimica Acta, 458*, 15–27.

Bakker, J., & Timberlake, C. F. (1997). Isolation, identification and characterization of new color-stable anthocyanins occurring in some red wines. *Journal of Agricultural and Food Chemistry, 45*, 35–43.

Bosso, A., Guaita, M., Vaudano, E., & Di-Stefano, R. (2000). Influenza dell' ossigeno sull' evoluzione dei composti fenolici durante la conservazione dei vini rossi. *Industrie delle Bevande, XXIX*, 630–640.

Castel, C., Morand, A., Pujol, G., & Naudin, R. (2001). Influence de la micro-oxygénation sur la composition phénolique et les caractères sensoriels des vins rouges. *Industrie delle Bevande, XXX*, 271–276.

Castellari, M., Matricardi, L., Arfelli, G., Gallassi, S., & Amati, A. (2000). Level of single bioactive phenolics in red wine as a function of the oxygen supplied during storage. *Food Chemistry, 69*, 61–67.

Ferrarini, R., Girardi, F., De-Conti, D., & Castellari, M. (2001). Esperience di applicazione della microossigenazione come tecnica d'affinamento dei vini. *Industrie delle Bevande, XXX*, 116–122.

Fulcrand, H., Benabdeljalil, C., Rigaud, J., Cheynier, V., & Moutounet, M. (1998). A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry, 47*, 1401–1407.

Glories, Y. La couleur des vins rouges (1984). 2 e partie. Mesure, origine et interprétation. *Connaissance de la Vigne et du Vin* 18, 253–271.

González San José, M. L. (1989). Comportamiento de compuestos del metabolismo secundario en la maduración de la uva de Vitis vinifera. Ph.D. thesis, University Autonomous of Madrid, Spain.

González San José, M. L., Diez, C., Santa-Maria, G., & Garrido, J. L. (1988). Análisis por CLAE de los pigmentos antociánicos de uvas de *Vitis vinifera L. Anales de Quimica C, 84*, 290–294.

Mateus, N., Silva, A. M. S., Rivas-Gonzalo, J. C., Santos-Buelga, C., & Freitas, V. (2003). A new class of blue anthocyanin-derived pigments isolated from red wines. *Journal of Agricultural and Food Chemistry, 51*, 1919–1923.

Mazza, G., Fukumoto, L., Delaquis, P., Girard, B., & Ewert, B. (1999). Anthocyanins, phenolics and color of Cabernet Franc, Merlot, and Pinot Noir wines from British Columbia. *Journal of Agricultural and Food Chemistry, 47*, 4009–4017.

Moutounet, M. (2003). La técnica de microoxigenación. In ACE *Enología*. Available from www.acenología.com.

Moutounet, M., Mazauric, J. P., Ducournau, P., & Lemaire, T. (2001). Micro-oxygénation des vins. Principe et applications technologiques. *Industrie delle Bevande, XXX*, 253–258.

OIV, (1990). Récueil des Méthodes Internationales d'Analyse des vins et des moûts. Office International de la Vigne et du Vin, Paris, France.

Otto, C. (2003). The Vasse Felix approach to micro-oxygenation. *Wine Industry Journal, 18*, 40–46.

Parish, M., Wollan, D., & Paul, R. (2000). Micro-oxygenation – a review. *Australian Grapegrower and Winemaker, 438*, 47–50.

Paronetto, L. (1977). *Polifenoli e tecnica enologica*. Milan, Italy: Selepress.

Pérez-Magariño, S. (1999). Caracterización de vinos tintos jóvenes y rosados de la D.O. Ribera del Duero. Ph.D. thesis, University of Burgos, Spain.

Pérez-Magariño, S., & González-Sanjosé, M. L. (2002). Influencia de aportes controlados de oxígeno sobre la calidad de vinos tintos de crianza. *Viticultura-Enología Profesional, 82*, 49–54.

Pérez-Magariño, S., & González-Sanjosé, M. L. (2004). Evolution of flavanols, anthocyanins and their derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening. *Journal of Agricultural and Food Chemistry, 52*, 1181–1189.

Pour-Nikfardjam, M. S., & Dykes, S. I. (2003). Micro-oxygenation research at Lincoln University. Part 3: Polyphenolic analysis of Cabernet Sauvignon wine under the application of micro-oxygenation. *Australian New Zealand Grapegrower and Winemaker, 468*, 41–44.

Revilla, I., Pérez-Magariño, S., González-Sanjosé, M. L., & Beltrán, S. (1999). Identification of anthocyanin derivatives in grape skin extracts and red wines by liquid chromatography with diode-array and mass spectrometric detection. *Journal of Chromatography A, 847*, 83–90.

Ribéreau-Gayón, P., & Stonestreet, E. (1966). Dógase des tannins du vin rouges et détermination du leur structure. *Chemia Analityczna, 48*, 188–196.

Roig, G. & Yerle, S. (2003). Balance y perspectivas de 10 años de microoxigenación. In ACE *Enología*. Available from www.acenología.com.

Singleton, V. L. (1987). Oxygen with phenols and related reactions in musts, wines and model systems: observations and practical implications. *American Journal of Enology and Viticulture, 38*, 69–77.

Swain, T., & Hillis, W. E. (1959). The phenolics constituents of *Prunus domestica* I. The quantitative analysis of phenolics constituents. *Journal of the Science of Food and Agriculture, 10*, 63–69.

Vivar-Quintana, A., Santos-Buelga, C., & Rivas-Gonzalo, J. C. (2002). Anthocyanin-derived pigments and colour of red wines. *Analytica Chimica Acta, 458*, 147–155.