Consideration of the influence of aging process, type of wine and oenological classic parameters on the levels of wood volatile compounds present in red wines

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Abstract

The contents of the volatile compounds extracted from wood: *cis*- and *trans*-whiskylactones, vanillin, syringaldehyde, furfural, 5-methylfurfural, eugenol, guaiacol, *p*-ethyl-phenol and *p*-ethylguaicol have been studied in 12 single-variety wines aged in new American oak barrels. The concentration of these compounds were determined after the following combinations of time in wood and time in bottle: 0 months in wood plus 18 months in bottle, 4 months in wood + 14 months in bottle, 9 months in wood + 9 moths in bottle and 12 months in wood + 6 months in bottle. According to the composition of the wines, it is possible to conclude in general, that, the extraction of these compounds was faster during the first four or 9 months of aging in wood, depending on the wines. After 12 months of oak maturation, the extraction of these compounds slowed down. Furthermore, the final concentrations of these volatile compounds were different among studied wines. These differences could be related to some of the values of some of their oenological parameters, such as the contents of tartaric acid and sulphur dioxide of each wine.
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1. Introduction

Maturation of wine in oak barrels is a common practice in red wine making and it is a complex process, in which several phenomena occur, producing organoleptic changes in wines that generally improve its flavour and taste (Ribérau-Gayon, Glories, Muajean, & Dubourdieu, 1999).

During the wood aging process, in which wine is in contact with wood, several physical and chemical processes take place: in the first place, oak wood contributes with volatile and phenolic compounds which improve wine quality. Furthermore, wines aged in barrels are constantly exposed to small amounts of air that penetrate the wood pores and bunghole (Vivas & Glories, 1996). This natural micro-oxygenation improves the polymerization and condensation reactions among flavonoid compounds (tannin–tannin and tannin–anthocyanin). These reactions directly affect colour and astringency of wines, and modify their organoleptic characteristics (Glories, 1992; Gómez-Cordovés & González-Sanjosé, 1995; Vivas, Glories, & Francois, 1991).

It is well known that wines, to be suitable for aging in wood, should have good structure and body, together with antioxidant capacity, to counterbalance the negative effect of oxygen. The phenolic composition of a wine is quite correlated with these characteristics (Dufour & Bayonove, 1999; Escalona, Birkmyre, Piggot, & Paterson, 2002; Glories, 1990). So, and especially according to the different phenolic compositions of grape varieties, not all single-variety wines are capable of withstanding a prolonged oxidative aging, even not all that have wines evolved in the

same way. The stability of the colour and the evolution of flavour and palatability during and after their contact with wood barrels, are the main differences reported.

The volatile compounds that are extracted from the wood, have different origins and contribute to wine flavour with different notes.

The *cis* and *trans* isomers of β-methyl-γ-octalactone (most commonly called whiskylactones or oak lactones), are already present in the green wood (Moutounet, Puech, Keller, & Feuillat, 1999) but their concentrations increase with wood toast (Weeks & Sefton, 1999) since their origin seems to be related to the thermal degradation of the lipids present in wood (Chatonnet, 1995). Furthermore, another known mechanism of their formation is the dehydration of 2-methyl-3-(3,4-dihydroxy-5-methoxybenzo)-octanoic acid present in oak wood (Otsuka, Sato, & Yamashita, 1980).

The volatile phenols, vanillin, syringaldehyde, guaiacol and eugenol, are formed by degradation of lignin, although vanillin is also present in green wood and its concentration is increased by seasoning and toasting (Pollnitz, 2000; Weeks & Sefton, 1999). Furfural compounds (furfural, 5-methyl-furfural and 5-OH-methylfurfural) are formed by thermolysis of the cellulose and hemicelluloses, together with Maillard reactions which take place during the process of elaboration of barrels (Cutzach, Chatonnet, Henry, & Dubourdieu, 1999; Sefton, Francis, & Williams, 1990; Sefton, Francis, Pocock, & Williams, 1990; Sefton et al., 1993; Weeks et al., 1999).

The volatile phenols, 4-ethylguaicol, 4 vinylguaicol, 4-vinylphenol and 4-ethylphenol, can be released from the wood (Chatonnet, Dubourdieu, Boidron, & Pons, 1992; Chatonnet, Dubourdieu, Boidron, & Lavigne, 1993), but usually their presence in wines has been associated with the action of *the Brettanomyces/Dekkera* genus (Chatonnet, Dubourdieu, Boidron, & Pons, 1992; Chatonnet et al., 1995; Pollnitz et al., 2000a). These yeasts are able to contaminate wood, and the presence of the cited compounds has been commonly attributed to the maturation of wines in used barrels (Chatonnet, Boidron, & Dubourdieu, 1993; Towey & Waterhouse, 1996a).

There are several factors that determine the final concentrations of the wood extracted compounds in wines and most of them have been quite well studied. This is the case with factors related to intrinsic characteristics of the wood: oak species, geographical origin, individual trees and barrel-to-barrel variations (Chatonnet & Dubourdieu, 1998; Doussot, De Jéso, Quideau, & Pardon, 2002; Marco, Artajona, Larrechi, & Rius, 1994; Moio, Prete, Diana, & Valentino, 1999; Mosedale, Puech, & Feuillat, 1999; Suavageot & Feuillat, 1999; Towey & Waterhouse, 1996b); seasoning and toasting (Cadahía, Fernández de Simón, & Jalocha, 2003; Chatonnet, Cutzach, Pons, & Dubourdieu, 1999; Chatonnet & Dubourdieu, 1999; Hale, MC Cafferty, Larmie, Newton, & Swan, 1999; Moio et al., 1999; Sefton et al., 1993); age of the barrel and volume (Garde-Cerdán, Torrea-Goñi, & Ancín-Azpilicueta, 2002; Grade-Cerdán, Rodríguez-

Mozaz, & Ancín-Azpilicueta, 2003; Pérez-Prieto, López-Roca, Martínez-Cutillas, Pardo-Mínguez, & Gómez-Plaza, 2002; Towey & Waterhouse, 1996b). Other studied factors are those related to the aging process, such as the length of the time the wine is in contact with the wood (Escalona et al., 2002; Pérez-Prieto, López-Roca, Martínez-Cutillas, Pardo-Mínguez, & Gómez-Plaza, 2003) and the conditions of the cellar (temperature and humidity). However, the factors related to the intrinsic characteristics of wine (pH, alcoholic grade, redox potential, total phenolic levels), have been poorly studied, or few published papers focussed on this group of factors have been found (Ancin, Garde, Torrea, & Jimenez, 2004; Díaz-Plaza, Reyero, Pardo, Alonso, & Salinas, 2002).

For these reasons, this work was focussed on some volatile compounds extracted from wood, during diverse aging processes of 12 different red single-variety wines. Three different combinations of wood aging (barrels) and bottle storage periods, for a total time of 18 months, were studied. These combinations were chosen in order to reproduce the most usual aging processes applied to Spanish wines.

2. Materials and methods

2.1. Samples

Twelve red single-variety wines made from different *Vitis vinifera* red grape varieties were studied.

The most important Spanish red grape variety is Tempranillo, which is given different names depending on the geographical region where it is cultivated. Three Tempranillo wines were studied, one made from grapes cultivated in the Ribera del Duero region (TRD), the second one obtained from grapes grown in the Cigales region (TC), and the third one elaborated from grapes from the Rueda region (TR).

Two single-variety wines, made from grapes of Tinta de Toro (TT), from the Toro region, and Tinta del País (TP) from Ribera del Duero, were also studied. These two varieties are considered by many authors to be the same variety as Tempranillo, named in different ways only by traditional and cultural factors typical of each region in which they are cultivated (Rubio, Yuste, Martín, & Yuste, 2005). A wine elaborated from Mencía (MC) grapes grown in the Bierzo Region was also studied. The grape varieties cited so far are the main varieties used to elaborate the wines of the Appellations of Origin of the Autonomous Community of Castilla y León of Spain.

Furthermore, wines make from two of the varieties used to make the special quality wines, named "Vinos de Calidad", of the same Community were also studied. These were: Rufete (RF), from the Sierra de Salamanca, and Juan García (JG), from the Arribes de Duero.

In addition, three single variety wines were obtained from the French grape varieties Malbec (MB), Merlot (MT), and Cabernet Sauvignon (CS) which were cultivated

in the Ribera del Duero region. The twelfth single-variety wine studied was a wine made from Garnacha Tinta (GT) grapes also cultivated in the Ribera del Duero region.

All the wines were elaborated and aged in the winery and cellars of the Castilla y León Enological Station situated in Rueda (Valladolid).

2.2. Elaboration process

The grapes were harvested in the different production areas and quickly transported to the Enological Station in Rueda in 25 kg plastic boxes.

The wines were elaborated according to the following process: the grapes were de-stemmed, crushed and 50 mg/l of SO_2 was added. Fermentation took place in stainless steel tanks without yeast inoculation and with a daily pumping-over. The temperature was controlled not to rise above 25 °C. Once alcoholic fermentation finished (sugar level under 4 g/l), the wines were strained-off from the tanks and transferred to stainless steel tanks, two per each single variety wine elaborated, where they underwent the malolactic fermentation. When this phase was over, the wine content in each "malolactic tank" was racked off to another stainless steel tank where it was kept at 15 °C until it was transferred to new, medium-toasted American oak barrels. All the casks used were made in the same cooperage and to the same specifications.

Taking into account the different types of wines currently produced in the different D.O. of Castilla y León, three lengths of time in barrel were chosen, completed with aging in bottle up to a total of 18 months. Thus, the next three different types of wines were elaborated:

(a) 4 months of aging in barrel and 14 months in bottle, simulating the so-called "young oak wines". These wines were labelled as (4 + 14),
(b) 9 months of aging in wood plus 9 months in bottle, wines labelled as (9 + 9),
(c) 12 months of aging in wood plus 6 months in bottle, wines labelled as (12 + 6).

The (b) and (c) wines correspond to the most traditional wood aging periods in Spain, which are related to the Spanish traditional category of "crianza" wines.

The so-called control wines were samples of each wine stored in tanks, which were bottled at the same moment in which the wines were transferred to barrels. These wines were labelled as (0 + 18), 0 months in barrel + 18 months in bottle, and they showed the evolution of wine volatile compounds during their storage in reduction conditions.

The samples of the wines of each kind of "aging combination" were formed by two replicates; each one was obtained from the mixture of the content of the three barrels which had been filled with the wine contained in each one of the malolactic tanks.

2.3. Reagents

Dichloromethane (HPLC grade) and the chemical standards used for quantitative analysis were purchased from Sigma–Aldrich (Sigma–Aldrich Corporation, St. Louis, Missouri, USA).

2.4. Analytical methods

2.4.1. Classic enological parameters

The most usual enological parameters analyzed in wineries and Enological Stations were selected, which are: titrable acidity (TA as g/l of tartaric acid), volatile acidity (VA as g/l acetic acid) and tartaric acid (TarcAc g/l), analyzed by following the EC methods (García-Barceló, 1990), ethanol (% v/v) and total SO_2 and free SO_2 (T-SO_2 and F-SO_2 as mg/l), pH and potassium (K^+ mg/l), determined according to the OIV methods (OIV, 1990). These parameters were determined in duplicate.

2.4.2. Volatile compounds analysis

The volatile compounds were isolated from wine by liquid-liquid extraction, following the method described by Moio et al. (1995). Two hundred millilitres of wine, 5 ml of dichloromethane and 50 μl of an internal standard were placed in a special flask in which oxygen had been previously removed by nitrogen. The flask was placed in an ice bath and stirred at 500 rpm for 3 h. After that, phase separation was carried out and the organic phase was kept and stored at −80 °C prior to analysis. Each sample was extracted twice.

Each extract of volatile compounds was analyzed by gas chromatography. A GC (Hewlett–Packard 6890), equipped with a FID detector, was used for quantification of the volatile components. The column was a 50 m × 0.25 mm carbowax BTR with a 0.33 μm thick stationary phase. The injector temperature was kept at 220 °C and the FID was kept at 250 °C. The carrier gas was helium at 1.3 ml/min. The temperature programme was 40 °C held for 8 min, raised to 85 °C at 10 °C min⁻¹ and held for 1 min, raised to 110 °C at 2 °C min⁻¹ and held for 1 min and finally raised to 200 °C at 3 °C min⁻¹ and held for 40 min (Ortega-Heras, González-Sanjosé, & Beltrán, 2002).

Isolated peaks were identified by comparison of their retention times with the retention times of their respective standards, and using their mass spectra data. A Hewlett–Packard 5973 mass detector, fitted with a Hewlett–Packard 6890 GC, was used. The ionization of the samples was achieved at 70 eV under the SCAN mode The mass range studied was from 30 to 250 m/z. A Hewlett–Packard Chem-station, equipped with the Wiley 275 library, was used for interpretation of MS spectra.

The quantification was carried out, following the internal standard quantification method. Quantitative data of the identified compounds were obtained by interpolation of the relative areas versus the internal standard area in the calibration curves built with the respective standards.

Since the repeatability of the chromatographic method was very good (with variation coefficients below 0.5%), only one injection of each dichloromethane extract was carried out.

2.5. Statistical analysis

ANOVA of the data, as well as the linear correlations among the different oenological parameters and the oak volatile compounds studied, were carried out with the Statgraphic Plus programme for windows (Manugistics Inc., 1999).

3. Results and discussion

3.1. General

First, it is important to note that RF wines, after ten months in barrel were not suitable for consumption. At this time, the RF wines showed important losses of colour, oxidative flavours and other negative mouthfeel notes. For that reason, the (12 + 6) RF wines were not studied.

Volatile compounds studied were grouped according to their origin and chemical family. In this way, data from the following groups of compounds are to be considered: whiskylactones; furfural and 5-methylfurfural; vanillin and syringaldehyde; eugenol and guaiacol; and 4-ethyl phenol and 4-ethyl guaiacol.

Two studies of variance were carried out, since two different factors were studied: aging treatment and type of wine (variety and vineyard factors together). The results obtained are shown in each one of the figures.

3.2. cis- and trans-β-Methyl-γ-octalactone (whisky or oak lactones)

The presence of whiskylactones in wine is clearly a result of the contact of the wine with the wood barrel. For that reason, in the control wines, (0 + 18), which were bottled without wood aging, these compounds were not detected (Figs. 1 and 2). In general, the levels of oak lactones increased with the time of wood aging; however, some differences were observed among wines. Furthermore, the concentrations of the two lactones were also different among wines of the same ages or same aging period.

The most common tendency was that the concentration of the cis-lactone increased linearly during the first 9 months of aging in oak wood. However, some exceptions were found, such as the wines made from RT, TC, TT and RF grapes, in which the extraction of this isomer was very fast during the first months, and slower from the 4th to 9th months.

From 9 to 12 months, in general, a slower increase of the cis-isomer content was observed, although some differences among wines were detected. So, the levels of cis-lactone of GT and MB wines did not show a statistical significant increase whereas, in the TC wines, an important increase was detected. It must be pointed out that the JG and MC wines showed statistically significant lower concentrations in the (12 + 6) wines than in their counterparts of (9 + 9).

So, TC, TRD and CS wines aged for 12 months in barrel and 6 months in bottle showed the highest levels of cis-lactone, while for (4 + 14) wines, the highest concentrations were found in the TC wines, followed by RF and TT and
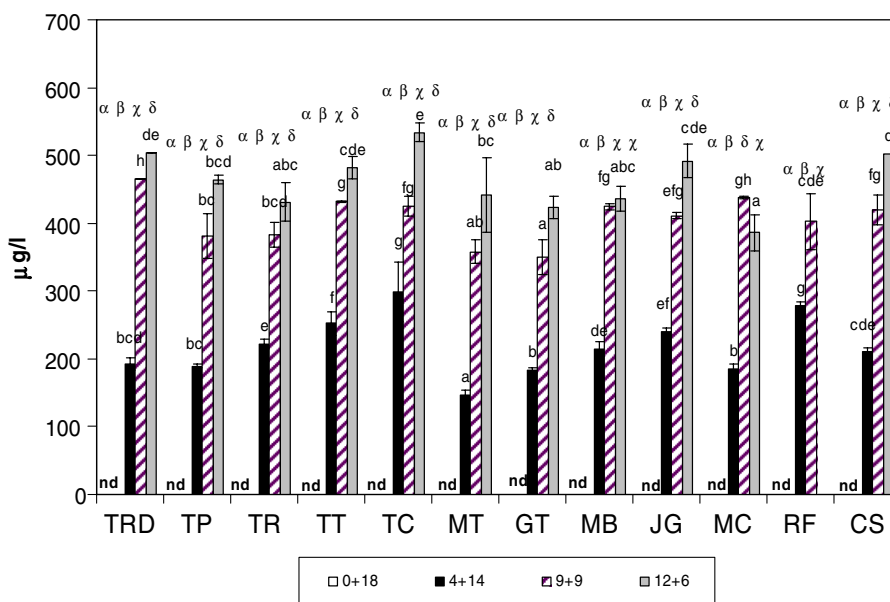

Fig. 1. cis-Whiskylactone levels of the twelve red single-variety wines studied in each different aging treatment applied. nd: not detected. The error bars show +/− the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.
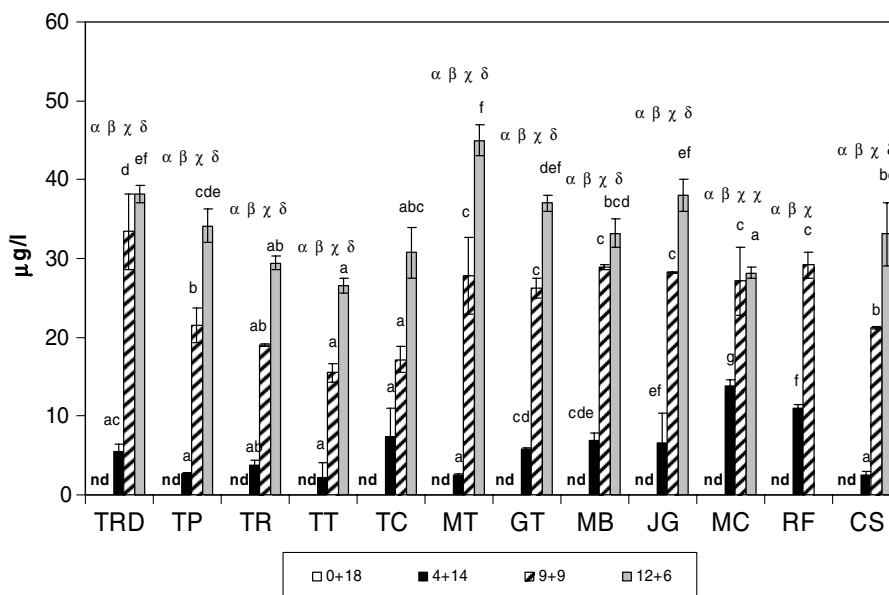
Fig. 2. *trans*-Whiskylactone levels of the 12 red single-variety wines studied in each different aging treatment applied. nd: not detected. The error bars show +/− the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.

JG; in the (9 + 9) wines the richest ones were TRD, followed by MC and TT.

Data for *trans*-whiskylactone led to the conclusion that the extraction of this isomer also increased with the length of the wood contact (Fig. 2) although, again, not all the wines evolved in the same way. In general, the highest amounts of *trans*-isomer were found in the wines aged 12 months in barrels (except for MC and JG wines). The dynamic extraction of this compound was maximum during the first 9 months; however, it was more intense during the second phase, which means from the 4th to the 9th months. Important and statistically significant differences among wines were also found for this compound (Fig. 2). So, (4 + 14) MC, RF and JG wines showed the highest levels of *trans*-lactone; the richest (9 + 9) wines were the TRD and, in the case of (12 + 6) wines, the highest concentrations of this isomer were found in the MT, JG and TRD wines.

It is well known that, in general, levels of *cis*-whiskylactone in wine are higher than the levels of the *trans*-isomer. Since Pollnitz (2000) found that *cis/trans*-isomerization hardly occurred in a weak acidic medium, such as wine, this could mean that the *cis*-isomer was easier extracted than the *trans*-isomer, the first one being released at the beginning of the maturation period. After that, and as long as the time of storage in wood increased, the rate of *trans*-lactone extraction also increased, in spite of the fact that the *cis*-isomer remained in the majority, the ratio *cis/trans* decreased. Similar results were found by Pérez-Prieto et al. (2003).

Some authors have defined this ratio as a parameter for distinguishing between wines aged in barrels with different origin (Waterhouse & Towey, 1994). Ratios found by other authors for wines aged in American oak barrels varied from 5 to 8 (Waterhouse & Towey, 1994) from 7.78 to 8.67 (Díaz-

Plaza et al., 2002) and from 8 to 10 (Pollnitz, Pardon, & Sefton, 2000b). These values are very far from the values obtained in this work, which ranged from 11 to 18 in the (12 + 6) wines, from 13 to 27 in the (9 + 9) wines; and from 13 to 82 in the (4 + 14) wines (Fig. 3). This variability in the results seems to indicate that it is quite difficult to establish a critical value for the ratio *cis/trans*, which could be used as an index to determine the origin of the barrels in which wines were aged. According to the results obtained, it is possible to conclude that the length of the contact between wine and wood induces important changes in the value of this ratio. Furthermore, not all the wines showed similar extraction capacities, leading to significant differences in the *cis/trans* ratio among wines. These differences were quite important, mainly in the wines aged for only four months in oak barrels, and values were really high in the TT, CS and TP wines and even in the TR and MT wines. In relation to the data considered previously, the considerable extraction of *trans*-lactone from the 4th to the 9th month explains the important reduction of the value of this ratio in the wines aged for 9 and 12 months in wood.

In general, the *cis*-isomer concentration in all the wood-aged wines was over its threshold level, 92 μg/l, contributing with oaky, coconut and vanilla notes to the wine aroma (Suavageot & Feuillat, 1999). However, in none of the wines studied, did the concentration of the *trans*-isomer exceed its threshold value of 460 μg/l (Chatonnet, Dubourdieu, & Boidron, 1992).

3.3. Furfural and 5-methylfurfural

The results obtained for the furfural and 5-methyl-furfural levels are summarized in Figs. 4 and 5. These com-
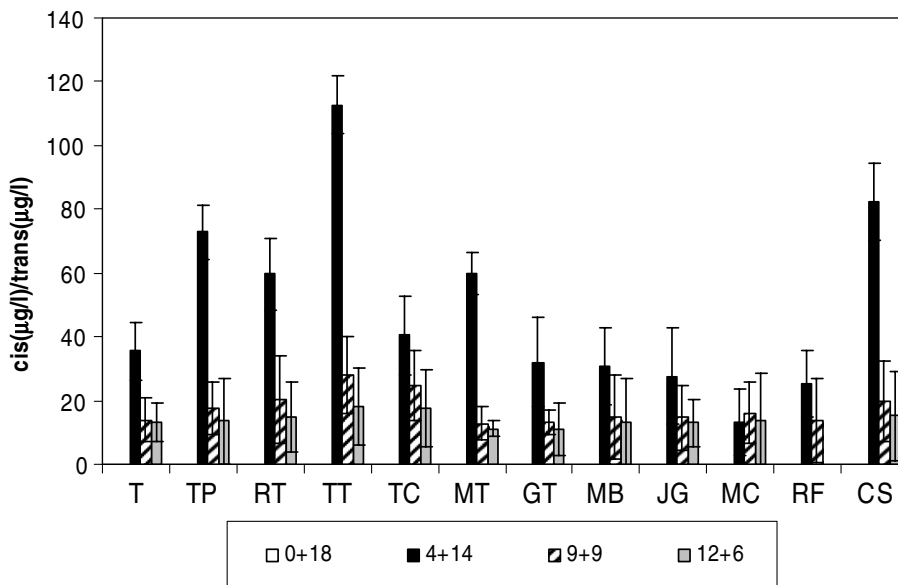
Fig. 3. *cis/trans*-Whiskylactone ratio of the twelve red single-variety wines studied in each different aging treatments applied. The error bars show ± the standard deviation values.

pounds were present in all the wines studied with only some exceptions. Furthermore, important and significant differences were found among wines, so, aging treatment and type of wine effects were detected. Each wine evolved in a different way, it being quite difficult to establish a general tendency for the extraction of these compounds. However, the highest concentration seemed to be reached during the first 9 months, and mainly from the 4th to the 9th month.

During the last three months in which the wines were in barrel, the extraction of these compounds was quite poor, with only some exceptions, such as for MC, TC, or MT wines. So, some of the (12 + 6) wines showed lower levels of these compounds than did their counterparts of 9 months in barrel + 9 months in bottle. A possible explanation of this fact is the degradation or transformation of furfural into furfuryl alcohol (2-furanmethanol), previously described by others authors (Marsal & Sarre, 1987; Spillman, Pollnitz, Liacopoulos, Parden, & Sefton, 1998a). This transformation is correlated with different wine parameters, such as the
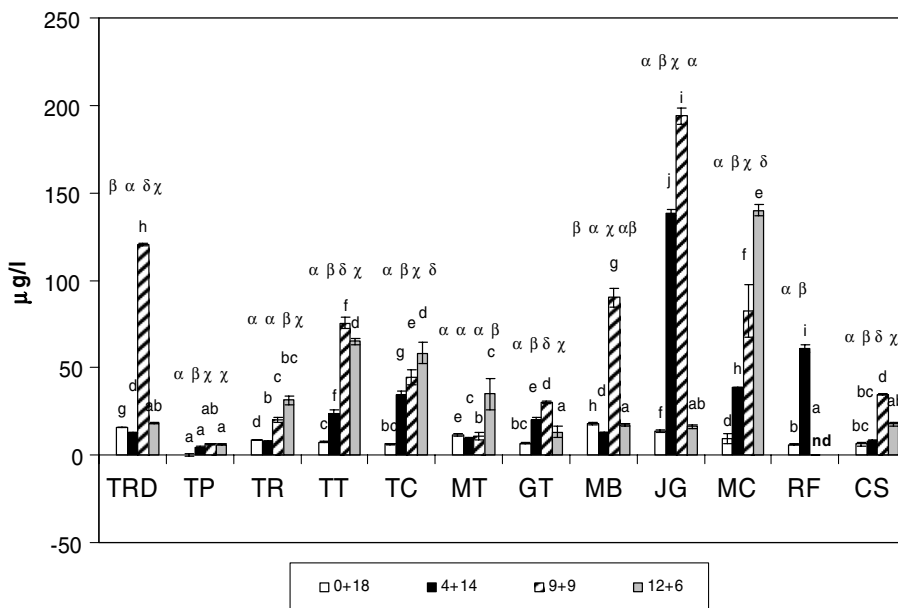

Fig. 4. Furfural levels of the 12 red single-variety wines studied in each different aging treatment applied. The error bars show ± the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.
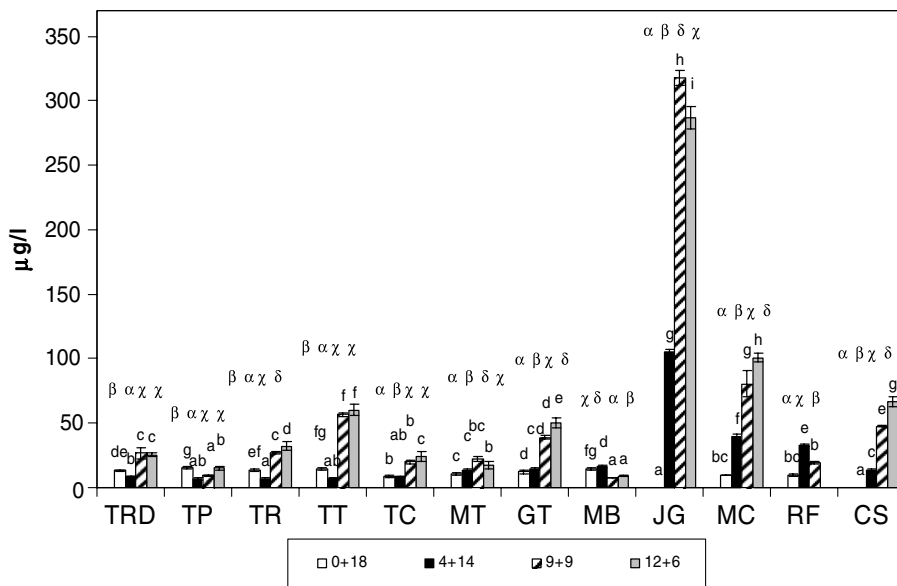
Fig. 5. 5-Methylfurfural levels of the 12 red single-variety wines studied in each different aging treatment applied. The error bars show ± the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.

presence/absence of enzymes responsible for this reaction, which seems to be correlated with varietal and cultural grape factors. Garde-Cerdán et al., 2002 also found an important decrease in the concentration of these compounds after twelve months of aging, although this study was carried out using twice-used French oak barrels.

The threshold level in red wines of furfural is 15 mg/l and, of 5-methylfurfural, 16 mg/l (Chatonnet, Dubourdieu, & Boidron, 1992; Cutzach et al., 1998), with an almond-like aroma. Some of the wines studied showed concentra-

tions below their thresholds and seem not to have an important role in the aroma of these wines. However, it is important to consider that they may strengthen the aroma of the lactones (Reazin, 1991).

3.4. Vainillin and syringaldehyde

In general, as the time of contact between wine and wood increased, the levels of vanillin and syringaldehyde increased (Figs. 6 and 7).
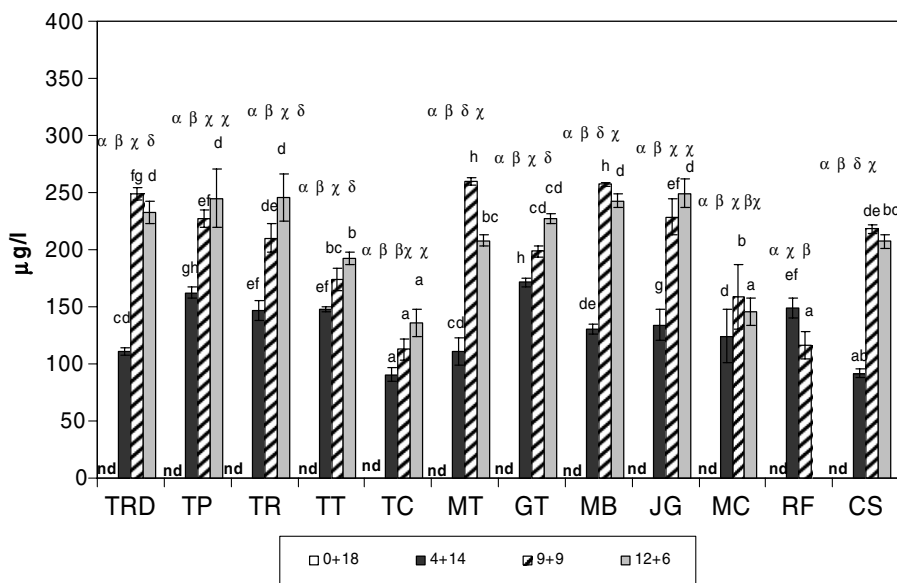

Fig. 6. Vanillin levels of the 12 red single-variety wines studied in each different aging treatment applied. nd: not detected. The error bars show ± the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.
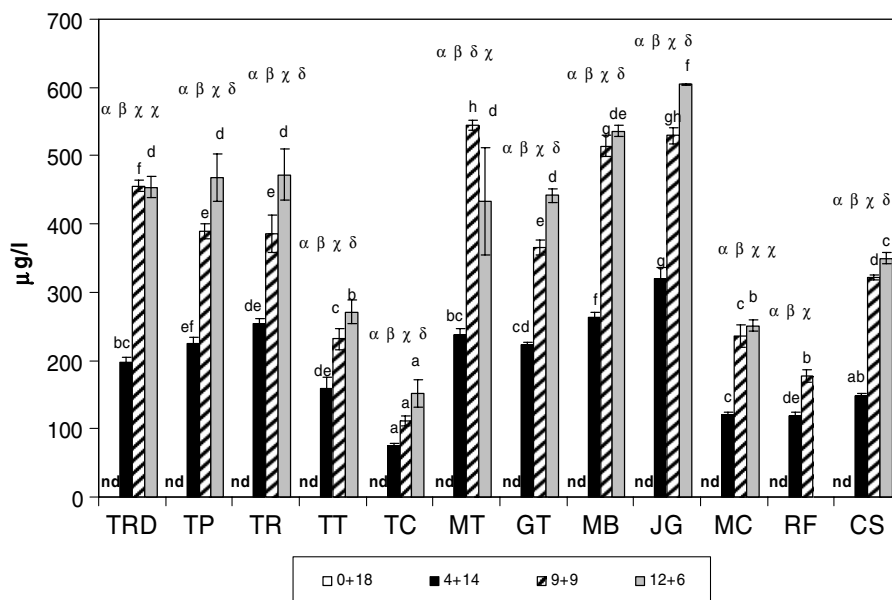
Fig. 7. Syringaldehyde levels of the 12 red single-variety wines studied in each different aging treatment applied. nd: not detected. The error bars show ± the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.

The extraction of vanillin was quite fast and very important during the first four months, and only in the TRD, MT, MB and CS wines, was the extraction also important during the following five months (until the 9th month of aging in wood). After the 9th month, the vanillin extraction slowed and even stopped. These results agreed with the data found by Spillman, Iland, and Sefton (1998b), who reported that the vanillin extraction was very fast during the first 20 weeks of oak storage in a hydro-alcoholic medium. It was also observed that the levels of this compound in some of the final $(12 + 6)$ wines were lower than their $(9 + 9)$ counterpart wines. This fact could have been due to its transformation into vanillic acid and its ethyl ester (Pollnitz, 2000; Spìllman, Pollnitz, Liacopoulos, Skouroumounis, & Sefton, 1997) or due to its reduction to vanillin alcohol (Chatonnet, Dubourdieu, & Boidron, 1992).

Similar results were obtained for the syringaldehyde levels. In this case, in the TRD, MT, MB and JG wines, the extraction was strong until the 9th month of aging in wood. Furthermore, again, $(12 + 6)$ wines, elaborated from MT and JG wines, showed significantly lower levels than did their counterparts, $(9 + 9)$ wines.

According to these results, important differences in the concentrations of these compounds were found among the different wines studied. So, in the final $(12 + 6)$ wines, the highest concentrations of vanillin were found in the TP, TR, TRD, JG and MB wines, whereas the lowest concentrations of this compound were found in the TC and MC wines. As for the syringaldehyde, the richest $(12 + 6)$ wines were again MB and the poorest one was TC. In the $(9 + 9)$ wines, the highest concentrations of vanillin were found in MT, TRD and MB wines whereas the

richest ones in syringaldehyde were MT, JG and MB. Regarding $(4 + 14)$ wines, the highest contents of vanillin were determined in GT wines, followed by TP, JG, TR, TT and RF wines. However, the richest $(4 + 14)$ wines in syringaldehyde were JG and MB, followed by RT and MT wines.

The perception thresholds reported for vanillin and syringaldehyde are 0.32 mg/l (Boidron, Chatonnet, & Pons, 1988) and 25 mg/l (Singleton, 1995). None of the wines studied exceeded these limits in any of the different aging systems studied, so it appears that none of these compounds played an important direct role in the final aroma of the wines.

3.5. Eugenol and guaiacol

Small quantities of eugenol were found in the control wines, $(0 + 18)$ and, in general, their levels increased significantly with the time of contact with the wood (Fig. 8). Generally, the extraction was faster during the first four months of aging, although the MT, MB, TRD, TP, MC and CS wines showed a stronger extraction also from the 4th to the 9th month of aging. After the 9th month, the extraction of this compound went on but more slowly or even stopped during the last three months in barrel, so, in the $(12 + 6)$ wines, the concentrations of this compound were only slightly higher than those found in the $(9 + 9)$ wines and, even in the GT, JG and MC $(12 + 6)$ wines, the contents of this compound were lower than in their $(9 + 9)$ counterpart wines. Data showed that the levels of this compound in the $(4 + 14)$ wines were more than 50% of the final level of eugenol in the $(9 + 9)$ or $(12 + 6)$ wines.
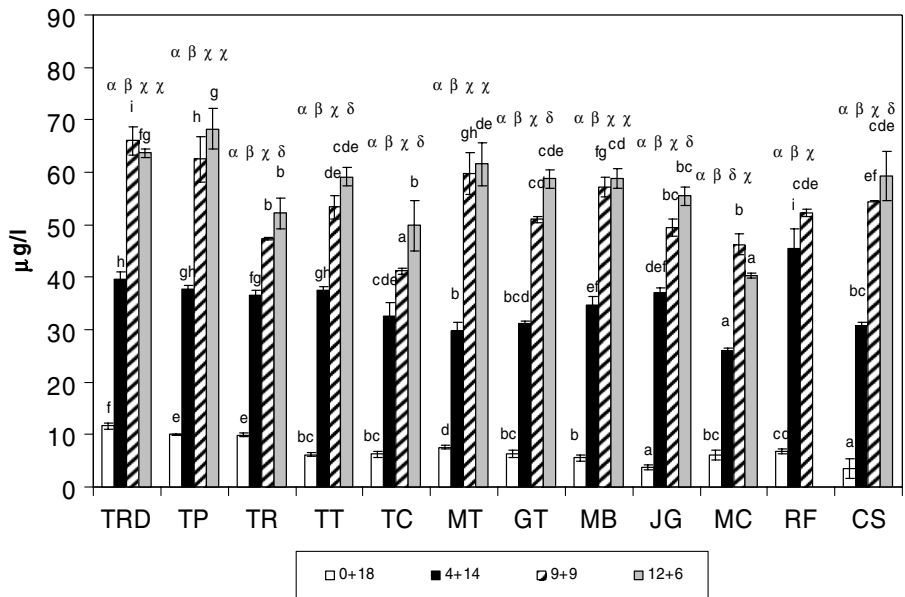
Fig. 8. Eugenol levels of the 12 red single-variety wines studied in each different aging treatment applied. The error bars show ± the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.

Once more, significant differences in the concentrations of this compound were found among wines, so, in general, the richest wines were the TRD, followed by the TR and MT wines. Among the (4 + 14) wines, RF ones were the richest, showing a strong potential of extraction.

Very low quantities of guaiacol were detected in the wood-aged wines (Fig. 9). Again, in most of the wines studied, the maximum extraction of guaiacol occurred during

the first four months; during the remainder of the oak aging, the extraction of this compound slowed down. However, some important exceptions were detected, so, in the MT and T wines, the rate of extraction was constant until the 9th month of oak maturation and decreased during the last three months. A similar evolution was found in MB and CS, with a slight increase during the second phase of the study (from the 4th to the 9th month of aging). Another

Fig. 9. Guaiacol levels of the 12 red single-variety wines studied in each different aging treatment applied. nd: not detected. The error bars show ± the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.

remarkable fact was that, in the GT and MC wines, no concentrations of this compound were found until the 9th month of aging and, in the TC wines, this compound only appeared in the wines stored for 12 months in barrel.

So, like in the rest of the oak volatile compounds studied so far, important differences regarding the concentration and accumulation of these two compounds were found among the different wines studied. Once again, T and MT wines were the richest ones, with the highest levels of guaiacol.

Sensory thresholds of eugenol and guaiacol have been determined as 15 and 75 µg/l, respectively (Boidron et al., 1988). All the wines aged in wood showed levels of eugenol above the threshold level, so, this compound should have contributed with clove notes to the wine aroma. However, none of the wines studied showed concentrations above the guaiacol threshold value.

3.6. 4-Ethylguaiacol and 4-ethylphenol

These compounds were only detected in some of the wood aged wines studied (Figs. 10 and 11). The results seem to show an important effect of the length of wood aging and of the type of wine in the extraction process.

In the RT, TT, TC, and MB wines, neither 4-ethylphenol nor 4-ethylguaiacol could be detected. In the rest of the wines, these compounds appeared in the (12 + 6) wines and in some of the (9 + 9) wines. The highest concentrations of these compounds were found in the RF wines, followed by GT, and CS.

The fact that the volatile phenols, *p*-ethylguaiacol and *p*-ethylphenol, appeared in such high concentrations in the Rufete (RF) wines might have been due to contamination with *Dekera/Brettanomycess* yeast. However, this kind of alteration has been found mainly in used barrels (Chatonnet, Dubourdieu, Boidron, & Pons, 1992; Pollnitz et al., 2000b), while new barrels were used in this study.

Furthermore, it is important to take into account that 4-ethylphenol and 4-ethylguaicol are formed from *p*-coumaric and ferulic acids (Chatonnet, Dubourdieu, Boidron, & Pons, 1992; Chatonnet et al., 1995), whose levels show important variability among grapes varieties, origin and cultural practices (Izcara, 2004.) Therefore, the differences found among wines could be explained according to the different grapes used to elaborate each wine, which were from different varieties, from different region, and which were grown with different cultural factors. The relationship between grape variety and 4-ethylphenol and 4-ethylguaiacol levels has previously been described in the literature (Pollnitz et al., 2000b).

Chatonnet, Dubourdieu, Boidron, and Pons (1992) determined individual detection thresholds of 605 and 110 µg/l for 4-ethylphenol and 4-ethylguaiacol, respectively, in a red wine. However, the sensory impact of these two compounds is additive and, for a mixture of 4-ethylphenol and 4-ethylguaicol in a ratio of 10:1 (typical of the ratio commonly found in a red wine), the detection threshold was 335 µg/l for 4-ethylphenol plus 34 µg/l for 4-ethylguaiacol. Above these concentrations, these compounds contribute to the wine aroma with unpleasant horsey, sweaty and leather notes. Since the addition of the concentrations of these two compounds in the (9 + 9) RF wine was above 400 µg/l, these compounds could have contributed to the negative aroma notes found in these wines.

Overall, results seem to show that not all the wines had the same capacity to extract the volatile compounds from

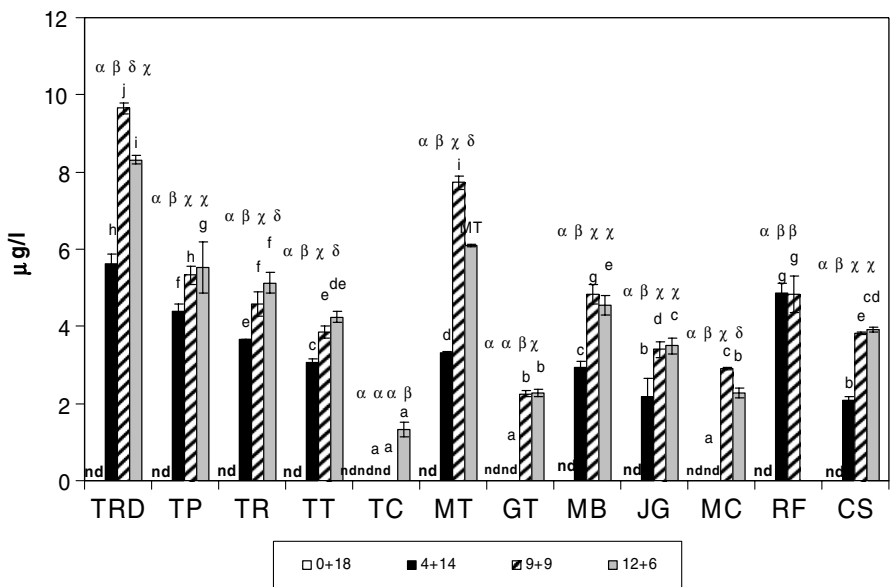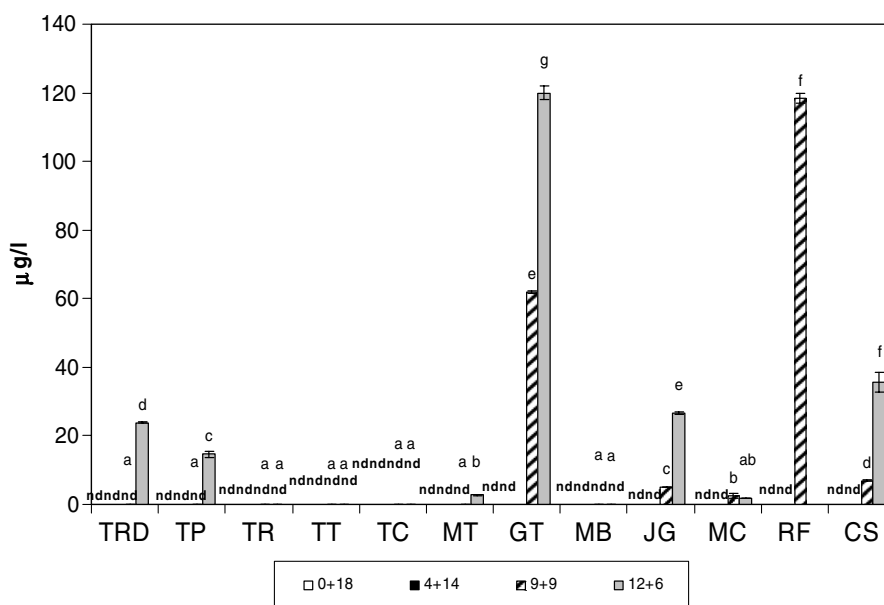Fig. 10. *p*-Ethylguaiacol levels of the 12 red single-variety wines studied in each different aging treatment applied. nd: not detected. The error bars show ± the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$.
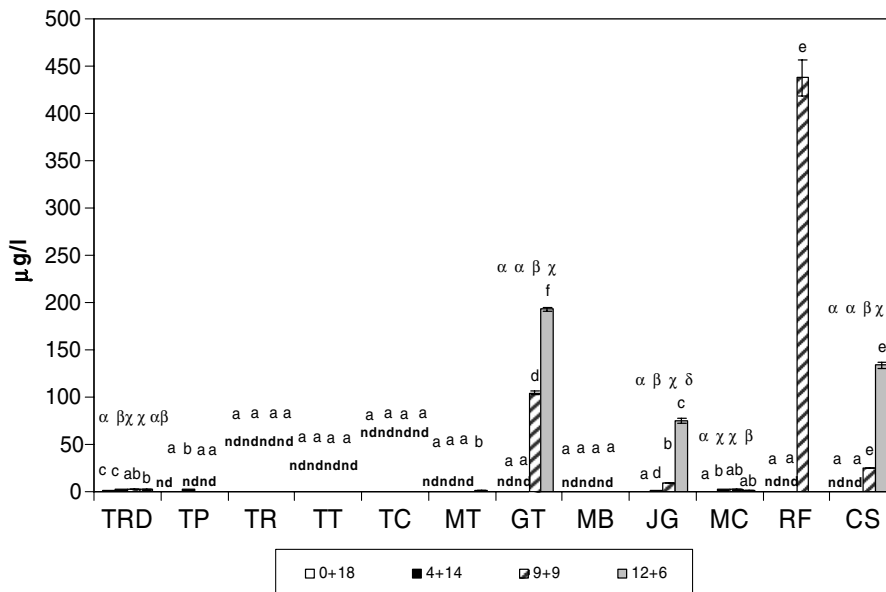
Fig. 11. *p*-Ethylphenol levels of the 12 red single-variety wines studied in each different aging treatment applied. nd: not detected. The error bars show ± the standard deviation values. Values with the same Arabic letter indicate no statistical significant difference among wines of different varieties with the same aging treatment for a $p \leqslant 0.05$. Values with the same Greek letter indicate no statistical significant difference among wines of the same variety but with different aging treatment for a $p \leqslant 0.05$.

wood. These differences can be seen in the wines aged 4 months in barrel + 14 months in bottle, but were getting higher as the length of time in contact with the wood increased (9 and 12 months). The (9 + 9) TRD MB and MT wines showed the highest levels of these volatile components, which were much higher than those of TC, which were the lowest. In the case of the wines aged 12 months in barrel + 6 months in bottle, the differences were slightly reduced, the wines of TC and MC standing out for their low levels. These differences could not be attributed to the content of alcohol or to the differences in pH, as will be discussed in the next paragraphs.

The wines studied showed significant differences for different oenological parameters (Tables 1a and 1b). Oenological classic parameters were studied for different reasons: first, some of them are directly connected with wine quality, such as volatile acidity (VA) and ethanol content, or with wine stability, such as pH, titrable acidity (TA), levels

of tartaric acid and levels of sulphur dioxide (SO_2); second, they are closely correlated with the extractive capacity of wines. It should be remembered that pH, ethanol and titrable acidity have a direct influence on ethanolysis of wood components. SO_2 has a solvent effect on the phenols, and modifies the redox potential of the medium. Tartaric acid and potassium are directly connected with the formation and precipitation of potassium hydrogen tartrate, whose crystals can impede the contact of the wine with the wood, and can even obstruct the wood pores, interfering with the diffusion of wood components to wine.

Data showed unexpected results for the extractive potential of the wines. So, for example, Mencía (MC) wines, which showed low pH and high alcohol content, also showed a low extraction capacity. For that reason, the statistically significant linear correlation was studied as a criterion to check and to clarify the possible effect of oenological parameters on the extractive capacity of each wine. Different

Table 1a

Mean values of the oenological classic parameters determined in the initial wines (just before being transferred to the barrels)

Wine	pH	Ethanol (%v/v)	TA (g/l)	VA (g/l)	T-SO_2 (mg/l)	TarAc (g/l)	K^* (mg/l)	TarAc/K^+
CS	3.91	12.8	5.5	0.63	51	1.5	1470	0.0010
GT	3.82	12.4	6.3	0.57	42	1.9	1260	0.0015
JG	3.63	13.3	7.5	0.39	31	2.9	1580	0.0018
MB	3.96	12	5.7	0.64	42	1.4	1660	0.0008
MC	3.55	13.5	4.9	0.43	26	2.8	1000	0.0028
MT	3.90	13.9	5.0	0.71	45	1.4	1320	0.0011
TRD	3.34	14.5	4.9	0.80	55	0.8	2210	0.0004
TC	3.85	13.4	5.2	0.42	58	2.9	1000	0.0029
TP	4.13	13.8	4.9	0.63	51	1.5	1470	0.0010
TT	3.90	14.0	4.3	0.53	58	1.3	1360	0.0010
RF	3.86	12.4	4.7	0.39	38	2.4	1200	0.0020
RT	4.36	14.0	3.8	0.38	43	1.7	2020	0.0008

Table 1b
Mean values of the oenological classic parameters determined in the wines aged 9 months in barrel and 9 months in bottle

Wine	pH	Ethanol (%v/v)	TA (g/l)	VA (g/l)	T-SO$_2$ (mg/l)	TarAc (g/l)	K^* (mg/l)	TarAc/K$^+$
CS	3.65	12.7	5.3	0.73	43	1.3	1270	0.0010
GT	3.45	12.2	6.1	0.66	39	1.9	1110	0.0017
JG	3.59	12.9	5.4	0.82	34	0.7	1410	0.0005
MB	3.66	11.8	5.7	0.74	39	1.3	1460	0.0009
MC	3.33	13.2	4.3	0.64	38	1.8	630	0.0029
MT	3.70	13.6	5.0	0.78	40	1.5	1190	0.0013
TRD	3.98	14.2	5.5	0.93	46	0.5	2100	0.0002
TC	3.50	13.7	4.9	0.83	40	1.7	800	0.0021
TP	3.91	13.8	4.7	0.77	46	0.7	1180	0.0006
TT	3.74	13.8	4.3	0.69	32	1.1	1350	0.0008
RF	3.36	12.3	4.7	0.81	35	1.7	910	0.0019
RT	3.99	13.6	4.0	0.77	35	1.0	1780	0.0006

studies were carried out, one considering all the wines together and others grouping the wines by aging treatments. The global linear matrix formed from data, of all the wines studied, only showed the following significant correlations: between volatile acidity and *cis*-whiskylactone ($p = 0.001$), *p*-ethyl guaiacol ($p = 0.001$) and 5-methyl furfural ($p = 0.001$); between titrable acidity and syringaldehyde ($p = 0.001$), and between potassium and *c*-whiskylactone ($p = 0.001$).

Obviously the whole group of studied wines contains many sources of variability, which can disturb the results. For that reason, it was decided to study the correlation matrix between more homogeneous samples, and new correlation matrices were obtained, gathering (or grouping) the wines according to the time of aging in barrel.

The new linear correlation matrices obtained ($4 + 14$; $9 + 9$ and $12 + 6$) showed a high number of associations between the oenological parameters and the volatile compounds extracted from wood, although most of them were only significant for α values higher than 0.05. Expected correlations, such as those relative to pH, titrable acidity, tartaric acid and potassium, were found, as well as correlations with the total and free SO$_2$. However, no significant correlations were found between the ethanol

content and the levels of any of the volatile compounds studied. These correlations were also expected, since it is well known that ethanol plays a very important role in the extraction of the wood compounds (Boulton, Singleton, Visón, & Kunkee, 2002; Cantagrel, Lurton, Vidal, & Galy, 1995). The last results could be due to the fact that the variability of the ethanol levels among the wines studied was very small, about 2 °Alc (alcoholic degrees), while the ethanol effect has been usually described at higher ranges, for differences equal to or higher than 5 °Alc.

Taking into account that the most important differences among wines and the higher levels of the wood volatile compounds were found in the wines aged 9 months in barrel plus 9 months in bottle, the results corresponding to these wines will be discussed in more detail.

Table 2a shows the linear correlation matrix between the values of the oenological parameters of the wines just before being transferred to barrels and the volatile compounds extracted from wood in the (9 + 9) wines. Table 2b shows the correlation matrix between the values of the oenological parameters and the volatile compounds in the (9 + 9) wines. Both matrices gave complementary information; the first one (Table 2a) shows the influence of the

Table 2a
Correlation matrix: oenological classic parameters of the wines at the moment of being transferred to the barrels versus levels of volatile compounds of these wines after 9 months of maturation in barrels plus 9 months of aging in bottle

Compound	pH	TA (g/l)	F-SO$_2$ (mg/l)	T-SO$_2$ (mg/l)	Ethanol (%v/v)	VA (g/l)	TarAc (g/l)	K$^+$ (mg/l)	TarAc/K
cis-Whiskylactone	ns	ns	ns	ns	ns	ns	ns	ns	ns
trans-Whiskylactone	−0.641 (0.025)	ns	ns	ns	ns	ns	ns	ns	−0.831 (0.002)
Furfural	ns	ns	ns	ns	ns	ns	ns	ns	−0.840 (0.011)
5-Methylfurfural	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vainillin	ns	ns	ns	ns	ns	ns	−0.721 (0.008)	0.628 (0.029)	−0.714 (0.014)
Syryngaldehyde	ns	ns	ns	ns	ns	ns	ns	ns	ns
Eugenol	ns	ns	ns	ns	ns	0.669 (0.013)	ns	ns	−0.670 (0.017)
Guaiacol	ns	ns	ns	ns	ns	0.597 (0.040)	ns	0.636 (0.026)	−0.601 (0.050)
p-Ethylphenol	ns	ns	ns	ns	ns	ns	ns	ns	ns
p-Ethylguaiacol	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: no significant correlations was found between the two studied parameters for $\alpha \leqslant 0.05$.
Only the coefficients with a *p* value lower than 0.05 have been shown (*p* value is shown in parentheses).

Table 2b
Correlation matrix: oenological classic parameters versus levels of volatile compounds of the wines aged 9 months in barrels plus 9 months in bottle

Compound	pH	TA (g/l)	F-SO$_2$ (mg/l)	T-SO$_2$ (mg/l)	Ethanol (%v/v)	VA (g/l)	TarAc (g/l)	K$^+$ (mg/l)	TarAc/K
cis-Whiskylactone	ns	ns	ns	ns	ns	ns	ns	ns	ns
trans-Whiskylactone	ns	ns	ns	ns	ns	ns	ns	ns	ns
Furfural	ns	ns	ns	ns	ns	ns	ns	ns	−0.702 (0.016)
5-Methylfurfural	ns	ns	ns	ns	ns	ns	ns	ns	ns
Vanillin	0.616 (0.033)	ns	−0.703 (0.011)	ns	ns	ns	ns	0.666 (0.018)	−0.906 (0.001)
Syryngaldehyde	ns	ns	−0.628 (0.029)	ns	ns	ns	ns	ns	−0.756 (0.007)
Eugenol	0.566 (0.055)	ns	−0.640 (0.025)	0.583 (0.046)	ns	ns	ns	0.652 (0.022)	−0.773 (0.005)
Guaiacol	0.597 (0.040)	ns	ns	ns	ns	ns	ns	0.637 (0.026)	−0.854 (0.001)
p-Ethylphenol	ns	ns	ns	ns	ns	ns	ns	ns	ns
p-Ethylguaiacol	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns: no significant correlations was found between the two studied parameters for $\alpha \leqslant 0.05$.
Only the coefficients with a *p* value lower than 0.05 have been shown (*p* value is shown in parentheses).

initial wine composition on the extraction of the volatile compounds. The second one (Table 2b) shows the correlations between the oenological parameters and the volatile studied compounds for a determined time of aging.

The first correlation matrix indicated that the pH effect negatively correlated with the extraction of *trans*-whisky-lactone, which was extracted in smaller amounts in the wines with initially higher pH. The second correlation matrix (Table 2b), shows how, in general, the wines with higher pH, showed higher concentrations of guaiacol, eugenol, and vanillin. According to these results, it is impossible to establish the influence of pH, and therefore more intensive studies will be necessary in order to find its effect on the extraction of the wood volatile compounds.

The first matrix (Table 2a) also showed a positive effect of the initial "higher" volatile acidity on the extraction of eugenol and guaiacol, and of the initial "higher" levels of potassium on the extraction of vanillin and guaiacol, showing a negative effect of the levels of tartaric acid on the levels of the vanillin. On the other hand, in the second matrix (Table 2b), there was no linear correlation between the volatile acidity or the tartaric acid levels and the concentrations of the volatile compounds in the (9 + 9) wines. However, correlations among the contents of potassium and the concentrations of guaiacol, vanillin, and eugenol were also found. All these results seem to indicate that the effects of the tartaric acid and the potassium on the extraction of the volatile wood compounds should be explained considering both parameters together. It is well-known that a higher initial concentration of tartaric acid in wine increases the probability of the formation and precipitation of potassium hydrogen tartrate. Then, the crystals formed can modify the capacity of the barrel to release substances, by blocking the exchange surface (Vivas, 1999). In the same way, it is quite clear that the levels of the potassium ion play a very

important role in the precipitation of the potassium hydrogen tartrate. These considerations seem not to agree with the results found in the correlation matrices; however, studying the composition of the initial wines (Table 1), it can be observed that the wines with the highest concentrations of potassium were also those with the lowest contents of tartaric acid. Therefore, in these wines, the formation of potassium hydrogen tartrate crystals was minimized, due to the lack of tartaric acid, and thus, the blocking of the wall of the barrels was reduced and the extraction was not impeded. This hypothesis agrees with the results of some of the wines, such as TT or TRD, which were those in which a smaller amount of potassium was lost during their maturation in barrels (10 and 110 mg/l respectively).

In the light of these results, it was decided to study the variation of the ratio of tartaric acid/potassium and its correlations with the other parameters studied. The linear correlation coefficients obtained showed, in general, high values, although with a negative sign. This fact clearly supports the previous cited hypothesis. A higher ratio of tartaric acid/potassium ion in the initial wine (just before the maturation process) increases the probability of formation of potassium hydrogen tartrate and therefore the extraction capacity might be reduced due to physical obstacles caused by the potassium hydrogen tartrate crystals formed. It would also be convenient to consider the influence of other factors, such as pH, on this process.

Finally, a possible explanation of the correlations found among some volatile compounds and the SO$_2$ in the (9 + 9) wines could be protection against oxidation due to higher levels of SO$_2$. Furthermore, this positive correlation could also be due the fact that the SO$_2$ favours extractions of some volatile compounds (such as phenols). On the other hand, SO$_2$ combination with phenols reduced the levels of free SO$_2$, which explains the negative correlation found

between this parameter and the levels of volatile compounds. Recently, a similar effect of the sulphur dioxide was described in model wine systems (Ancin et al., 2004).

4. Conclusions

The results obtained in this work indicate, once more, the well-known fact that the extraction of the volatile compounds from oak barrels increased in general with the length of the wood aging period. However it was clearly shown that this increase was generally faster during the first 4 or 9 months and, afterwards, the extraction was slower. When aging period is so long, some degradation reactions can occur, reducing the levels of some of the components extracted from the wood.

Furthermore, from the comparative study of the twelve single variety wines studied, it has been clearly shown that not all the wines studied had the same capacity to extract the volatile compounds present in oak barrels. This generally well-known fact among the winemakers has not previously been analytically and objectively demonstrated. The different capacities of extraction of different wines seemed not to be correlated with their differences in pH, ethanol level or total acidity values. This fact could be due to the small range of variability of the cited parameters in the studied wines.

Tartaric acid and potassium levels seem to play an important role in the extraction of the phenols derived from the lignin degradation.

Sulphur dioxide levels showed a positive effect on the extraction.

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