

Importance of Chip Selection and Elaboration Process on the Aromatic Composition of Finished Wines

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The evolution of volatile compounds extracted from wood while being macerated for 1 month with four different commercial chips (different geographical origins and toasting degrees) was studied. Furthermore, the effect of the microoxygenation process between alcoholic and malolactic fermentation also was studied. The wood volatile compounds in wines macerated with the four types of chips evolved in the same way. However, the amounts of compounds extracted depended on the type of chip used. There were differences in the levels of vanillin, *cis*-whiskey lactone, furfural, *trans*-isoeugenol, and *cis*-isoeugenol in wines in accordance with the type of wood chips (French or American), and the last two compounds along with 5-methyl furfural presented differences that were directly related to the toast level. However, no effects of microoxygenation treatment on the extraction of volatile compounds extracted from chips were observed. Therefore, the results obtained in this study highlight the importance of chip selection on the aromatic characteristics of finished wines.

KEYWORDS: Volatile compounds; wine; oak chips; microoxygenation

INTRODUCTION

Aging wines in barrels is a traditional practice that improves wine quality and modifies its composition due to compounds that are extracted from the wood (tannins, phenolic acids, and volatile compounds) and to the chemical reactions that take place when oxygen passes through the wood pores or staves.

However, aging wines in wood requires long periods of time and is very costly. For this reason, new inexpensive and simpler techniques have been used for some years. Thus, sensory properties similar to those of wines aged in barrels could be attained through the use of oak chips. This practice recently was approved and legislated by the European Community (CE 2165/2005 and CE 1507/2006), but in some countries such as Australia, South Africa, and Chile, this practice has been used for several years. Nowadays, there are a great number of these kinds of products available on the market, and the resulting wine characteristics depend on several factors (chip size, botanical and geographical origin of the oak, type of toast, doses, contact time with wine, moment of application, variety, woods other than oak, etc). Furthermore, to imitate the oxidation process that occurs in barrels, the combination of oak chips and

microoxygenation should be used for what is called industrial aging (*1*). Microoxygenation also can be applied at the end of alcoholic fermentation and before the beginning of malolactic fermentation to improve the intensity and stability of the wine color as well as palatability and structure (*2–8*).

There are several factors that determine the final characteristics of wines macerated with chips, and some of these factors have been studied in recent years. Some papers have been written after studying the effect of chips on the phenolic composition of different wines (*9–13*), whereas others have focused on volatile compounds, mainly on those extracted from wood. Thus, Guchu et al. (*14*) evaluated the influence of the geographical origin of the oak (American or Hungarian), the toasting degree (toasted and nontasted), and the time of contact on volatile compounds in a white wine. Arapitsas et al. (*15*) also studied the effect of the contact time, but only for 14 days, and the size of the chips on red wines. Frangipane et al. (*16*) studied four types of chips from four different French forests, added to a red wine stored in 2 year old barrels. Campbell et al. (*17*) studied the origin of the wood and the type of heating in model wines. Rodríguez-Bencomo et al. (*18*) studied 14 different commercial types of chips. An analysis of the results discussed in these papers indicates a great variability in the results, which is probably due to the fact that there are many factors that affect wood composition and its interaction with wine.

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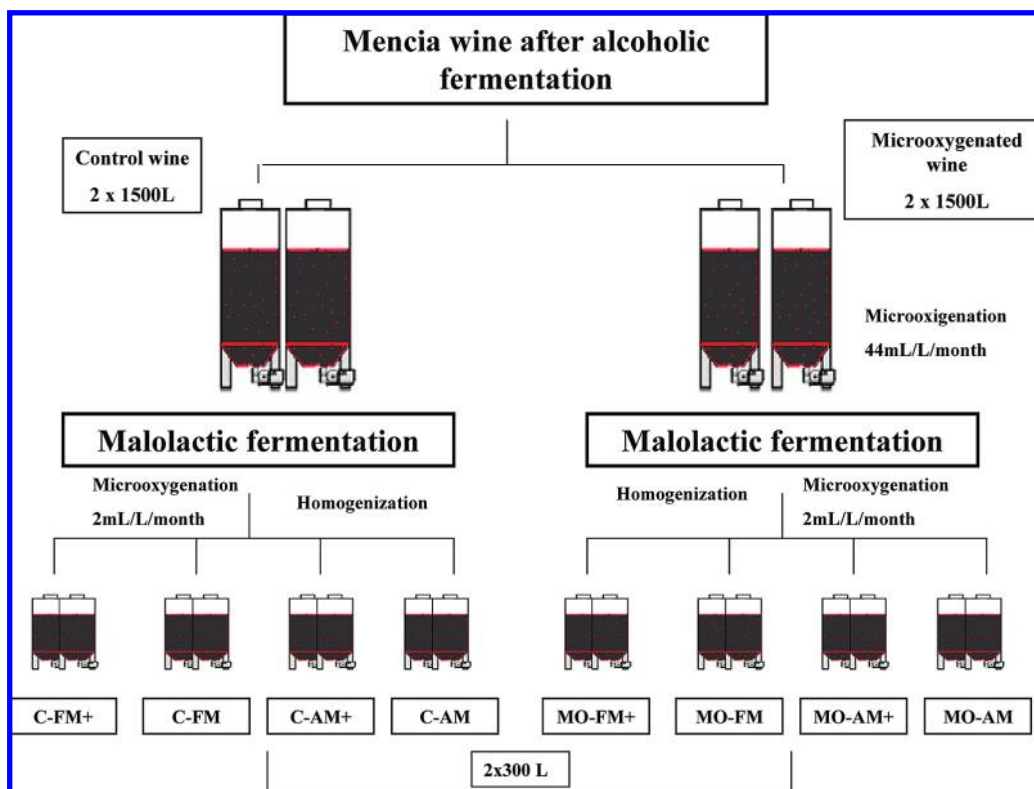


Figure 1. Scheme of experimental design.

No paper has been published in which the effect of microoxygenation applied before malolactic fermentation and during maceration with chips and the use of chips has been studied. So, the main purpose of this study was to show the effect of microoxygenation and wood factors such as botanical and geographical origin and toasting degree along with contact time on the extraction of volatile compounds present in oak chips. Therefore, from our point of view, this is the first scientific paper in which different factors, one being microoxygenation, are studied together and in which all assays were carried out on a semi-industrial scale.

MATERIALS AND METHODS

Samples. A 2005 vintage red single-variety wine was elaborated from the autochthonous Spanish *Vitis vinifera* Mencia grape variety. This wine was elaborated following a traditional red winemaking method. After alcoholic fermentation, the wine was divided into four 1500 L tanks (3 m in height and 0.80 m in diameter). Two of them were microoxygenated (MO) with 44 mL/L/month (25 mL/L of oxygen applied over 17 days). The oxygen doses were implemented through sensory analysis by a group of expert winemakers and technicians according to the initial characteristics of the wine (structure, presence of green tannins, and herbaceous and reductive aromas). The end of the treatment also was determined through sensory analysis, when the tasters believed that the wines had attained the desired characteristics: removal of reductive notes, change of green tannins into hard tannins, and increase in body and structure of the wine. The microoxygenation equipment used was supplied by Oenodev.

After the microoxygenation treatment, the control (C) and MO wines underwent malolactic fermentation, and when it was finished, the two C tanks and the two MO tanks were homogenized. Thus, ~3000 L of C and MO wines were divided into 16 different 300 L stainless steel tanks (3 m in height and 0.35 m in diameter). Two tanks out of each set (C and MO) were macerated with 4 g/L of four different types of chips so the study could be carried out in duplicate (Figure 1). Each chip was obtained from a different commercial supplier and was chosen according to the results obtained in a previous study on 14 different kinds of chips (18). All the chips used were similar in size (5–30 mm

× 3–25 mm × 1–4 mm), but each one was obtained from wood of different origin and degree of toast. Thus, the chips studied had these characteristics: *Quercus alba* medium toast chip (AM) and *Q. alba* medium plus toast chip (AM+), both of them from the U.S., and medium plus toast chip (FM+) and medium toast chip (FM) from a mixture of *Quercus petraea* and *Quercus robur*. The degree of toast considered was the one specified by the manufacturer. No data regarding the toasting process were provided by the manufacturers.

Furthermore, to imitate the oxidation process that takes place in the barrel, the maceration tanks were microoxygenated with 2 mL/L/month during the 28 days that the wines were in contact with the chips. To study the rate of extraction of the volatile compounds released from each kind of chip, samples from each tank were taken once a week during the 28 day treatment.

Chemicals and Reagents. The chemical standards were purchased from Fluka (Buchs, Switzerland), Aldrich (Steinheim, Germany), and Lancaster (Strasbourg, France). Ethanol (HPLC-grade) was from Scharlau (Barcelona, Spain), dichloromethane (HPLC-grade) was from Merck (Darmstadt, Germany), and Milli-Q water was from a Millipore system (Bedford, MA).

Analysis of Volatile Compounds from Wood. The volatile compounds were extracted by liquid–liquid extraction based on the method developed by Ortega-Heras et al. (19). Two hundred and fifty milliliters of wine, 5 mL of dichloromethane, and 75 μ L of a mixture of three internal standards (IS) (450 mg/L of 2-octanol and 3,4-dimethylphenol and 550 mg/L of γ -hexalactone) were added to a flask. Extraction lasted for 3 h with continuous stirring (150 rpm). Then, the organic phase was separated and stored at -20°C until analysis, without being concentrated before being injected. Each sample was extracted twice.

The chromatographic analyses were performed with an HP-6890N GC instrument coupled to an HP-5973 inert MS detector equipped with a J&W Scientific Innowax capillary column (60 m length, 0.32 mm i.d., and 0.25 μ m thick film). The injection was carried out in splitless mode, and the temperature of the injector was set to 240 $^{\circ}\text{C}$. The oven column program was set at 40 $^{\circ}\text{C}$ (held for 10 min), raised to 240 at 2 $^{\circ}\text{C}/\text{min}$, and held at this temperature for 45 min. Detection was in EI mode (70 eV), and the identification was carried out using the spectra obtained with commercial standard compounds

Table 1. Suppliers, Retention Times, IS, and Quantification Ions of Each Compound Analyzed

compound	chemical standard	quantification ion and [qualifiers (<i>m/z</i>)]	IS	retention time (min)
2-octanol (IS = 1)	Aldrich	45 [55;97]		37.72
furfural	Fluka	96 [95;39]	1	40.60
5-methylfurfural	Aldrich	110 [109;53]	2	47.73
γ -hexalactone (IS = 2)	Fluka	85 [57;70]		55.08
guaiacol	Fluka	109 [124;81]	2	64.35
<i>trans</i> -whiskey lactone	Aldrich	99 [71;87]	1	65.71
<i>cis</i> -whiskey lactone	Aldrich	99 [69;87]	2	69.35
eugenol	Fluka	164 [149;131]	3	79.20
3,4-dimethylphenol (IS = 3)	Lancaster	107 [122;77]		82.73
<i>cis</i> -isoeugenol	Aldrich	164 [149;103]	3	84.40
<i>trans</i> -isoeugenol	Aldrich	164 [149;131]	3	88.70
vanillin	Fluka	152 [81;109]	3	97.90
syringaldehyde	Fluka	182 [181;111]	3	112.11

Table 2. Calibration Parameters, Reproducibility, and % Recovery for Determination of Volatile Compounds Studied

compound	calibration		reproducibility (RSD %)	recovery							
	range ($\mu\text{g/L}$)	R^2		amounts added ($\mu\text{g/L}$)		recovery W1 (%)		recovery W2 (%)		recovery W3 (%)	
				add 1	add 2	add 1	add 2	add 1	add 2	add 1	add 2
furfural	35–2850	0.9969	5.40	278	1110	114	99	87	97	113	102
5-methylfurfural	45–2850	0.9963	2.58	192	1540	45	51	48	70	43	59
<i>cis</i> -whiskey lactone	10–610	0.9937	4.63	41	325	103	116	112	104	101	110
<i>trans</i> -whiskey lactone	6–842	0.9971	4.30	56	449	102	114	111	102	96	107
guaiacol	4–131	0.9970	2.64	9	70	98	105	95	100	89	109
eugenol	2–141	0.9994	3.40	24	94	90	95	101	102	92	96
<i>cis</i> -isoeugenol	1–7	0.9913	1.40	2	4	46	48	59	58	60	66
<i>trans</i> -isoeugenol	2–54	0.9968	5.55	4	29	54	59	41	46	51	65
vanillin	30–523	0.9984	3.59	48	380	98	106	105	96	98	80
syringaldehyde	45–755	0.9836	2.74	94	377	106	106	95	96	107	108

and from the NIST library. Quantification was carried out following the IS quantification method through interpolation of the relative areas (total area/IS area) on a calibration curve built with the respective standards. To build the calibration curves, a synthetic wine containing known amounts of volatile compounds (13% v/v) ethanol, 5 g/L of tartaric acid, and pH adjusted to 3.5 with 1 M NaOH was extracted and analyzed following the proposed procedure. Suppliers, quantification ions, retention times, and IS chosen for each compound studied are shown in **Table 1**. Recovery was calculated in three different wines: a white wine (W1), a young red wine (W2), and a red wine aged in new oak barrels for 12 months to take into account differences in matrix structure (pH, alcohol content, polyphenol composition, etc.). The three wines were spiked with known amounts of the compounds studied at two different concentrations. Each one was analyzed in triplicate. The concentrations added to the samples for the recovery experiments appear in **Table 2**.

Sensory Analysis. A tasting panel made up of 12 expert judges (7 male and 5 female)—specialists in wines and members of the tasting panels of various regulatory councils for different Spanish appellations of origin—carried out the quantitative descriptive analysis (QDA) of the wines studied. The QDA procedure established by the international standard ISO 6564:1985 was applied. According to the requirement established in the aforementioned standard, the aptitudes and qualifications of the tasting judges were evaluated applying ISO 6658:1985 and ISO 8586:1993 standards. The judges' complementary olfactory abilities were tested using a certified collection of wine aromas (20).

A profile of 13 basic olfactory descriptors was developed and used. The descriptors evaluated, which were selected from the flavor wheel for food (21), were as follows: olfactory intensity, grassy, vegetal, fruity, woody, vanillin, coconut, spicy, nutty, animal, coffee, chocolate, and burnt or roasted. Structured seven-point scales were used to evaluate each descriptor. Training in the adequate use of the scale was carried out according to ISO 4121:1987. The standards used were those described in UNE 87013:1996, which include compounds such as eugenol, vanillin, and so on.

Statistical Analysis. Data were tested by a box and whiskers graphic representation, which allows easy detection and quick anomalous data.

No outlier was detected. Analysis of variance (ANOVA) was applied to test factor effects such as the type of chips or microoxygenation treatment. Since several factors were studied, a multifactor ANOVA was carried out. Furthermore, data of final wines (after 28 days of maceration) were analyzed applying the least significant difference test (LSD) to determine significant differences, considering confidence levels of 95%.

Multivariate analyses such as the factorial analysis and discriminant analysis were used to evaluate the overall effect of maceration. A principal components analysis (PCA) was used as a factoring technique, and varimax rotation was applied to simplify the interpretation of the data. When selecting the number of factors, the decision was made to choose as many factors as necessary to explain 80% of the total variance. The discriminant analysis followed the forward step wise method, which begins with no variables in the model and adds them one at a time to obtain a final model. The statistical *F* function was used as the criteria for variable selection. Statistical analyses were carried out using the Statgraphic Plus program for Windows.

RESULTS AND DISCUSSION

Method Validation. The following wood volatile compounds were studied: furanic aldehydes, furfural and 5-methylfurfural; the two isomers of β -methyl- γ -octalactone, *cis* and *trans* (commonly known as oak lactones or whiskey lactones); the volatile phenols guaiacol, eugenol, and *cis*- and *trans*-isoeugenol; and the aldehyde phenols, vanillin and syringaldehyde.

The results obtained from the validation process of the method are shown in **Table 2**. To calculate the reproducibility of the method, a red wine aged for 6 months in oak barrels was extracted 5 times on five different days. All the resulting RSD values were below 6%, which means that the method proposed is reproducible. The linear calibration interval and R^2 values also are shown in **Table 2**. The data in this table clearly show that the linearity is satisfactory in all cases, with the only exception being syringaldehyde with an R^2

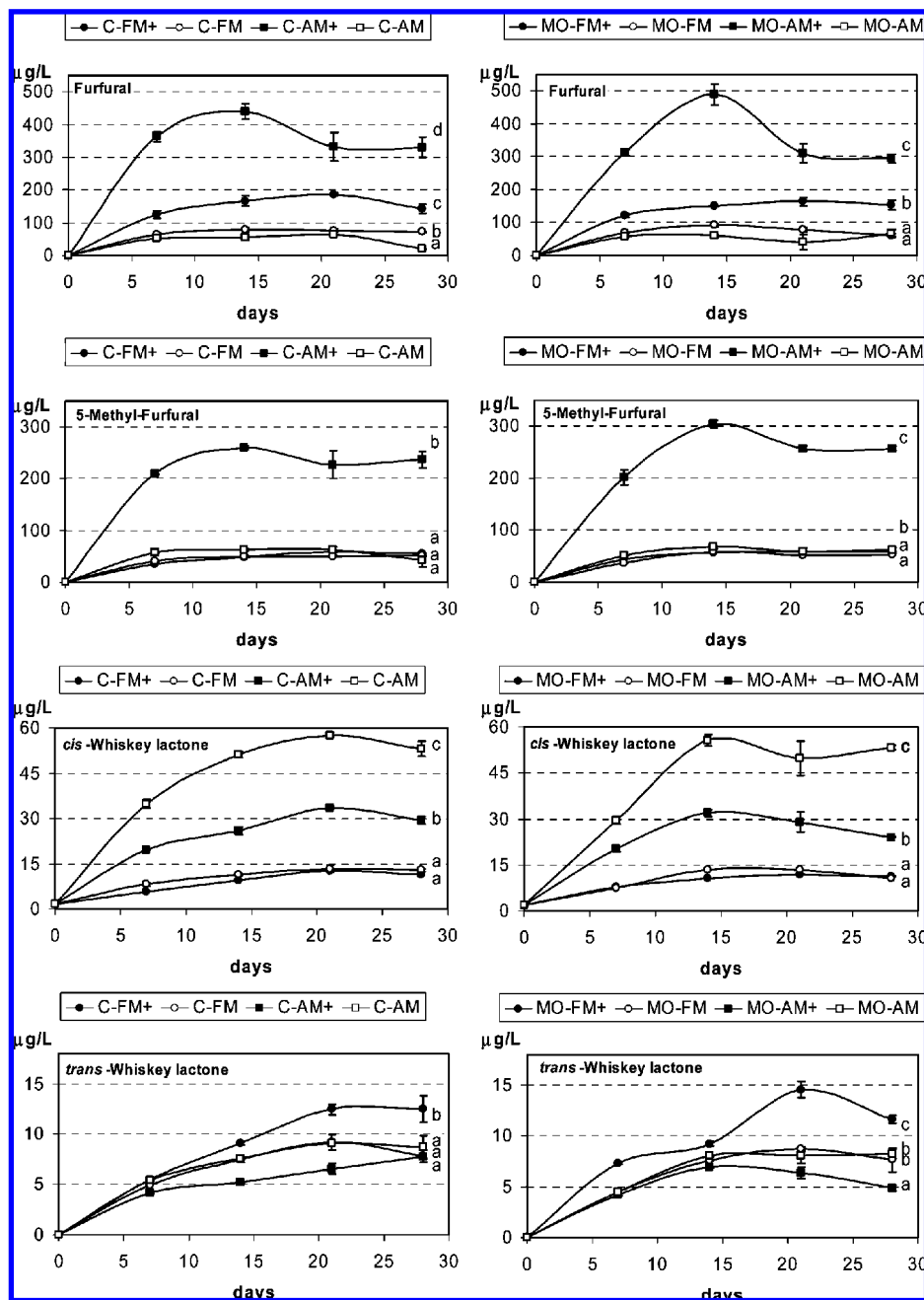


Figure 2. Evolution during maceration process with the four different types of chips studied of furfural, 5-methyl furfural, and *cis*- and *trans*-whiskey lactone in C and MO wines. FM+: medium plus toast French chip; FM: medium toast French chip; AM+: medium plus toast American chip; and AM: medium toast American chip. The error bars show \pm SD values. At 28 days of maceration with chips, values with the same letter indicate no statistically significant differences among the different types of chips studied for $\alpha = 0.05$.

value of 0.9836. Furthermore, the linear ranges include the normal concentrations of these compounds in wines. The average recovery for each compound ranges between 96 and 108% for all compounds studied with the exception of 5-methylfurfural and the *cis* and *trans* isomers of isoeugenol with average recoveries of 53, 56, and 53%, respectively. In this case, a correction factor according to the mean recovery obtained was applied to the concentrations of these compounds to obtain their real concentration in wine. To apply this correction factor, the concentration value obtained by the direct interpolation of the relative peak area in the corresponding calibration graph is corrected by the corresponding recovery, taking into account that the expected recovery should have been 100%. Therefore, the linearity,

accuracy, and precision of the method are satisfactory, and it can be used to determine volatile compounds extracted from wood.

Volatile Compounds Extracted from Wood during Maceration Process. The levels of wood volatile compounds extracted during wine maceration were quantitatively different depending on the contact time and type of chip (Figures 2, 3, and 4). The influence of this and other factors such as microoxygenation treatment being applied before malolactic fermentation was analyzed applying a multifactor ANOVA test (Table 3). Geographical origin, toasting degree, and time contact factors were detected for all compounds studied. An exception was the effect of the time contact factor on the levels of furanic aldehydes. In contrast, the effect of microoxygenation being

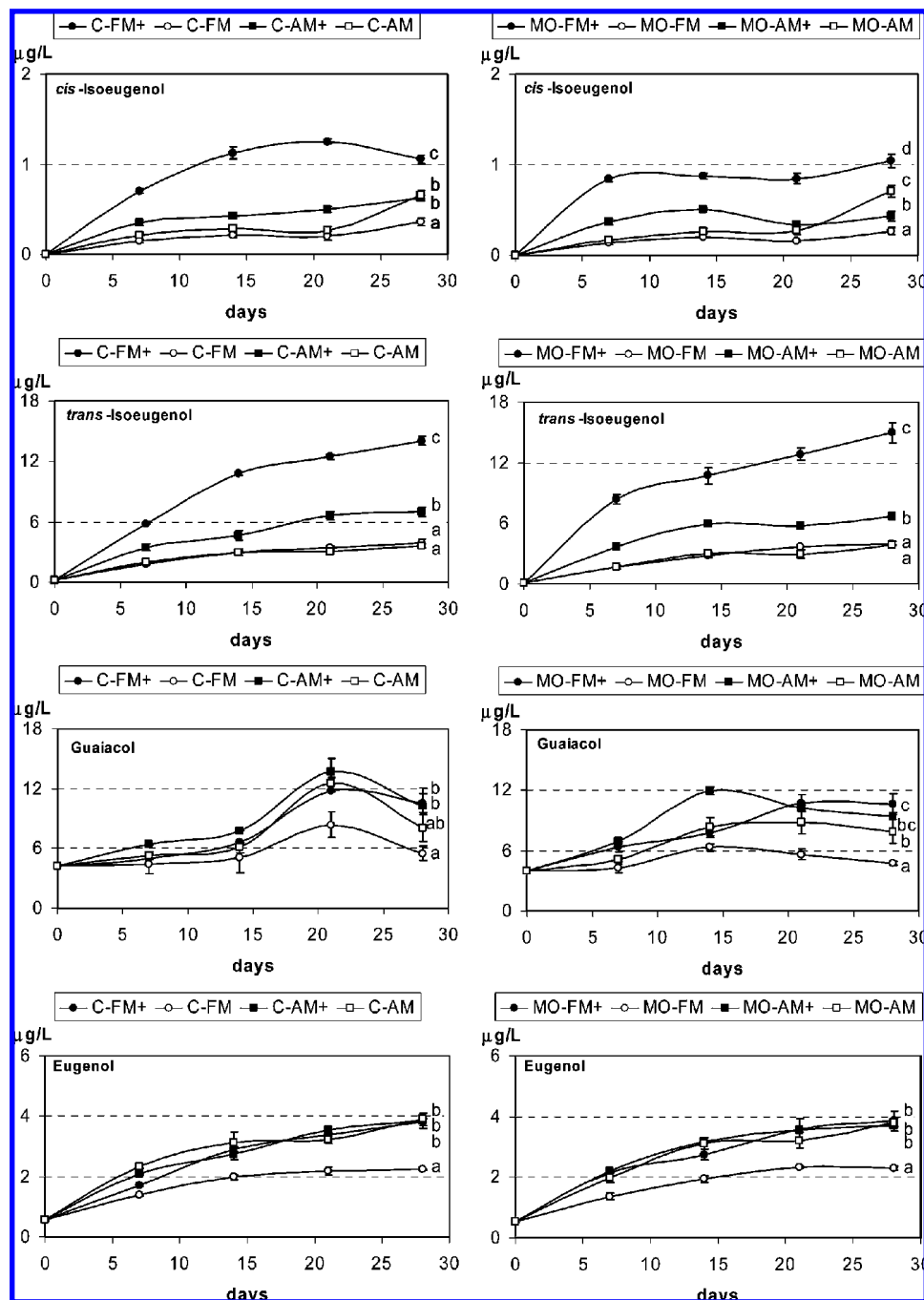


Figure 3. Evolution during maceration process with the four different types of chips studied of *cis*- and *trans*-isoeugenol, guaiacol, and eugenol in C and MO wines. FM+: medium plus toast French chip; FM: medium toast French chip; AM+: medium plus toast American chip; and AM: medium toast American chip. The error bars show \pm SD values. At 28 days of maceration with chips, values with the same letter indicate no statistically significant differences among the different types of chips studied for $\alpha = 0.05$.

applied before malolactic fermentation was only detected for two compounds, vanillin and syringaldehyde.

The effect of time contact can be easily observed in **Figures 2, 3, and 4**, which show the kinetics extraction for each compound studied. In general, the two phenolic aldehydes analyzed, along with the two isomers of whiskey lactones and the volatile phenols (with the exception of guaiacol), displayed a continuous ascending tendency throughout the entire maceration process. However, *cis*-whiskey lactone in MO wines showed the highest values after 14 days of maceration, and *cis*-isoeugenol in wines macerated with American chips (C and MO) remained constant until the third week of treatment to then increase in the last week.

Furanic aldehydes were mainly extracted during the first 2 weeks and decreased or remained constant during the next 2 weeks. Guaiacol levels showed a similar evolution to the furanic aldehydes, reaching a maximum concentration after the third week of maceration with the exception of the MO wines macerated with FM, AM, and AM+ chips, in which the highest concentrations were reached at 14 days.

In general, no effect from the microoxygenation treatment after alcoholic fermentation and before malolactic fermentation was observed in the final concentration of the volatile compounds extracted from wood in the wines; therefore, the results that are being discussed refer to both C and MO wines. Statistically significant differences were found among the

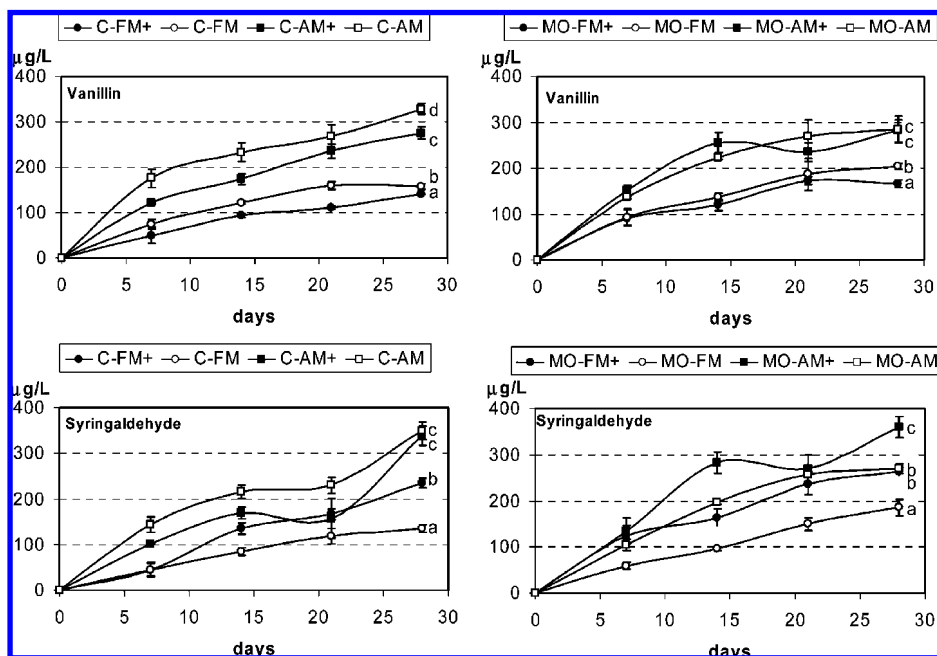


Figure 4. Evolution during maceration process with the four different types of chips studied of vanillin and syringaldehyde in C and MO wines. FM+: medium plus toast French chip; FM: medium toast French chip; AM+: medium plus toast American chip; and AM: medium toast American chip. The error bars show \pm SD values. At 28 days of maceration with chips, values with the same letter indicate no statistically significant differences among the different types of chips studied for $\alpha = 0.05$.

Table 3. Multifactor ANOVA with All Data and Wines

compound	microoxygenation treatment before malolactic fermentation		origin		toasting degree		time in contact with chips	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
furfural	0.030	0.860	28.3	0.000	121	0.000	1.65	0.189
5-methylfurfural	0.400	0.529	62.6	0.000	55.6	0.000	0.880	0.455
cis-whiskey lactone	0.170	0.682	261	0.000	46.8	0.000	9.13	0.000
trans-whiskey lactone	0.030	0.860	26.6	0.000	2.65	0.109	20.2	0.000
guaiaicol	0.150	0.696	17.1	0.000	42.8	0.000	27.3	0.000
eugenol	0.150	0.703	46.3	0.000	35.1	0.000	54.3	0.000
cis-isoeugenol	1.37	0.246	13.5	0.000	66.9	0.000	4.90	0.004
trans-isoeugenol	0.37	0.544	43.0	0.000	144	0.000	12.2	0.000
vanillin	6.60	0.005	256	0.000	15.1	0.000	70.3	0.000
syringaldehyde	9.49	0.003	73	0.000	12.0	0.001	53.6	0.000

different types of chips studied. These differences depend on the origin and toast level of the chip.

The decrease observed in furanic aldehydes in the final days of the maceration process was especially significant in the case of the wine treated with the AM+ chip. A possible explanation for this is the degradation or transformation of furfural into furfuryl alcohol (2-furan-methanol), which has been previously described by other authors (22, 23). As for the continuous extraction of whiskey lactones, Arapitsas et al. (15) also observed an increase in oak lactone levels during the maceration time (2 weeks) in red wines treated with chips, with this increase occurring particularly quickly in the first seven days. Similar results were obtained by Guchu et al. (14) in white wines macerated with American toasted chips. However, when they used nontoasted American chips, an ascending tendency of the concentration of these compounds with maceration time was not observed.

Maceration with AM+ chips produced wines with the highest values of furanic aldehydes, and wines macerated with FM+ chips also showed higher levels of furfural than wines macerated with AM and FM chips (Figure 2). Therefore, as was expected, the toasting level was highly influential on the furfural concentration. These differences are associated with the fact that furanic

compounds are mainly formed during wood toasting through the thermal degradation of cellulose and hemicellulose present in the wood (24) and that their concentrations increase with the toast level (25). The results obtained correlate with those found by Pérez-Coello et al. (26), who observed that hydroalcoholic solutions macerated with American chips presented higher levels in furanic derivatives than the hydroalcoholic solutions macerated with French oak. In this study and according to the results obtained (Figure 2), it can not be asserted that American chips with a medium toast level are richer in these compounds than those macerated with French chips.

The concentrations of furfural and 5-methylfurfural found in the wines after 28 days of treatment were lower than their threshold levels: 15 mg/L for furfural and 16 mg/L for 5-methylfurfural (27, 28). Nevertheless, it is important to note that they may strengthen the aroma of the lactones (29). Moreover, the concentrations of furfural at the end of the maceration treatment were similar to those found in other similar studies (14, 30). However, they were lower than those found by other authors working with wines aged for several months in barrels (15, 16). But they were similar to those found in a study in which a wine of the same variety was aged for 12 months in new American medium toast oak barrels (31).

Levels of *cis*-isoeugenol and *trans*-isoeugenol produced similar results as the furanic aldehydes, (Figure 3). However, in this case, the highest values were detected in wines macerated with FM+ chips, followed by those macerated with AM+ ones.

One effect of the origin of the oak (French or American) and the type of toast level was also found in the concentrations of the two isomers of whiskey lactones, although the differences due to the latter effect were only statistically significant in wines macerated with American oak chips for the *cis* isomer, and in wines macerated with French chips for the *trans* one (Figure 2). As was expected, the wines macerated with AM chips showed the highest values of *cis*-whiskey lactone, at approximately twice the levels of AM+ macerated wines, which also showed levels which were twice as high as in the wines macerated with French chips, regardless of the toasting degree. The differences among wines macerated with AM+ and AM chips are due to the outcome of heating the oak which is a combined process of volatile formation and possible losses and/or decomposition, including oxidation (17, 32). Thermal degradation can be more intense in American oak which is richer in *cis*-whiskey lactone which explains why the toasting effect was only detected in wines macerated with American chips. However, the *trans* isomer showed a different behavior, since in the C wines the highest concentration of this compound was found in the wine macerated with a French chip FM+.

The concentrations of whiskey lactones obtained were below their threshold levels, 74 µg/L and 32 µg/L for *cis* and *trans*, respectively (27). That means that these compounds could not contribute with oaky, coconut and vanilla notes to wine aroma. However, Mosedale et al. (33) stated that threshold values may not be reliable data in a complex medium such as wine and some compounds may influence wine aroma at lower levels than their individual threshold values by means of additive, synergistic or suppressive effects, therefore whiskey lactones could contribute to the coconut, woody and vanillin notes present in wine at the concentrations found in this study.

It is well-known that in general, levels of *cis*-whiskey lactone in wine are higher than the levels of its *trans* isomer (34). However, in the wines macerated with French chips, the differences found between the contents of the two lactones were not very significant (Figure 2). Ordoñez et al. (35) also found similar concentrations of the two isomers in wines macerated with French chips.

The levels of *cis*- and *trans*-whiskey lactone found in the wines studied were lower than the ones usually found in wines aged for long periods in barrels (31, 36–38), and lower than those found in wines macerated with small (1 cm × 1 cm × 0.1 cm) and big (3.4 cm × 2 cm × 1 cm) chips (15), but they were similar to the levels found by some authors in different kinds of wines macerated with American and French chips (14, 30, 35). Furthermore, it should be pointed out that Guchu et al. (14), were not able to detect these lactones in wines macerated with Hungarian chips.

Levels of eugenol also showed some effect associated with the type of chip (Figure 3). Wines macerated with FM chips showed the lowest concentration of this compound. An effect of the type of chip used (origin and toast level) also was found for vanillin, syringaldehyde, and guaiacol (Figures 3 and 4). Wines macerated with American chips showed higher levels of vanillin and syringaldehyde than those macerated with French ones; these results are consistent with data found by Pérez-Coello et al. (26) and Miller et al. (39). In general, wines macerated with M+ chips showed lower levels of vanillin than those macerated with the lower toasting degree chips. These

Table 4. Factor Loadings after Varimax Rotation

	factor 1	factor 2	factor 3
eigenvalue	4.285	2.787	1.912
percent variance	42.853	27.872	19.122
cumulative percentage	42.853	70.272	89.848
furfural	0.013	0.119	0.980
5-methylfurfural	0.253	-0.115	0.955
<i>trans</i> -whiskey lactone	0.196	0.834	-0.355
<i>cis</i> -whiskey lactone	0.865	-0.297	-0.091
vanillin	0.959	-0.105	0.114
syringaldehyde	0.847	0.317	0.210
guaiacol	0.546	0.540	0.392
eugenol	0.771	0.570	0.178
<i>cis</i> -isoeugenol	-0.048	0.916	0.061
<i>trans</i> -isoeugenol	-0.087	0.970	0.140

results agree with others found in the literature (40, 41), but they contrast with those found by Cadahía et al. (42) and Campbell et al. (17). However, in general, the lowest levels of syringaldehyde were detected in the wines macerated with the lowest toasted chips. A botanical and geographical origin factor was observed for guaiacol levels since statistically significant differences between the levels of this compound in wines macerated with American and French oak with the same degree of toasting were observed, especially among medium toasted chips.

Only the level of vanillin in the C wine macerated with AM chips exceeded the perception threshold (0.32 mg/L) (43), and no wine showed levels of syringaldehyde higher than its threshold (25 mg/L) (32). However, although it is known that syringaldehyde does not play an important role in wine aroma, vanillin can contribute to wine aroma even though its concentration is below its threshold due to additive or synergic effects as has been explained before (33). Furthermore, Spillman et al. (44) suggested that the vanillin character displayed in many wines aged in American oak correlates more closely with the concentration of *cis*-whiskey lactone than that of vanillin. The concentrations of vanillin and syringaldehyde found in wines macerated with chips were similar to those found by other authors (14, 30, 36) but significantly lower than those found by Arapitsas et al. (15) and Frangipane et al. (16). Furthermore, these concentrations were similar to those found by Ortega-Heras et al. (31) in wines aged for 12 months in new American barrels.

The results discussed indicate that the type of chip notably influenced the final concentration of the most significant volatile compounds released from wood. However, based on a single analysis, it is not easy to evaluate the real effect of the type of chip. Therefore, to complete the information obtained, it is necessary to evaluate the effect of the kind of chip on all compounds studied as a whole. This study could be carried out by using different multivariate statistical analyses such as factorial analysis. Factorial analysis showed that the distribution of the variables into three factors was enough to explain more than 80% of the total variability. Furthermore, the three factors showed an eigenvalue above 1. Table 4 shows the loading of each variable for each one of the factors, as well as the eigenvalue and variance for each factor.

The compounds associated in factor 1 (*cis*-whiskey lactone, the two aldehyde phenolics, and the volatile phenols eugenol and guaiacol) were the only compounds whose levels showed the most significant effect of the wood origin (American or French), with some of them also showing an influence from the toasting degree. It can be said that the geographical origin of the wood, American or French, affects the extraction of the

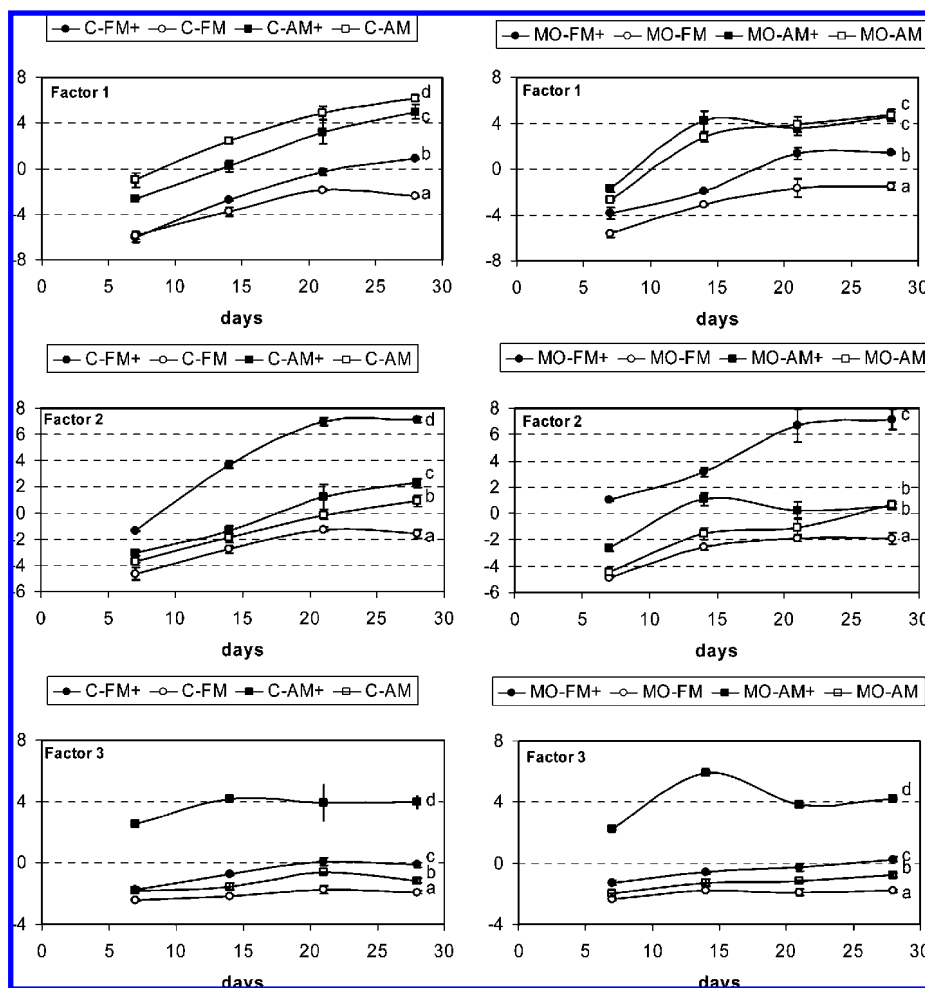


Figure 5. Evolution during the maceration process with the four different types of chips studied of the three factors obtained in the factorial analysis. FM+: medium plus toast French chip; FM: medium toast French chip; AM+: medium plus toast American chip; and AM: medium toast American chip. The error bars show \pm SD values. At 28 days of maceration with chips, values with the same letter indicate no statistically significant differences among the different types of chips studied for $\alpha = 0.05$.

volatile compounds associated with this factor from wood, with the wines macerated with American chips being richer in these compounds than those macerated with French chips (**Figure 5**). Again, the results obtained from the C wines were similar to those found in the MO wines. These results indicate the importance of selecting the type of chip in accordance with the desired finished wine aroma.

The variables mainly associated in factor 2, *trans*-whiskey lactone and *cis*- and *trans*-isoeugenol (**Table 4**), were the compounds for which the highest concentrations were found in wines macerated with FM+ chips, whereas in factor 3, the two furfuranic compounds were especially associated, the levels of which were maximum in the wines macerated with AM+ chips. Both factors showed the effect of toast degree. Factor 3 also indicated the effect of the wood origin. Therefore, and according to results obtained from factor 2, the toast degree effect was more significant with the French chips than with the American ones, and on the other hand, in factor 3, this effect was more significant for American chips than for French chips (**Figure 5**).

In general, factor analysis data showed differences among the wines macerated with the four types of chips, while no general statistically significant differences were detected between the MO wines and the C wines. That means that factorial analysis did not indicate any significant difference between the

C and the MO wines, as, in general, both kinds of wines presented similar values and evolutions for each factor.

Another way to study the effect of type of chip on all volatile compounds extracted from wood is through discriminant analysis. If each type of chip truly did produce different characteristics in the wines, they could be differentiated by discriminant analysis. Discriminant analysis was carried out taking into account all the samples, without considering the microoxygenation factor. When discriminant analysis was applied to classify the wines according to wood origin and toast degree, the final model selected six of the 12 variables considered. The selected variables listed in order from higher to lower discriminate power are 5-methylfurfural, *cis*-whiskey lactone, *trans*-isoeugenol, eugenol, *trans*-whiskey lactone, and *cis*-isoeugenol. The three first variables showed high *F* values being the most important ones for the differentiation of wines macerated with the four types of chips. The model obtained was satisfactory, with a global classification of 100% (**Figure 6**).

The most interesting result is the fact that the centroids of wines macerated with American chips showed a long distance between them with respect to the centroids of wines macerated with French chips, which showed closer distances between them (**Figure 6**). This fact seems to indicate that there are more possibilities of producing peculiar characteristics in the wines

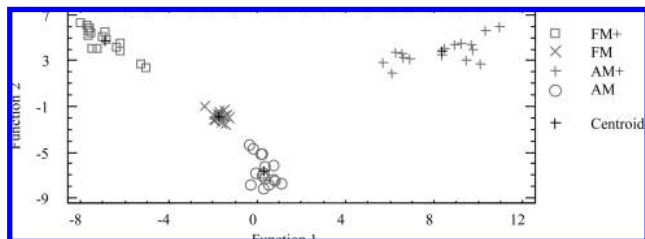


Figure 6. Distribution of wines studied in the plane defined by canonical functions 1 and 2 according to type of chip used.

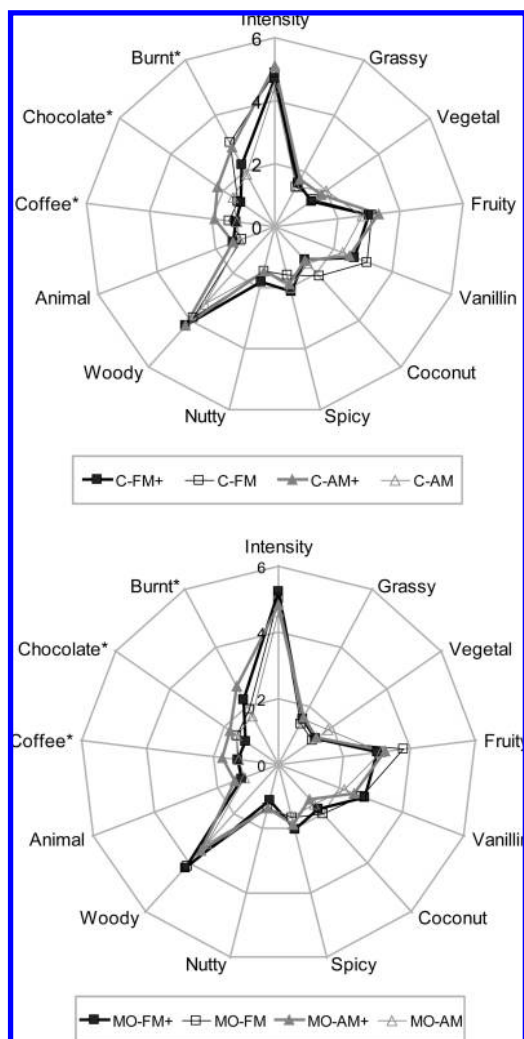


Figure 7. Sensory spider diagrams for wine for each type of chip studied. Mean values are shown. Attributes marked with an asterisk indicate that statistically significant differences were found for $\alpha = 0.05$.

when working with American rather than with French chips and also corroborates the fact that the type of chip is a very significant factor for determining the final aromatic composition of finished wines. Furthermore, since there was no difference between the MO and the C wines for each kind of chip studied, once more, it was observed that the chip effect is similar or independent of previous wine treatment.

The results obtained in the sensory analysis corroborated only some of the results found in chemical analysis. Thus, in both the C and the MO wines, the judges found statistically significant differences in the notes to burnt, chocolate, and coffee, the wines macerated with AM+ chips being the richest ones in these attributes (Figure 7). This fact agrees with the highest concentrations of furfural and 5-methylfurfural found in these wines

since these compounds are responsible for caramel, almond, and toast notes present in wines (27). However, the judges did not find statistically significant differences between the wines for the other olfactory attributes such as vanillin, coconut, or spice as could be expected according to the data obtained from the multifactor ANOVA results. These results are in line with those published previously by Guchu et al. (14), who noted that the global effect of the toasting degree of chips was more important than the effect of the geographical origin of oak. These authors pointed out that judges did not show any significant preferences between wines macerated with Hungarian oak chips and those macerated with American oak chips.

In summary, it can be said that the diffusion process of the volatile compounds present in oak wood chips to the wines is quite rapid and that, in general, the maximum concentration is reached in only 21 days. Furthermore, the kinetic extraction does not seem to depend on the type of chip used since the wines macerated with the four types of chips studied evolved in the same way. In addition, the extraction processes also were independent of the application of microoxygenation treatment before maceration with chips.

The final amount of compound extracted depends on the kind of chip used. Some of the differences found were related to the wood origin, as was the case of the two isomers of whiskey lactone and vanillin, while others were related to the toast degree, such as the levels of *cis*- and *trans*-isoeugenol, furfural, and 5-methyl furfural. However, in the majority of the cases, a mixture of both factors was detected.

Sensory analysis also showed remarkable differences, mainly in burnt, chocolate, and coffee notes. Therefore, chip selection must be carried out with extreme care, considering that each commercial supplier offers chips manufactured from wood of different origins and from diverse toasting processes and because they are not at all well-standardized, which could have a great influence on the final characteristics of the wines.

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Received for review November 6, 2007. Revised manuscript received April 21, 2008. Accepted April 28, 2008. We thank the INIA and ITACyL for funding provided for this study under Projects RTA04-084 and BU-02/2005, respectively. S.P.-M. and M.O.-H. are grateful to INIA for partially financing their work contracts. J.J.R.-B. also thanks the ITACyL for his technical grant.

JF800373D