

## Volatile Compounds of Red Wines Macerated with Spanish, American, and French Oak Chips

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The volatile composition of a red wine aged for 2 months with three different Spanish oak chips (*Quercus pyrenaica* and *Quercus petraea*) from different regions of Castilla y León was compared with that of wines aged with American and French chips. In general, the extraction kinetics showed that the maximum concentration of the volatile compounds extracted from wood can be reached in only 1 month. In the final wines, the levels of furanic aldehydes and eugenol were higher in the wines macerated with Spanish chips, whereas *cis*-whiskey-lactone, vanillin, and methyl vanillate showed higher levels in wines treated with American chips. Among the wines treated with the different Spanish chips, the differences observed in the volatile composition were more related to the geographical origin of the forest than to the botanical species. In general, the wines macerated with Spanish chips showed levels of oak-related volatile compounds that were more similar to those macerated with French chips than to those macerated with American chips.

**KEYWORDS:** Oak chips; volatile compounds; *Quercus pyrenaica*; wine

### INTRODUCTION

Oak barrels have been commonly used in aging wine because it improves wine quality and modifies its composition because of the compounds that are extracted from wood such as tannins, phenolic acids, and volatile compounds. The different volatile compounds extracted from wood during the aging process (lactones, furanic compounds, vanillin derivatives, and phenol derivatives) have important sensory properties and contribute to the overall aroma of the wine. The quantity and quality of the compounds extracted depend on several factors, including the botanical and geographic origin of the wood (1–3) and the toast level of the wood (4, 5).

Among all the species of *Quercus*, *Quercus petraea*, *Quercus robur*, and *Quercus alba* are the most used for aging wine. The first two species come mainly from French forests, while the last species comes from American forests. However, there are other countries in Europe such as Spain, Portugal, or Hungary where *Q. petraea* and *Q. robur* also grow.

In Spain, apart from the European species, there are other species of interest in oenology, *Quercus pyrenaica* Willd). Different works have studied the utility of oak wood from Spanish forests in oenology (6–9). All of these studies have concluded that oak wood from Spanish forests, regardless of the species, is suitable for aging wine to improve its quality.

However, barrel production is very costly and aging wine requires long periods of time. A more economic alternative to the use of oak casks is the use of oak wood fragments commonly

called chips. This practice was recently approved and legislated by the European Community (CE 2165/2005 and CE 1507/2006), although this practice has been used in some countries such as Australia and South Africa for several years. Nowadays, there are a great number of these kinds of products available on the market and the characteristics of the final wines will depend on several factors (chip size, botanical and geographical origin of the oak, type of toast, doses, amount of time it is in contact with the wine, moment of application, variety, etc).

In recent years, different papers on different aspects related to the use of chips have been published. Some papers have studied how macerating wine with oak chips affects its phenolic composition (10–12). As for the volatile composition, the evolution of the wine volatile compounds released from wood during the maceration process and maceration with chips of different origins and/or different toasting degrees have been studied (13–16): the effect of the size and contact time (17, 18), the effects of combining the use of chips with the microoxygenation of wine (16), and the volatile composition of white wines fermented with oak chips (19). Other works compare the aromatic composition of wines aged with chips and wine aged in oak barrels (18, 20).

However, in general these studies are focused on American and French chips, and very few papers present results regarding the use of chips from other countries or oak species. In particular, no paper has been published that study the effect of Spanish *Quercus petraea* and *Q. pyrenaica* Willd chips on the volatile composition of wines macerated with chips.

For this reason, the aim of this work was to assess the influence of the use of Spanish oak wood fragments on the oak-related volatile composition of wines and to compare the results obtained

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with those found in the same wine macerated with different French and American oak chips.

## MATERIALS AND METHODS

**Samples.** A 2006 vintage red single-variety wine was elaborated from autochthonous Spanish *Vitis vinifera* "Tinta del País" grape variety. This wine was elaborated following a traditional red wine making method. After malolactic fermentation, the wine was divided into fourteen 35 L stainless-steel tanks. Every two tanks of wine were macerated with 4 g/L of seven different kinds of chips, so the study was carried out in duplicate. The chips studied had the following characteristics: two *Quercus alba* American oak chips from two different commercial suppliers, A1 and A2, and with different toasting degrees, medium plus and medium, two French oak chips from a mixture of *Quercus petraea* and *Quercus robur* also from two different commercial suppliers, F1 and F2, and with two different toasting degrees, medium plus and medium, two *Quercus pyrenaica* Willd Spanish medium toast chips (S1 and S3), and a *Quercus petraea* Spanish medium toast chip (S2). The three Spanish chips came from the same supplier and from three different forests from Castilla y León Autonomous Community. Therefore, the manufacturing and toasting process was the same for the three of them. All the chips used were similar in size (5–30 mm × 3–25 mm × 1–4 mm). One sample from each tank was taken after 15, 30, and 60 days of maceration.

**Chemical Standards and Reagents.** The chemical standards were purchased from Fluka (Buchs, Switzerland) (furfural,  $\gamma$ -butyrolactone, guaiacol, eugenol, vanillin, and syringaldehyde), Aldrich (Steinheim, Germany) (methyl octanoate, 2-octanol, 5-methylfurfural, whiskey-lactone, isoeugenol, 2,6-dimethoxyphenol, and acetovanillone), and Lancaster (Strasbourg, France) (4-ethylguaiacol, 4-vinylguaiacol, 4-ethylphenol, 3,4-dimethylphenol, 4-vinylphenol, ethyl vanillate, and methyl vanillate). The ethanol (HPLC-grade) was from Scharlau (Barcelona, Spain), the dichloromethane (HPLC-grade) was from Merck (Darmstadt, Germany), and the Milli-Q water was from a Millipore system (Bedford, MA).

**Analysis of the Volatile Compounds of Wine.** The volatile compounds were extracted by liquid–liquid extraction with the method developed by Rodríguez-Bencomo et al. (16). Two-hundred-fifty milliliters of wine, 5 mL of dichloromethane, and 75  $\mu$ L of a mixture of three internal standards IS (450 mg/L of 2-octanol and 3,4-dimethylphenol; 550 mg/L of methyl octanoate) were added to a flask. The extraction was carried out for 3 h with continuous stirring (150 rpm). The organic phase was then separated and stored at –20 °C until it was analyzed. Each extraction was carried out twice.

The chromatographic analyses were performed with a HP-6890N GC coupled to a HP-5973 inert MS detector equipped with a Quadrex 007CWBTR capillary column (60 m length, 0.25 mm i.d., and 0.25  $\mu$ m film thickness). The carrier gas was helium at 0.8 mL/min. The oven column program was set to 40 °C (held for 10 min), raised to 240 °C by 2 °C/min, and held at this temperature for 45 min. Detection was in EI scan mode (70 eV), and identification was carried out using spectra obtained with commercial standard compounds and from the NIST library. Quantification was carried out following the internal standard quantification method. Then quantitative data of the relative areas (absolute areas/internal standard area) were interpolated in the calibration graphs built from results from pure reference compounds. The ion  $m/z$  [in brackets] and the internal standard selected for quantification of each compound were the following: *trans*-whiskey-lactone [71], *cis*-whiskey-lactone [87], 4-vinylphenol [120], *cis*-isoeugenol [164], and *trans*-isoeugenol [164] were determined using methyl octanoate [74] as the internal standard; 4-ethylguaiacol [137], eugenol [164], methyl vanillate [151], ethyl vanillate [151] were determined using 2-octanol [45] as internal standard; and furfural [96], 5-methylfurfural [110],  $\gamma$ -butyrolactone [86], guaiacol [109], ethylphenol [107], 4-vinylguaiacol [135], vanillin [152], acetovanillone [166], and syringaldehyde [182] were determined using 3,4-dimethylphenol [107] as internal standard. The internal standard for each compound was that for which the best the reproducibility was obtained in the validation study of the method.

**Statistical Analyses.** The statistical analysis of the data was carried out by analysis of variance (ANOVA). The least significant difference test (LSD) considering confidence levels of 95% was applied to determine

significant differences among the different chips studied at the end of the maceration treatment. Multivariate analysis factorial analysis and discriminant analysis were used to evaluate the overall effect of maceration. A principal component analysis (PCA) was used as a factoring technique, and a varimax rotation was applied to simplify the interpretation of the data. The discriminant analysis was carried out following the forward stepwise method. The prediction capacity of the discriminant models was studied by "cross-validation" with three cancellation groups in order to validate the models, that is, to know their stability. All the statistical analyses were carried out using the Statgraphics Plus program for Windows.

## RESULTS AND DISCUSSION

The volatile compounds studied were furanic aldehydes, furfural, and 5-methylfurfural, the two isomers of  $\beta$ -methyl- $\gamma$ -octalactone *cis* and *trans* (commonly known as oak lactones or whiskey lactones) and  $\gamma$ -butyrolactone, the volatile phenols guaiacol, eugenol, *cis*-isoeugenol, *trans*-isoeugenol, 4-vinylguaiacol, 4-ethylphenol, 4-ethylguaiacol, 2,6-dimethoxyphenol, and the aldehyde phenols and their derivatives, vanillin, ethyl vanillate, methyl vanillate, acetovanillone, and syringaldehyde. The concentrations of each compound in the final wines (after 60 days of maceration) macerated with the seven chips and the LSD test are shown in **Table 1**.

The extraction kinetics for the different compounds studied can be seen in **Figures 1** and **2**, which show the evolution of each compound during the maceration process for the seven chips studied. In the initial wine, the only compounds quantified were  $\gamma$ -butyrolactone, guaiacol, ethyl vanillate, acetovanillone, and 4-vinylguaiacol. The concentration of  $\gamma$ -butyrolactone and ethyl vanillate did not increase significantly during the maceration process. Furthermore, it should be also pointed out that the concentration of 4-vinylguaiacol found in the initial wine was significantly higher than those found in the wines macerated with chips. This fact could be due to the adsorption of this compound by the wood as it has been described by other authors (21) or to reactions of 4-vinylguaiacol with anthocyanins (22, 23). In general, the rest of the compounds studied displayed a continuous ascending tendency for the first 15 days of the maceration process (or 30 days in a few cases), and then the contents remained constant, although the concentration of each compound in the wines depended on the kind of chips used. However, furanic compounds furfural and 5-methylfurfural showed an important decrease in concentration after 15 days of contact with the chips. This decrease was continuous during the rest of the maceration process, although it was more intense during the third and fourth week. The results of the extraction kinetics agree with the results obtained in previous studies (16).

The important decrease observed in furanic aldehydes, furfural and 5-methylfurfural, after the first 15 days could be due to the oxidation of furfural to furfuryl alcohol (24, 25), to the formation of 2-furanmethanethiol, having a toasty aroma (26), or to the formation of brown products among the furanic aldehydes and the (+)-catechin of wine (27). This decrease had been also observed in a previous study of wines macerated with chips (16), but it has also been observed in wines aged in wood (6, 28, 29).

After 2 weeks of treatment, important differences among the wines elaborated with the different chips were observed in the wines macerated with the Spanish chips S2 and S3, the ones that showed the highest concentrations of furanic compounds. In the final wines the differences found were less relevant, although statistically significant differences were also found among them (**Table 1**). Thus, the wines macerated with Spanish chips showed concentrations of furanic compounds that were higher than those macerated with French and American chips with the exception of

**Table 1.** Concentrations ( $\mu\text{g/L}$ ) of the Different Compounds Analyzed in the Final Wines Macerated with the Seven Types of Chips (2 Months of Maceration)<sup>a</sup>

compound	F1 <sup>b</sup>	F2 <sup>b</sup>	A1 <sup>b</sup>	A2 <sup>b</sup>	S1 <sup>b</sup>	S2 <sup>b</sup>	S3 <sup>b</sup>	F ratio	p value
furfural	617 b	168 a	1592 c	90 a	1660 c	1848 c	2722 d	76	0.0000
5-methylfurfural	230 a	169 a	811 c	179 a	464 b	1005 d	758 c	55	0.0000
<i>trans</i> -whiskey-lactone	40 d	30 cd	18 ab	27 bc	124 e	37 cd	13 a	87	0.0000
<i>cis</i> -whiskey-lactone	60 b	66 b	127 d	188 e	92 c	86 c	31 a	72	0.0000
$\gamma$ -butyrolactone	11 (mg/L)	11 (mg/L)	10 (mg/L)	12 (mg/L)	10 (mg/L)	10 (mg/L)	11 (mg/L)	ns	
4-ethylphenol	1.7 ab	1.6 a	1.7 b	1.6 a	1.6 a	1.6 a	1.6 a	3	0.0270
4-vinylguaiaicol	8.0	7.7	8.5	10	6.7	9.1	8.4	ns	
4-vinylphenol	4.7	5.3	3.7	5.3	4.3	4.8	3.9	ns	
4-ethylguaiaicol	3.3 f	0.53 c	2.2 e	0.87 d	0.17 a	0.31 ab	0.36 b	424	0.0000
vanillin	786 c	774 c	991 d	988 d	246 a	220 a	364 b	131	0.0000
methyl vanillate	4.2 b	3.7 ab	5.5 c	7.0 d	2.6 a	2.9 a	2.7 a	15	0.0000
ethyl vanillate	31	31	29	33	30	32	29	ns	
acetovanillone	93 f	56 c	74 d	82 e	42 a	41 a	48 b	130	0.0000
syringaldehyde	3057 e	1449 c	3182 e	2541 d	694 ab	505 a	959 b	108	0.0000
guaiaicol	27 c	12 a	29 d	18 b	12 a	18 b	12 a	158	0.0000
eugenol	15 b	9.4 a	16 b	14 b	8 a	33 c	8.2 a	53	0.0000
<i>cis</i> -isoeugenol	6.4 d	1.2 ab	2.9 c	1.8 b	0.63 a	1.1 ab	0.76 a	58	0.0000
<i>trans</i> -isoeugenol	36 d	5.9 ab	16 c	9.8 b	3.6 a	7.4 ab	4.2 a	49	0.0000
2,6-dimethoxyphenol	94 e	16 ab	85 d	38 c	15 a	21 b	16 ab	299	0.0000

<sup>a</sup> Analysis of variance (ANOVA) and least significant difference test (LSD) ( $n=4$ ). ns: differences not statistically significant. <sup>b</sup> Type of chip. F1 and F2: French chips. A1 and A2: American chips. S1, S2 and S3: Spanish chips. Values with the same letter and in the same row indicate no statistically significant differences among the different chips studied for  $\alpha = 0.05$ .

wines treated with A1 chip which showed contents similar to those of S2 wines for furfural and similar to those of S3 wines for 5-methylfurfural. Among the wines with Spanish chips, the highest levels of furfural were observed in the wines with S3 chips and for 5-methylfurfural in the wines with S2; the wines with S1 chips showed the lowest levels. These compounds are mainly formed during wood toasting through the thermal degradation of cellulose and hemicellulose, and their levels increase with the toasting temperature (4, 30). In our case, the effect of the toasting degree could not be seen in the wines macerated with French chips for the 5-methylfurfural. Since the three Spanish chips had the same toasting process and came from the same supplier, the differences found among them could be due to their different composition in cellulose and hemicellulose because these compounds come from the thermodegradation of wood celluloses and hemicellulose (30). Therefore, the differences found among these chips could be due to the botanical species and to the geographical location of their forest.

The levels of furfural found in wines macerated with Spanish chips *Quercus pyrenaica* (S1 and S3) ranged between 1660 and 2722  $\mu\text{g/L}$ . These levels are similar to those found by Fernández-Simón et al. (9) in Spanish wines aged for 1 year in *Quercus pyrenaica* barrels (134–3560  $\mu\text{g/L}$ ). In the case of 5-methylfurfural, the concentrations found in wines treated with Spanish chips (S1 and S3) were also in the range observed in wines aged for 1 year in Spanish *Quercus pyrenaica* barrels (93–2470  $\mu\text{g/L}$ ) (9).

Regarding to the two isomers of whiskey-lactones studied, as expected, the highest concentrations of the *cis* isomer were found in the wines macerated with American chips (188  $\mu\text{g/L}$  for A2 and 127  $\mu\text{g/L}$  for A1). The wine treated with the S3 chip showed the lowest levels of *cis*-whiskey-lactones (31  $\mu\text{g/L}$ ), whereas the other two Spanish chips studied showed contents of this compound intermediate between those of the American and French chips. However, the wines macerated with S1 chips were the richest in the *trans* isomer (124  $\mu\text{g/L}$ ). The concentrations of *trans*-whiskey-lactone found in these wines were between 5 and 7 times higher than those found in the other wines. The differences found among the wines macerated with the rest of the chips studied were less relevant, though again, the wines macerated with S3 chips (13  $\mu\text{g/L}$ ) showed contents significantly lower than those of the

French chips. It is remarkable that wines treated with the Spanish *Q. petraea* chips (S2) showed levels of the *trans*-isomer similar to those of French chips (F2).

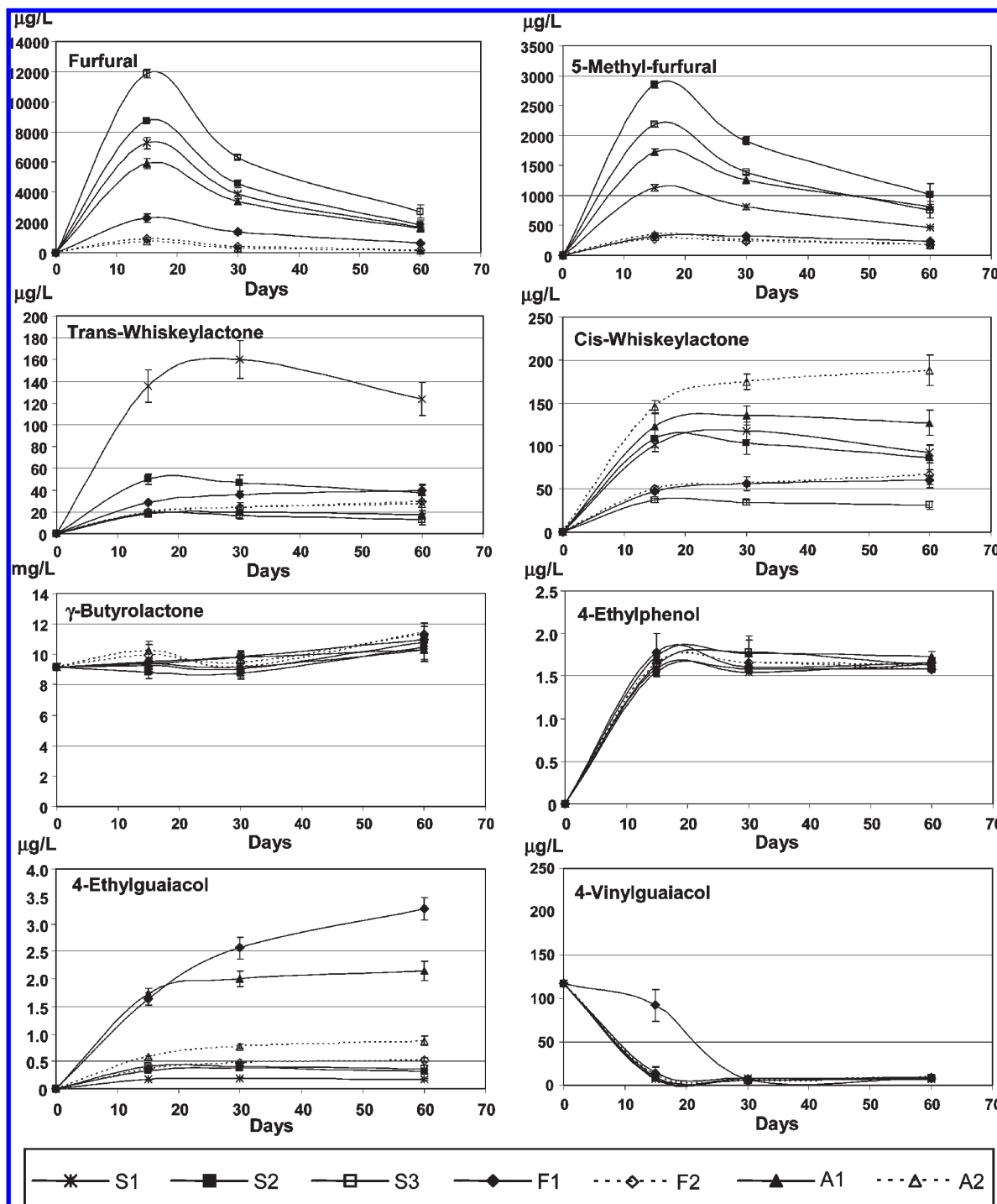
A toasting effect was only found in the wines macerated with American chips. These results agree with those found in other works (16).

According to some works, the *cis/trans* ratio can be used to distinguish between French and American wood (31). In our case (Figure 3), the two wines treated with American chips showed ratios around 7 whereas wines macerated with French chips showed ratios between 1.51 and 2.22. The wines macerated with Spanish chips showed values around 2.35 for S2 and S3 and 0.75 for S1. The ratios were therefore closer to those of French chips than those of American chips. Other authors have found values around 4 in wines aged in Spanish *Quercus Pyrenaica* oak barrels (9) and values of 2.0 for wines aged in Spanish barrels of *Quercus petraea* (6).

In general, in all the wines studied, the levels of both isomers were lower than those observed by other authors in wines aged in French, American, and Spanish barrels for 1 year (6, 9, 32). However, Ortega-Heras et al. (28) found values of the *trans* isomer in wines aged for 12 months in American oak casks similar to those found in this study. The levels of *cis*- and *trans*-whiskey-lactones in these wines were similar to the levels found by some authors in different kinds of wines macerated with American and French chips (13, 16, 20).

As for the other lactone analyzed,  $\gamma$ -butyrolactone, after 2 months of the wine being in contact with the chips, no statistically significant differences were found among the different chips studied. The concentrations ranged between 10.3 and 11.5 mg/L, which are values that are slightly higher than the initial one (9.14 mg/L). Studies carried out on wines stored in oak barrels showed that the extraction of  $\gamma$ -butyrolactone is carried out during the first months of the aging process because this compound is extracted from the more external surface of the wood (33).

Ethylphenols and vinylphenols can also be extracted from wood although in very low concentrations; they are mainly formed during wine aging by microbiological transformation of hydroxycinnamic acids of wines by *Brettanomyces/Dekkera* yeast (34). The low concentrations of these four volatile



**Figure 1.** Evolution during the maceration process of furfural, 5-methylfurfural, *cis*-whiskey-lactone, *trans*-whiskey-lactone,  $\gamma$ -butyrolactone, 4-ethylphenol, 4-ethylguaiaicol, and 4-vinylguaiaicol in the wines macerated with different oak chips: (S1, S2, and S3) Spanish chips; (F1 and F2) French chips; (A1 and A2) American chips. The error bars represent SD values.

compounds found in all the wines studied indicated the lack of microbiological contamination by these yeasts. No statistically significant differences were found among the different chips studied at the end of the maceration process for 4-vinylphenol and 4-vinylguaiaicol. The values of 4-ethylphenol found in these wines were lower than those obtained in wines aged in Spanish, American, and French barrels (6, 9, 33) and similar to those obtained in wines macerated with American chips for 3 months (20). As for 4-ethylguaiaicol, an effect of the kind of wood was observed; thus, at the end of the maceration process, the wines macerated with the three Spanish chips showed concentra-

tions of this compound that were significantly lower than those of American and French chips ( $<0.36 \mu\text{g/L}$  for Spanish and between 0.53 and  $3.3 \mu\text{g/L}$  for American and French) and wines macerated with F1 chip presented the highest values. In this case, an effect of the toasting degree was observed, since the wines macerated with the medium plus toasted chips showed higher concentrations of this compound than the wines macerated with medium toasted chips.

Levels of 4-ethylguaiaicol ( $<3.3 \mu\text{g/L}$ ) were lower than those determined in wines aged in Spanish, French, and American oak barrels for 12 months (9). Guchu et al. (13) obtained similar levels



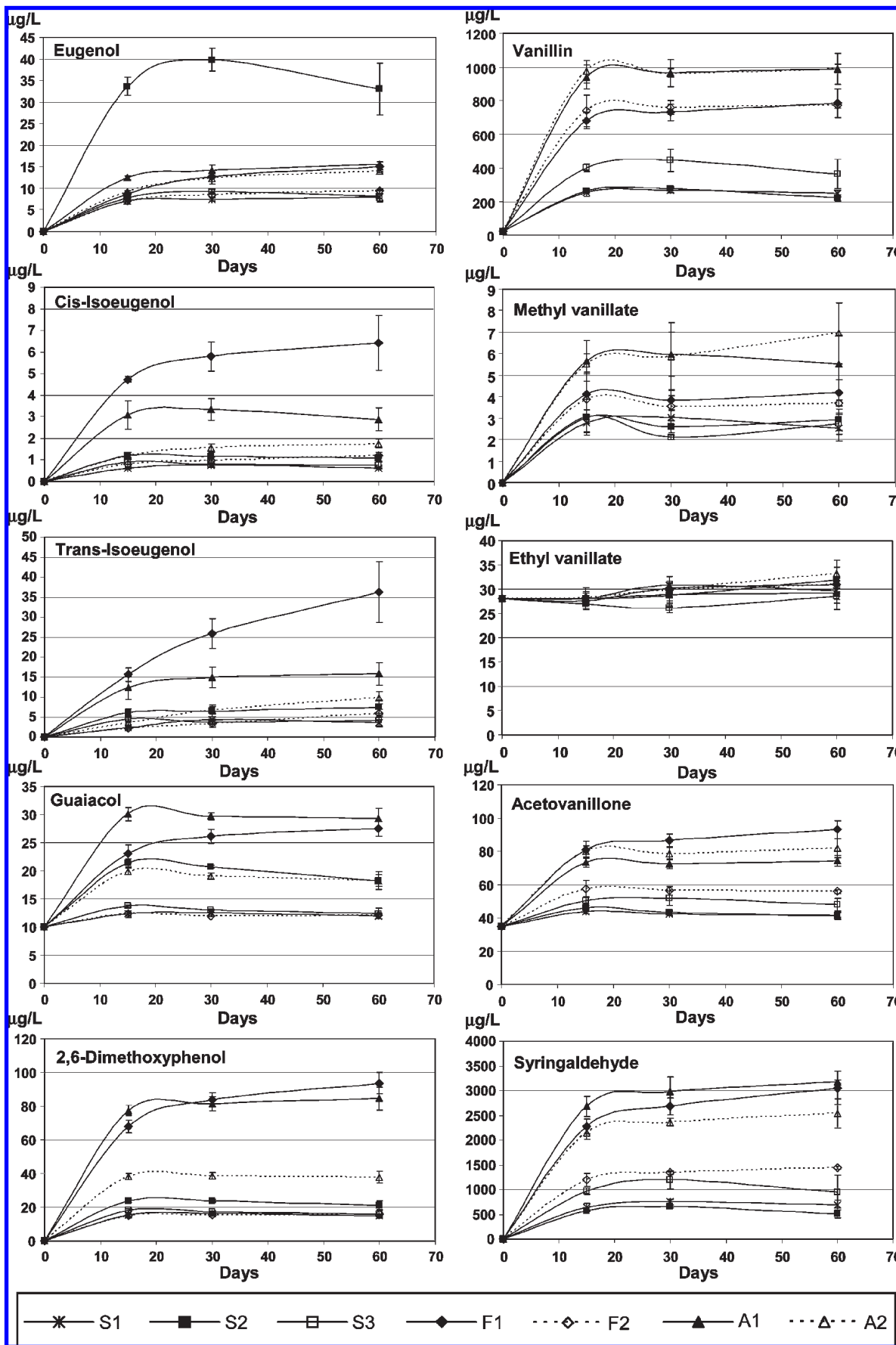
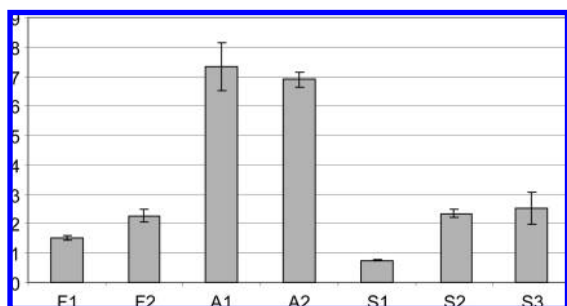


Figure 2. Evolution during the maceration process of eugenol, *cis*-isoeugenol, *trans*-isoeugenol, guaiacol, 2,6-dimethoxyphenol, vanillin, ethyl vanillate, methyl vanillate, acetovanillone, and syringaldehyde in the wines macerated with different oak chips: (S1, S2, and S3) Spanish chips; (F1 and F2) French chips; (A1 and A2) American chips. The error bars represent SD values.



**Figure 3.** The *cis*-*trans*-whiskey-lactone ratio of the wines macerated with different oak chips: (S1, S2, and S3) Spanish chips; (F1 and F2) French chips; (A1 and A2) American chips. The error bars represent SD values.

(0.3–5.8  $\mu\text{g/L}$ ) in white wines macerated with American and Hungarian oak chips for 25 days.

The levels in the wines of other oak-related volatile phenols (eugenol, *cis*- and *trans*-isoeugenol, 2,6-dimethoxyphenol, and guaiacol) were also related to the kind of wood and toasting degree. Only guaiacol showed detectable contents in the initial wine of 10  $\mu\text{g/L}$  which makes it clear that the rest of the volatile phenols are only present in red wines aged in wood. At the end of the maceration process, wines treated with Spanish *Quercus pyrenaica* chips (S1 and S3) showed statistically significant lower levels of these volatile phenols than those of wines macerated with the two American chips and with F1 chips. In general these wines macerated with Spanish chips showed a presence of these compounds that was similar to that of wines macerated with F2 chips, with the exception of S2, which presented contents of eugenol significantly higher than those of the rest of wines. These results do not coincide with those found by other authors who observed higher concentrations of eugenol and guaiacol in wines aged in Spanish *Quercus pyrenaica* oak casks than in French or American ones (9).

A toasted effect was also observed, since wines macerated with medium plus American and French oak chips showed higher concentrations of these five compounds than those treated with medium toast chips. The results are in accordance with the fact that these compounds are formed by the thermo degradation of lignin in the wood's toasting process (35).

Levels of guaiacol, 2,6-dimethoxyphenol, eugenol, and *trans*-isoeugenol were lower than those obtained in wines aged in oak barrels (American, Spanish, and French) for 1 year (9). For guaiacol, similar values were observed in wines aged in American and French oak barrels for 1 year (33), and wines aged in Spanish and French barrels showed values of guaiacol and eugenol similar to those found in the wines of this work (6). The contents observed for these last compounds in wines macerated with American oak chips (25 days and 2 months) were in the range of those found by other authors (13, 36).

The extraction of the phenolic aldehydes and their derivatives evaluated, methyl and ethyl vanillate, acetovanillone, vanillin, and syringaldehyde, was also related to the geographical origin of the chip. At the end of the maceration process, wines treated with Spanish chips showed the lowest concentrations of these compounds, with the exception of ethyl vanillate, for which no statistically significant differences were found among the different chips studied. In the case of vanillin and methyl vanillate, wines macerated with American chips showed contents that were significantly higher than those of French chips. Among the wines macerated with Spanish chips there were no relevant differences between *Quercus pyrenaica* and *Quercus petraea*, although levels of vanillin and acetovanillone were higher in wines treated with S3

**Table 2.** Odor Thresholds and Aroma Notes of Each Compound Analyzed

compd	odor threshold <sup>a</sup> ( $\mu\text{g/L}$ )	aroma notes
furfural	15000 (37) <sup>b</sup>	bread, almond, sweet
5-methylfurfural	16000 (38) <sup>b</sup>	almond, caramel, burnt sugar
<i>trans</i> -whiskey-lactone	32 (37) <sup>c</sup>	oaky, coconut, vanilla
<i>cis</i> -whiskey-lactone	74 (37) <sup>c</sup>	oaky, coconut, vanilla
$\gamma$ -butyrolactone	50000 (39) <sup>d</sup>	caramel, sweet, buttery
4-ethylphenol	620 (40) <sup>c</sup>	phenolic, animal, stable
4-vinylguaiacol	380 (41) <sup>c</sup>	clove
4-ethylguaiacol	140 (37) <sup>c</sup>	phenolic, smoked
vanillin	320 (41) <sup>c</sup>	vanilla
methyl vanillate	3000 (42) <sup>b</sup>	caramel, butterscotch, vanilla
ethyl vanillate	990 (42) <sup>b</sup>	flower, fruit, sweet, vanilla
acetovanillone	1000 (39) <sup>b</sup>	vanilla
syringaldehyde	25000 (43) <sup>b</sup>	vanilla
guaiacol	11 (44) <sup>b</sup>	smoke, sweet, medicine
eugenol	14.3 (45) <sup>b</sup>	clove, honey
<i>trans</i> -isoeugenol	6 (46) <sup>b</sup>	flower
2,6-dimethoxyphenol	57 (42) <sup>b</sup>	phenolic, medicine

<sup>a</sup> Reference from which the value has been taken is giving in parentheses.

<sup>b</sup> Odor threshold in model solution. <sup>c</sup> Odor threshold in red wine. <sup>d</sup> Odor threshold in water.

chips than in those treated with the other two Spanish chips. A toasted effect was only observed for acetovanillone and syringaldehyde and also for methyl vanillate in the case of French chips. However, a clear pattern was not observed because the effect of the toast depended on the compound and the botanical origin of the chip.

Fernández-de-Simón et al. (9) observed that the levels of vanillin were slightly lower than those observed in wines aged in Spanish *Quercus pyrenaica* barrels for 12 months. In the same study, the authors found the levels of ethyl and methyl vanillate in wines aged in American, French, and Spanish barrels to be lower than those found in the wine elaborate in this study. The levels of syringaldehyde were in the range of those found in that work. Regarding the results found in other wines elaborated with chips, the levels of vanillin were lower (for wines with Spanish chips) and similar (for wines with French and American chips) to those observed in red wines macerated with American chips for 2 months (36). However, the concentrations found in this work were higher than those observed in white wines macerated or fermented with American and French chips (13, 19). In the case of syringaldehyde, levels of wines of Spanish chips were similar to those observed by Martínez-García et al. (36) and higher in the case of wines of American and French chips. The levels of both compounds in wines with American and French chips were higher than those found in a previous work (9).

The odor thresholds of the compounds analyzed and the different notes that each one gives to the aroma of the wine can be seen in **Table 2**. Guaiacol was the only compound that showed contents higher than its odor threshold in all the wines at the end of the maceration process. However, in some of these wines, some compounds were also present in concentrations that were higher than their odor thresholds: *cis*-whiskey-lactone in wines macerated with S1, S2, and American chips, *trans*-whiskey-lactone in wines with S1, S2, and F1 chips, eugenol and *trans*-isoeugenol in wines with F1 and S2 chips, 2,6-dimethoxyphenol in wines with F1 and A2 chips, and vanillin in all wines except those with S1 and S2 chips. However, it should be taken into account that not only the compounds present in wine in concentrations higher than their odor thresholds contribute to wine aroma. The rest of the volatile compounds released from wood could also contribute because of synergic or additive effects and show sensory influences even with concentrations lower than their threshold (2).

Thus, for example, furanic compounds may strengthen the aroma of whiskey-lactones (47).

The results discussed appear to indicate that the geographical origin of the chip influences the final concentration of some of the oak-related volatile compounds studied. To corroborate this fact, it is necessary to carry out different statistical analyses such as factorial analysis and discriminant analysis. A factorial analysis was carried out taking into account all the compounds analyzed (18) and all samples (the seven different kinds of chips studied and the three samples taken along the maceration process). Factor analysis selected four factors with an eigenvalue that was higher than 1. These four factors explained 79.8% of the total variance. **Table 3** shows the loading of each variable for each one of the factors, as well as the eigenvalue for each factor.

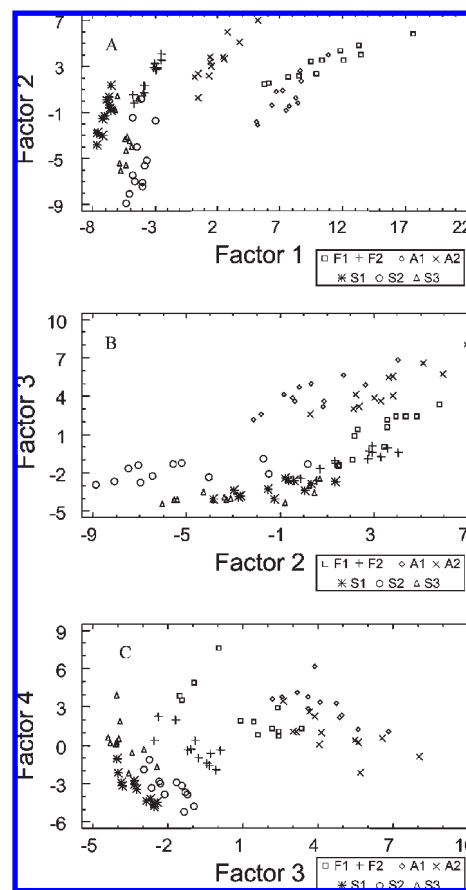
The representation of the seven kinds of chips in the plane defined by the two main factors shows different groups related to oak type and the kind of toast (**Figure 4A**). This figure shows that the three wines macerated with Spanish chips are very close, and it is not possible to separate wines macerated with S2 and S3 chips. Wines macerated with F2 chips are also near those macerated with Spanish chips. Wines treated with the chips with the highest toast degree, A1 and F1, appear separated from the rest. That means that the compounds mainly associated with factor 1 (guaiacol, 4-guaiacol, 2,6-dimethoxyphenol, acetovanillone, syringaldehyde, and the two isomers of isoeugenol) showed the effect of the kind of wood but also of the toasted degree, enhancing the toasting process the extraction of the volatile compounds associated with this factor. The compounds associated in factor 2 and factor 3 showed a significant effect of the wood origin, as can be seen in parts **B** and **C** of **Figure 4**. The compounds associated with factor 2 (furfural, 5-methylfurfural,  $\gamma$ -butyrolactone, and eugenol) showed the highest concentrations in wines macerated with Spanish chips. The compounds gathered in factor 3 (*cis*-whiskey-lactone, vanillin, and methyl vanillate) also showed a clear effect of the kind of wood. The wines macerated in American chips showed the highest concentrations of these compounds. It can be observed in **Figure 4C** that wines macerated with French chips fall between wines treated with Spanish and American chips. This fact points out that Spanish wood, *Quercus pyrenaica* or *Q. petraea*, gives aromatic characteristics to wine that are more similar to those given by French than by American wood. These results coincide with findings by other authors (6, 7). The compounds associated with factor 4 did not present a clear effect with respect to either the kind of wood or the toasted degree.

The stepwise linear discriminant analysis carried out corroborated the results obtained in the factorial analysis. When discriminant analysis was applied to classify the wines according to wood origin (Spanish, French, or American), the final model selected 11 of the 18 variables considered, classifying 100% of the samples correctly. The selected variables listed in order from highest to lowest discriminant power were vanillin, *cis*-whiskey-lactone, furfural, *trans*-whiskey-lactone, eugenol, guaiacol, *cis*-isoeugenol, syringaldehyde, 2,6-dimethoxyphenol, ethyl vanillate, and methyl vanillate. It is important to point out that vanillin presented an *F* value (667) that was much higher than the rest of variables, and this single variable was able to classify 96% of the samples correctly. **Figure 5** shows the distribution of the samples in the plane formed by canonical functions 1 and 2, according to the geographical origin of the chip used. The positions of the three centroids show a longer distance between wines macerated with American and Spanish chips than between wines treated with French and Spanish chips. Again, this fact seems to indicate that the Spanish chips are more similar to French chips than to American chips. The evaluation of the model was done by crossvalidation, obtaining a global

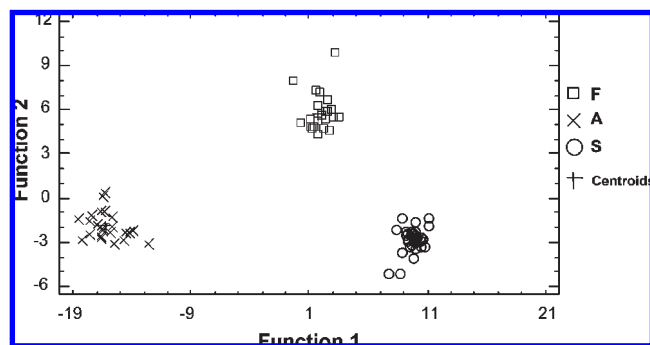
**Table 3.** Factor Loading after Varimax Rotation<sup>a</sup>

	factor 1	factor 2	factor 3	factor 4
eigenvalue	7.67829	2.92298	2.02504	1.73778
percentage of variance	42.657	16.239	11.250	9.654
cumulative percentage	42.657	58.896	70.146	79.801
furfural	-0.1707	<b>-0.7852</b>	-0.2360	0.0330
5-methylfurfural	-0.0922	<b>-0.9416</b>	-0.0158	-0.0887
$\gamma$ -butyrolactone	0.1301	<b>0.7124</b>	0.1118	-0.3618
guaiacol	<b>0.8504</b>	-0.2580	0.3819	0.1324
<i>trans</i> -whiskey-lactone	-0.2470	-0.0118	-0.2507	-0.5261
<i>cis</i> -whiskey-lactone	0.0014	-0.0234	<b>0.8874</b>	-0.1920
4-ethylguaiacol	<b>0.9586</b>	0.1210	0.0790	0.1540
4-ethylphenol	0.1740	-0.0103	0.0089	<b>0.7810</b>
4-vinylguaiacol	0.2781	0.1278	-0.2758	0.3612
2,6-dimethoxyphenol	<b>0.9315</b>	0.0209	0.2244	0.2068
vanillin	0.4108	0.4036	<b>0.6164</b>	0.4713
methyl vanillate	0.3465	0.2987	<b>0.7939</b>	0.1301
ethyl vanillate	0.1698	0.4930	0.3285	<b>-0.6735</b>
acetovanillone	<b>0.7277</b>	0.3676	0.3707	0.3394
syringaldehyde	<b>0.7344</b>	0.2711	0.4460	0.3865
eugenol	0.1463	<b>-0.6148</b>	0.1715	-0.4219
<i>cis</i> -isoeugenol	<b>0.9722</b>	0.1435	-0.0736	0.0659
<i>trans</i> -isoeugenol	<b>0.9653</b>	0.1329	-0.0464	-0.1030

<sup>a</sup> Boldface values indicate the larger factors.



**Figure 4.** Distribution of wines studied in the plane defined by the factors of the factorial analysis: (A) factor 2 vs factor 1 according to the type of chip used; (B) factor 3 vs factor 2 according to the origin of the chips used; (C) factor 4 vs factor 3 according to the origin of the chips used. S: Spanish chips (S1, S2, S3). F: French chips (F1, F2). A: American chips (A1, A2).



**Figure 5.** Distribution of wines studied in the plane defined by canonical functions 1 and 2 of discriminant analysis according to the origin of the chips used: (S) Spanish chips; (F) French chips; (A) American chips.

percentage of correct classification of 100% and of global prediction of 100% also. These results mean that the selected variables are useful to differentiate the different kind of chips used by their origin.

In summary, the results discussed indicate that in wines macerated with chips, the maximum concentration of the volatile compounds present in wood can be reached in only 1 month. The final concentration of these oak-related volatile compounds also depends on the geographical origin of the chip employed. Thus, wines macerated with Spanish chips are richer in furanic aldehydes and eugenol whereas those macerated with American chips have higher concentrations of *cis*-whiskey-lactone, vanillin, and methyl vanillate. In general, the wines macerated with Spanish chips show levels of oak-wood related volatile compounds more similar to those macerated with French ones. Significant differences were also found for some of the volatile compounds analyzed among the three Spanish chips studied, although these differences appear more related to the geographical origin of the forest than to the botanical species. However, it has also to be taken into account that there is a lot of variation in oak within the same stave, tree, forest, etc. (2, 48). Therefore, it can be concluded that Spanish chips, *Quercus pyrenaica* and *Quercus petraea*, are as suitable as French or American chips for the “alternative” aging of wine and that their use could give rise to wines with different characteristics from those elaborated with chips from the American and French wood that is traditionally used in cooperage.

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