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Analytical Methods

# Statistical differentiation of wines of different geographic origin and aged in barrel according to some volatile components and ethylphenols

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ABSTRACT

The purpose of this paper was to study the effect of wine composition and geographical origin on the accumulation of volatile compounds in barrel-aged wines. Therefore, 267 wines belonging to different origin appellations were analysed. In order to analyse the data, a multivariate statistical technique was applied, that can be quite useful in creating an overall view of the problem in which multiple variables are analysed. Statistical analysis gave four significant factors, accounting for 84.12% of the variance. These factors summarise the information of a group of variables closely correlated to each other. Factor 1 grouped together oak lactones and eugenol, so it was associated with oak barrel type and with toasting of barrels. Factor 2 was associated with ethylphenols, related to wine quality diminution. Factor 3 grouped together guaiacol, furfural, and 5-methylfurfural, and was thus associated with wood thermal degradation; factor 4 was associated with the *cis*/*trans* ratio, related to oak origin. Whereas geographical origin of wines affected the accumulation of volatile compounds, the *cis*/*trans* ratio was only affected by oak barrel type. Among the tested parameters, alcoholic degree was the enological parameter that had the greatest effect on the accumulation of volatile compounds in wines. Oak lactones were the compounds that were present in all wines, so they could be used as oak ageing indicators.



## 1. Introduction

Oak wood compounds extracted by the wines during barrel ageing are of great importance since they modify wine aroma remarkably (Díaz-Plaza, Reyero, Pardo, & Salinas, 2002a; Garde-Cerdán & Ancín-Azpilicueta, 2006a; Pérez-Prieto, López-Roca, Martínez-Cutillas, Pardo-Mínguez, & Gómez-Plaza, 2003). Until the present, studies on ageing of wines have focused fundamentally on the oak wood used to make the barrels. It is known that the quantity of compounds potentially extractable from oak barrels by wine depends mainly on the geographical origin and on the species of oak (Miller, Howell, Michaelis, & Dickmann, 1992; Mosedale, Puech, & Feuillat, 1999), on the seasoning of the staves (Cadahía, Muñoz, Fernández de Simón, & García-Vallejo, 2001; Sefton, Francis, Pocock, & Williams, 1993), on the toasting of the barrel (Chatonnet, Boidron, & Pons, 1989; Hale, McCafferty, Larmie, Newton, & Swan, 1999) and on the age of the barrel (Chatonnet, 1991; Garde-Cerdán, Rodríguez-Mozaz, & Ancín-Azpilicueta, 2002a; Pérez-Prieto, López-Roca, Martínez-Cutillas, Pardo-Mínguez, & Gómez-Plaza, 2002). Once extracted, the compounds from oak wood can undergo chemical or biochemical transformations in the wine (Boidron, Chatonnet, & Pons, 1988) and, as a consequence they can modify their concentration (Garde-Cerdán, Torrea-Goñi, & Ancín-Azpilicueta, 2002b; Spillman, Iland, & Sefton, 1998). However, only two studies have been found which dealt with the effect of wine composition on the accumulation of volatile compounds originating from oak wood (Garde-Cerdán, Torrea-Goñi, & Ancín-Azpilicueta, 2004; Ortega-Heras, González-Sanjosé, & González-Huerta, 2007). In both of these studies the number of samples used was insufficient to determine this effect, and they concluded that more studies were necessary (Garde-Cerdán & Ancín-Azpilicueta, 2006b; Ortega-Heras et al., 2007). In addition, we found no papers dealing with the influence of geographical origin of wines on the accumulation of volatile compounds coming from wood.

For these reasons, the purpose of this paper was to study the correlation between geographic origin, some general components (alcoholic degree, pH, and total acidity) and the presence of volatiles coming from wood, and ethylphenols, for red wines aged in oak barrels. For this purpose, samples of red Spanish wines aged in wood and coming from different geographic areas were analysed to focus on the reality of the market and the consumer. In order to better exploit the large sample number, we also tried to relate the volatile composition of wine with the type of wood used in ageing. As data numbers were very high, we were able to apply an efficient multivariate statistical treatment, a technique that can be very helpful in

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creating an overall view of the problem in which multiple variables are analysed. In this way, it was possible to have a clearer view of the problem analysed while allowing us to determine the relationships between the variables and the potential factors that influence them.

## 2. Material and methods

### 2.1. Samples

One of the most tedious tasks of this study was the selection of samples, as the authors wanted to have the widest and realistic wine sample distribution from the consumer point of view. The starting point was the information afforded by the Spanish Ministry of Agriculture, Fisheries and Food (MAPA, 2004) about domestic trade and wine production volume, which affirmed that 95% of the Spanish Origin Designations (OD) produced more than 30,000 hl. Wines selection was carried out in local supermarkets of eight Spanish cities according to the obtained data, where four OD (Rioja, Mancha, Ribera del Duero and Valdepeñas) accounted for 72% of all commercialised red wines. The other Spanish OD selected in this study had been chosen by the information given by the MAPA but was also justified as an attempt to extend the study in relation to the different geographical wine production areas. Due to the great number of samples and the exhaustive consumption study of red wines obtained through AcNielsen consulting, all OD have been grouped according to the following areas (AcNielsen, 2004) which are defined as follows: zone 1 included the following origin appellations: Ribera del Duero, Navarra and Rioja; zone 2: La Mancha, Valdepeñas, Toro and Vinos de Madrid; zone 3: Priorato, Terra Alta, Costers del Segre, Cariñena, Penedés and Somontano, and zone 4: Jumilla, Valencia and Utiel-Requena.

For this study, 267 *crianza* wines (aged in oak barrels for 6 months at least) were analysed (Table 1). The total number of wine brands was 89 (three bottles of each brand that belonged to different lots). General wine information (e.g. oak barrel type, grape variety, etc) was obtained from the labels on the bottles. The greatest number of wines (234: 87.6%) were aged in American oak barrels, 30 were aged in American and French oak barrels (11.3%), and three were aged in French oak barrels (1.1%). The barrels capacity was 225 l. Wines were elaborated with a single variety of grape (41%), two varieties (31%), or three varieties (28%). The most commonly used varieties were *Tempranillo* and *Cabernet Sauvignon*, being used, respectively, in 94% and 39% of the wines.

### 2.2. Enological parameters

The pH and the total acidity of the wines were determined with the method described by the Office International de la Vigne et du Vin (1990). The alcoholic degree of the wines was determined according to the official method established by the ECC (1990).

### 2.3. Analysis of volatile oak compounds and ethylphenols by gas chromatography

The volatile compounds furfural, 5-methylfurfural, *cis*-oak lactone, *trans*-oak lactone, guaiacol, eugenol, 4-ethylphenol, and 4-ethylguaiacol (Sigma-Aldrich, Madrid, Spain) were analysed following the method described by Marín, Zalacain, De Miguel, Alonso, and Salinas (2005). Compounds were extracted by introducing the polymethylsiloxane coated stir bar (0.5 mm film thickness, 10 mm length, Twister, Gerstel, Mülheim and der Ruhr, Germany) into 10 ml of sample, to which 100 μl of internal standard γ-hexalactone solution at 1 μl/ml in absolute ethanol (Merck, Damstard, Germany) was added. Samples were stirred at 700 rpm at room temperature for 60 min. The stir bar was then removed from the sample, rinsed with distilled water and dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC–MS analysis.

In the thermal desorption tube, the volatile compounds were desorbed from the stir bar at the following conditions: oven temperature at 330 °C; desorption time, 4 min; cold trap temperature, −30 °C; helium inlet flow 45 ml/min. The compounds were transferred into the Hewlett-Packard LC 3D mass detector (Palo Alto, USA) with a fused silica capillary column (BP21 stationary phase 50 m length, 0.22 mm i.d., and 0.25 μm film thickness; SGE, Ringwood, Australia). The chromatographic program was set at 50 °C (held for 5 min), raised to 180 °C at 2.5 °C/min (held for 2 min) and to 230 °C (5 °C/min) and held for 20 min. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used. The mass range varied from 35 to 500 u and the detector temperature was 150 °C. Identification was carried out using the NIST library and by comparison with the mass spectrum and retention index of chromatographic standards designed by us and data found in the bibliography. Quantification was based on five-point calibration curves of respective standards ($R^2 > 0.94$) in a 12% ethanol (v/v) solution at pH 3.6.

### 2.4. Statistical analysis

A factor analysis was applied using statistical software SPSS 14.0. The number of variables was nine (furfural, 5-methylfurfural, *cis*-oak lactone, *trans*-oak lactone, ratio *cis*/*trans*, guaiacol, eugenol, 4-ethylphenol, and 4-ethylguaiacol). All variables were mean centred and scaled to unit variance prior to the analysis. The factor analysis has been used to concentrate the information in a reduced number of new variables (named factors) that represent the original variables and collect the major part of total variability (Hair, Anderson, Tatham, & Black, 1995). The principal components method has been used as a factor extraction method and posterior to that a varimax rotation was carried out to obtain a better interpretation of the factors.

Data for the volatile compounds were processed by analysis of variance with alcoholic degree, total acidity, geographic area, and oak barrel type as independent variables. Volatile compounds and the score factors obtained in the factor analysis have been used as dependent variables. It was previously verified that all the assumptions, which the statistical method needed, were fulfilled. The method used was the LSD (least significative difference) test for multiple comparisons ($\alpha < 0.05$).

**Table 1**
Number of bottles of "*crianza*" red wines analysed from the different areas

| | Number of bottles analysed |
|---|---|
| Zone 1 | 150 |
| Zone 2 | 60 |
| Zone 3 | 30 |
| Zone 4 | 27 |
| Total | 267 (89 brands) |

We analysed three bottles from different lots of each of the 89 wines studied.

## 3. Results and discussion

### 3.1. Characteristics of wines

The average pH of the red wines analysed was 3.5, the maximum being 3.9 and 2.98 the minimum. The average value was within the pH range (3.1–3.6) recommended by Amerine and Ough (1976) for a product safe against bacterial contamination and for a wine with good sensory characteristics. The total acidity presented an average of 5.40 g tartaric acid/l, with a maximum of 7.44 g tartaric acid/l and a minimum of 4.02 g tartaric acid/l. The average

value of the alcoholic degree of the wines was of 12.80% (v/v), with a maximum of 13.50% (v/v) and a minimum of 12.00% (v/v).

### 3.2. Content of volatile oak compounds and ethylphenols in the wines

Furfural was present in all the samples from zones 1, 3, and 4 (Table 2), and the maximum value of this compound in the wines studied from zone 1 was above its perception threshold (20 mg/l; Boidron et al., 1988). Nevertheless, 5-methylfurfural was not over its perception threshold (45 mg/l; Boidron et al., 1988) in any of the samples studied, with a minimum value of 0 in all the zones (Table 2). These two compounds, which possess a grilled almond aroma, enhance the perception of oak lactones (Reazin, 1981). In the case of guaiacol, its total average value was close to its perception threshold (0.075 mg/l; Boidron et al., 1988), and this compound was not present in some samples (Table 2). Eugenol was below its perception threshold (0.5 mg/l; Boidron et al., 1988) in the samples studied, with this compound being present in all wines from zones 1, 3, and 4 (Table 2). These two volatile phenols, guaiacol and eugenol, add smoky and spicy aromas to the wine.

The only compounds coming from oak wood that were present in all the wines studied were the two isomers, *cis* and *trans*, of β-methyl-γ-octalactone (Table 2). Therefore, these compounds could be used as indicators to show that a wine has been aged in presence of oak wood. *cis*-Oak lactone was above its perception threshold (0.046 mg/l; Wilkinson, Elsey, Prager, Tanaka, & Sefton, 2004) in all the wines (Table 2); in the case of the *trans* isomer, its average value for all the samples (0.19 mg/l) was below its perception threshold (0.46 mg/l; Chatonnet, Boidron, & Pons, 1990). These two lactones produce woody and coconut-like aromas in the wine, which add quality. The average value of 4-ethylphenol (4-EP) and 4-ethylguaicol (4-EG) in wines from the different zones was, in all cases, over the values from which these compounds can contribute negatively to wine quality (0.620 mg/l and 0.140 mg/l, respectively; Chatonnet, Dubourdieu, Boidron, & Pons, 1992), since these are responsible for the medicinal and horsey off-odours found in some red wines.

The total average of the ratio *cis*/*trans* (7.43) confirmed that most of the "*crianza*" wines studied were aged in American oak barrels, since American oak species give a greater quantity of *cis*-oak lactone to the wine as compared to European oaks. Several studies confirm this (Díaz-Plaza, Reyero, Pardo, Alonso, & Salinas, 2002b; Garde-Cerdán et al., 2002a; Gómez-Plaza, Pérez-Prieto, Fernández-Fernández, & López-Roca, 2004; Marco, Artajona, Larrechi, & Rius, 1994; Pérez-Prieto et al., 2002; Towey & Waterhouse, 1996). The total average of the ratio 4-EP/4-EG was 2.19, a value below the 8.0 value found by Pollnitz, Pardon, and Sefton (2000). These authors observed that this ratio is a function of the grape variety, observing values from 3.5 for the *Pinot Noir* variety to 10.1 for the *Cabernet Sauvignon* variety. In our samples, the dominant variety was *Tempranillo*, which is why the ratio found in our samples can correspond to this variety. Within the wines, a wide range of concentrations of 4-ethylphenol and 4-ethylguaiacol was observed, consistent with the results of Pollnitz et al. (2000) and Chatonnet et al. (1992), who have demonstrated the importance of winemaking practices in the formation of these compounds.

### 3.3. Multivariate statistical analysis

Factor analysis was applied to reduce the number of variables. This gave four significant factors with eigenvalues greater than unity, accounting for 84.12% of the total variance (Table 3). The

**Table 2**
Maximum, minimum and average values (expressed in mg/l) of the volatile compounds from the different geographic areas

| | | Zone 1 | Zone 2 | Zone 3 | Zone 4 | Total zones |
|---|---|---|---|---|---|---|
| | Maximum | 22.72 | 14.97 | 11.61 | 13.10 | 22.72 |
| Furfural | Minimum | 0.86 | 0.00 | 0.40 | 0.85 | 0.00 |
| | Average | 5.17 | 3.75 | 3.14 | 4.56 | 4.56 |
| | Maximum | 1.06 | 0.78 | 0.59 | 0.77 | 1.06 |
| 5-Methylfurfural | Minimum | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Average | 0.22 | 0.17 | 0.09 | 0.22 | 0.19 |
| | Maximum | 0.37 | 0.59 | 0.23 | 0.31 | 0.59 |
| Guaiacol | Minimum | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Average | 0.07 | 0.11 | 0.03 | 0.08 | 0.07 |
| | Maximum | 0.33 | 0.33 | 0.24 | 0.15 | 0.33 |
| Eugenol | Minimum | 0.02 | 0.00 | 0.03 | 0.02 | 0.00 |
| | Average | 0.11 | 0.09 | 0.08 | 0.06 | 0.10 |
| | Maximum | 5.03 | 2.79 | 3.74 | 2.47 | 5.03 |
| *cis*-Oak lactone | Minimum | 0.21 | 0.26 | 0.19 | 0.17 | 0.17 |
| | Average | 1.48 | 1.07 | 1.15 | 0.82 | 1.29 |
| | Maximum | 0.58 | 0.61 | 0.56 | 0.31 | 0.61 |
| *trans*-Oak lactone | Minimum | 0.05 | 0.05 | 0.04 | 0.03 | 0.03 |
| | Average | 0.20 | 0.16 | 0.18 | 0.15 | 0.19 |
| | Maximum | 5.78 | 5.58 | 3.50 | 4.64 | 5.78 |
| 4-Ethylphenol | Minimum | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Average | 1.76 | 1.05 | 0.91 | 1.23 | 1.45 |
| | Maximum | 3.23 | 2.27 | 1.50 | 2.37 | 3.23 |
| 4-Ethylguaiacol | Minimum | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Average | 0.73 | 0.41 | 0.33 | 0.62 | 0.60 |
| | Maximum | 14.81 | 13.14 | 14.04 | 19.48 | 19.48 |
| Ratio *cis*/*trans*[a] | Minimum | 1.85 | 1.84 | 2.64 | 1.63 | 1.63 |
| | Average | 7.39 | 7.87 | 6.99 | 7.15 | 7.43 |
| | Maximum | 3.84 | 5.33 | 4.23 | 3.33 | 5.33 |
| Ratio 4-EP/4-EG[b] | Minimum | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| | Average | 2.28 | 2.12 | 2.07 | 1.94 | 2.19 |

[a] Ratio *cis*/*trans*, concentration of *cis*-oak lactone/concentration of *trans*-oak lactone.
[b] Ratio 4-EP/4-EG, concentration of 4-ethylphenol/concentration of 4-ethylguaiacol.

**Table 3**
Factor analysis

| Factor number | Eigenvalue | Percent of variance | Cumulative percent |
|---|---|---|---|
| 1 | 3.36 | 37.34 | 37.34 |
| 2 | 1.76 | 19.57 | 56.91 |
| 3 | 1.27 | 14.06 | 70.97 |
| 4 | 1.18 | 13.16 | 84.12 |

loadings for each variable on the four selected factors are displayed in Table 4. As the data are autoscaled, each loading is the correlation between the variable and the respective factor.

Factor 1 explained 37.34% of total variance (Table 3). This factor was associated with *cis*-oak lactone, *trans*-oak lactone, and eugenol (Table 4). These compounds come from oak wood. *cis*-Oak lactone and *trans*-oak lactone are produced by the dehydration of acids present in oak wood (Otsuka, Zenibayashi, Itoh, & Totsuka, 1974). Chatonnet et al. (1989) and Maga (1989) showed that the concentrations of oak lactones increased with the toasting of the wood; with these products being among the most important volatile substances coming from wood. Eugenol forms from the thermal degradation of lignin at high temperature (Sefton et al., 1993).

Factor 2 grouped together the ethylphenols (Table 4) and explained 19.57% of total variance (Table 3). Ethylphenols, 4-ethylphenol and 4-ethylguaiacol, are produced by the contaminant yeasts *Brettanomyces*/*Dekkera*, from grape-derived phenolic acids, and are considered detrimental to wine quality (Chatonnet et al., 1992).

Factor 3 was associated with guaiacol, furfural, and 5-methylfurfural (Table 4), compounds coming from oak wood, and explained 14.06% of total variance (Table 3). Guaiacol forms from the thermal degradation of lignin at high temperature, while furanic compounds (furfural and 5-methylfurfural) are formed through thermal degradation of carbohydrates (Boidron et al., 1988).

Finally, factor 4 was associated with the *cis*/*trans* ratio (Table 4), and explained 13.16% of total variance (Table 3). This ratio can be used to distinguish those wines aged in American oak barrels and those wines aged in French oak barrels (Waterhouse & Towey, 1994).

Each one of the factors obtained summarises the information from a group of variables, which are all correlated to each other. The fact that these volatile compounds show correlated values leads us to believe that external and internal characteristics exist (e.g. geographic area, type of wood, alcoholic degree or total acidity), which may influence these wines. The information found in this study will allow us to research the possible causes of the variation of the four significant factors, previously obtained from the factor analysis applied, as a function of the geographic area, type of wood, alcoholic degree, and total acidity of the different wines. The value that a sample shows in each of the four factors is called the *score factor*.

**Table 4**
Factor loading matrix after varimax rotation

| | Factor 1 | Factor 2 | Factor 3 | Factor 4 |
|---|---|---|---|---|
| Eugenol | 0.92 | 0.10 | 0.09 | 0.14 |
| *cis*-Oak lactone | 0.91 | 0.24 | 0.13 | 0.18 |
| *trans*-Oak lactone | 0.84 | 0.20 | 0.10 | −0.39 |
| 4-Ethylphenol | 0.16 | 0.97 | −0.04 | −0.00 |
| 4-Ethylguaiacol | 0.23 | 0.95 | 0.05 | 0.03 |
| 5-Methylfurfural | 0.04 | 0.16 | 0.86 | −0.05 |
| Furfural | 0.29 | 0.02 | 0.75 | −0.05 |
| Guaiacol | −0.02 | −0.14 | 0.70 | 0.10 |
| Ratio *cis*/*trans* | 0.06 | 0.03 | 0.02 | 0.98 |

### *3.4. Effect of the enological parameters on the accumulation of volatile oak compounds and ethylphenols in the wines*

Of the enological parameters of the wines (pH, alcoholic degree, and total acidity), only the alcoholic degree presented correlation with the four score factors (Fig. 1). Hence, among the considered parameters, it could be said that the alcoholic degree of the wine was the parameter that had a greater effect on the accumulation of volatile compounds in wines aged in oak barrels. The accumulation of volatile compounds coming from oak wood (factors 1 and 3) was more favoured in the wines with lower alcoholic degrees (12% and 12.5%) than in the wines with greater alcoholic degree (13% and 13.5%; Fig. 1a and c). The extraction of volatile compounds from oak wood can depend, among other factors, on the alcoholic degree of the wine (Garde-Cerdán et al., 2004), although these compounds, once extracted, can evolve in the wine (Garde-Cerdán

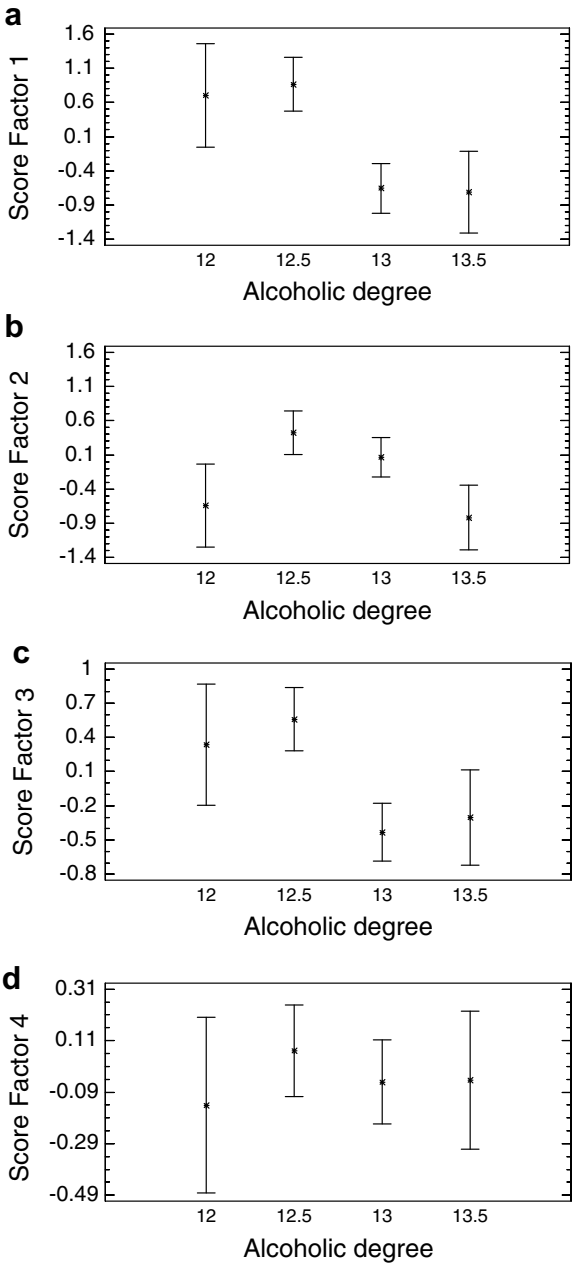


**Fig. 1.** Relationship between each of the four score factors and alcoholic degree (%, v/v) of wines: means and 95.0 percent LSD intervals.

et al., 2002b; Spillman et al., 1998). Thus, their accumulation could have been favoured in wines with lower alcoholic degrees (Fig. 1a and c). In the case of ethylphenols (factor 2), their accumulation in the wines diminished as the alcoholic degree of wines increased from 12.5% to 13.5% (Fig. 1b). This is in agreement with the data found by Dias, Pereira-da-Silva, Tavares, Malfeito-Ferreira, and Loureiro (2003), who observed that 4-ethylphenol production was inhibited by increasing concentrations of ethanol. The *cis*/*trans* ratio (factor 4) in the wines was independent of the alcoholic degree (Fig. 1d), which confirmed that this factor was affected mainly by the oak type used for ageing.

As regards pH, no correlation with the four score factors was observed, and total acidity only showing correlation with factor 2. In this sense, the concentration of ethylphenols (factor 2) was higher in the wines with a total acidity of 4.68–4.93 g tartaric acid/l (Fig. 2). The values on the *X*-axis for the three groups defined for total acidity are the ones that generate the maximum differentiation among the groups.

### 3.5. Effect of the geographical origin of wines on the accumulation of volatile oak compounds and ethylphenols in wines

The accumulation of *cis*-oak lactone, *trans*-oak lactone, and eugenol (factor 1) in the zone 1 wines was superior to the presence of these compounds in the other zones, where significant differences among the wines were not present (Fig. 3a). The same happens to the presence of 4-ethylphenol and 4-ethylguaiacol (factor 2) in the wines studied (Fig. 3b). The accumulation of guaiacol, furfural, and 5-methylfurfural (factor 3) was lower in the zone 3 wines than in those of zone 1 (Fig. 3c). Therefore, zone 1 was different from the other three zones, all three of which presented a more similar behaviour to each other. Besides other factors, this could be because zone 1 presents climatic differences with respect to the other zones (http://www.winesfromspain.com; MAPA, 2004). In the case of the *cis*/*trans* ratio, significant differences between wines of the four geographic areas were not observed (Fig. 3d), thus indicating that this parameter depends fundamentally on the oak barrel type used in ageing.

### 3.6. Effect of the oak barrel type on the accumulation of volatile oak compounds and ethylphenols in wines

The immense majority of the barrels used for the ageing of wines were made from American oak (87.6%). This probably indicates that, for the ageing of this type of wines (*crianza*), the favourite oak is the American oak, probably due to economic reasons, since the barrels of French oak are more expensive than those of American oak. For that reason, the number of wines aged in French oak barrels was very low in comparison with those aged in American oak and in a combination of both (American and French).

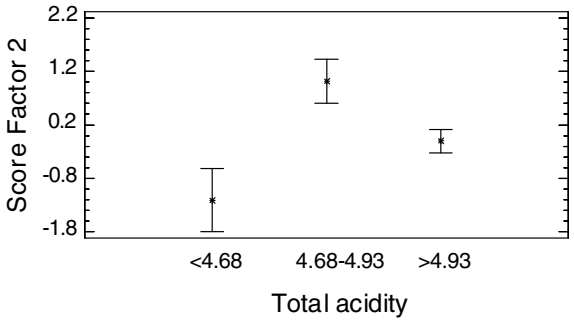


**Fig. 2.** Relationship between the score factor 2 and the total acidity (g tartaric acid/l) of wines: means and 95.0 percent LSD intervals.

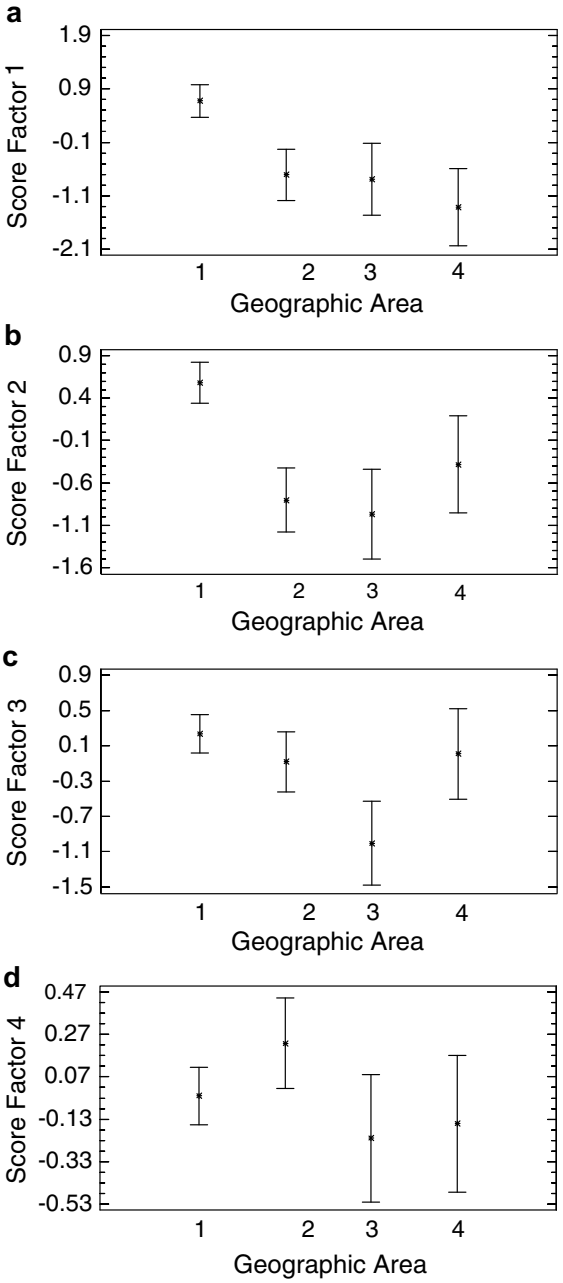


**Fig. 3.** Relationship between each of the four score factors and the geographical origin of wines: means and 95.0 percent LSD intervals.

The factor 1 (*cis*-oak lactone, *trans*-oak lactone, and eugenol), the factor 2 (4-ethylphenol, and 4-ethylguaiacol), and the factor 3 (guaiacol, furfural, and 5-methylfurfural) did not present significant differences based on the oak barrel type used for the ageing of the wines (Fig. 4a–c). Nevertheless, the *cis*/*trans* ratio (factor 4) presented significant differences between wines aged in American oak and combination of American and French oaks and the ones aged in French oak (Fig. 4d), being lower in this last one, as other authors have found (Díaz-Plaza et al., 2002b; Garde-Cerdán et al., 2002a; Pérez-Prieto et al., 2002; Towey & Waterhouse, 1996). The wines aged in a combination of American and French oaks presented behaviour similar to the aged ones in American oak; thus indicating that the contribution of the American oak was more important than the contribution of the French oak.

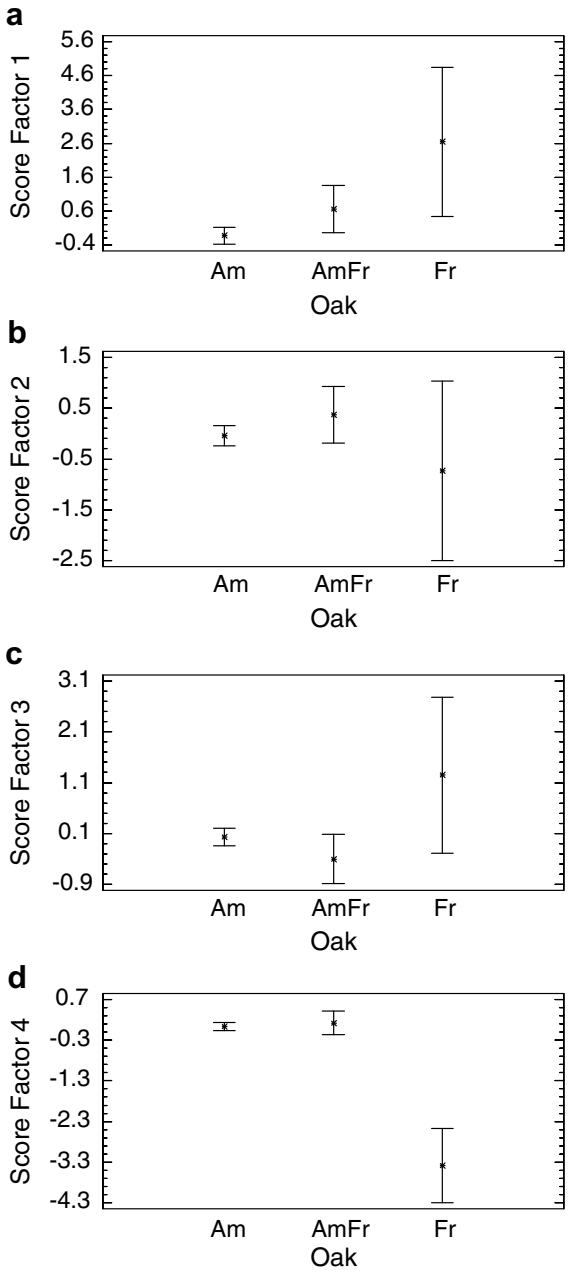


**Fig. 4.** Relationship between each of the four score factors and the oak barrel type: means and 95.0 percent LSD intervals.

## 4. Conclusions

Statistical analysis gave four significant factors, accounting for 84.12% of the total variance. Each one of these factors summarises the information of a group of variables closely correlated to each other. Factor 1 grouped together *cis*-oak lactone, *trans*-oak lactone, and eugenol, therefore it was the factor associated with the wood barrel type and with the toasting of the barrel. Factor 2 was associated with ethylphenols (4-ethylphenol and 4-ethylguaiacol), and thus related to the diminution of wine quality. Factor 3 grouped together guaiacol, furfural, and 5-methylfurfural, so it was associated with the thermal degradation of oak wood, and factor 4 was associated with the ratio *cis*/*trans* related to the origin of oak. The geographical origin of the wines affected the accumulation of volatile compounds (factors 1–3) but did not affect the *cis*/*trans* ratio (factor 4), while the oak barrel type only affected the *cis*/*trans* ratio in the wines. *cis*- and *trans*-Oak lactones were the only compounds that were present in all the "*crianza*" wines, so these compounds could be used as indicators that a wine has been aged in presence of oak wood. Among the tested parameters, alcoholic degree was the enological parameter that had the greatest effect on the accumulation of volatile compounds in the wines aged in oak barrels.

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