Criteria to discriminate between wines aged in oak barrels and macerated with oak fragments

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ABSTRACT

Wine ageing in barrels is carried out to increase stability and achieve more complex aromas. In the last few years, however, the practice of macerating wine with small fragments of toasted oak (chips) has become increasingly common. This conveys similar tastes, aromas, and wooden notes to the wine as those obtained with traditional barrel ageing, but much faster and at a fraction of the cost. Without proper regulation, this could lead to fraud if wine macerated with chips is offered as barrel aged wine.

In the present study, 75 volatile compounds have been determined by applying gas chromatography–mass spectrometry (MS) and flame ionization detection (FID). It has been found that compounds directly related to the wood have greater discriminative power for telling apart wines aged in barrels from those macerated with oak fragments, but no single compound permits flawless classification. Therefore, we have studied the effect of the addition of oak fragments of different origins, different oak types, different formats and subjected to different toasting processes on a set of 231 samples from 6

Spanish Denominations of Origin wines (DOs), and compared them to those same wines aged in oak barrels. In light of the results, we have developed a set of criteria which allows to distinguish with high degree of accuracy between wines which have been aged in barrels and those macerated with oak fragments. The application of these criteria to different wines allows correct classification in over 90% of cases.

Keywords

Discriminate wines, barrel oak, oak chips, aroma compounds

1. Introduction

Wine ageing is a technique commonly used in wineries to increase the stability of wines, spontaneously clarify them, and achieve more complex aromas. Normally, oak barrels are used. The composition of the wine in direct contact with the barrel is modified as the wine extracts compounds from the wood such as tannins, phenolic acids and volatile compounds. Moreover, the coloring elements in wine stabilize due to the micro-oxygenation produced when air flows through the barrel staves, increasing the quality of the wine. However, this method is expensive and requires long periods of time. In the last few years, the practice of macerating wine with small fragments of toasted oak has become increasingly common, as it conveys similar tastes and aromas to the wine as those obtained with traditional barrel ageing, but much faster and at a fraction of the cost (wine macerated with oak fragments can be up to 10 times cheaper than the same wine aged in barrel). The increased surface area of the fragments accelerates the extraction of the compounds.

The use of oak fragments for macerating wines is already an alternative to oak barrel ageing. New wine-producing countries such as Chile, Argentina, South Africa, Australia or the United States have been using these techniques for several years. A great variety of systems are used to elaborate wines this way, all based on adding pieces of oak of different sizes, wood types and degree of toasting to the wine. Some of them are introduced directly in the tank, and some of them to reuse old barrels.

Oak fragments can be found in a variety of forms (del Alamo Sanza, 2006). These include shavings, known as *oak fragments*; cut into dices, named *cubes* or *oak beans*; *oak powder*; pieces of granulated wood called *pencil shavings* or *granulates*; *dominoes*; or square pieces referred to as *blocks* or *segments*. Additionally, bigger pieces designed

to be placed in the tank can also be found on the market, usually in the form of staves, hence being called *tank staves*, *winewood* or *infusion staves*. Old barrels can also be used by adding wooden pieces such as *oak chains*, *sticks*, or *barrel inserts*.

All the above-mentioned products are made from different kinds of oak wood (American, French, Hungarian, Pyrenean) and are subjected to a variety of toasting methods (fire, hot air, infrared radiation) and degrees of toasting (in addition to the well known light, medium and strong levels, toasting is also offered as simple or double, or performed at specific temperatures).

The effects produced by the addition of wooden pieces into wine depend on several factors, which define the characteristics of the wine. These include the origin of the wood (Chatonnet & Dubourdieu, 1998; Fernandez de Simon, Cadahia, & Jalocha, 2003; Frangipane, De Santis, & Ceccarelli, 2007), the type of drying, (Masson, Baumes, Moutounet, & Puech, 2000; Vivas & Glories, 1996) the toasting process (Fernandez de Simon, Cadahia, del Alamo, & Nevares, 2010; Fernandez de Simon et al., 2003; Franco, Castells, Martínez, & Pérez, 2007), the amount of fragments added to the wine (Fan, Xu, & Yu, 2006), the contact time between wine and oak (Bautista-Ortin et al., 2008), the size of the wooden pieces, and the age of the barrel (Arapitsas, Antonopoulos, Stefanou, & Dourtoglou, 2004; Mosedale, Puech, & Feuillat, 1999; Singleton, 1995).

The aromas that the wood conveys to the wine come from the degradation of compounds from the wood during its toasting process, or from the wood itself. Eugenol and oak lactones add spicy character and oak flavor. When the lignin degrades during the toasting process, volatile phenols such as guaiacol and aromatic aldehydes such as vanillin and syringaldehyde are generated (Chatonnet, Cutzach, Pons, & Dubourdieu, 1999; Diaz-Maroto, Sanchez-Palomo, & Perez-Coello, 2004). Also, the degradation of hemicelluloses produces furanic compounds such as furfural and 5-methyl furfural (Garde-Cerdan & Ancin-Azpilicueta, 2006; Perez-Coello, Gonzalez-Vinas, Garcia-Romero, Cabezudo, & Sanz, 2000) which are reminiscent of toasted almond and nuts. These compounds appear preferentially at a specific temperature so if the toasting is precise and homogeneous, clearly definable aromatic characteristics can be achieved. If wooden pieces toasted at different temperatures are mixed, the compounds conveyed by the wood will be more diverse.

In Europe the use of oak fragments to macerate wines is an alternative to oak barreling. This enological practice is approved by EU regulations (CE) Nº 2165/2005 and (CE) Nº 1507/2006 which define the terms of use of oak fragments in wine.

Oak fragments are able to give wine a wooden touch without the need to use barrels. Without proper regulation, this could lead to fraud if such wine is offered as barrel aged wine. European regulations on wine protect specific labelings (crianza, reserva) for wines which have obtained exclusively through aging in barrels. OIV resolutions in this matter explicitly forbid wines with particular indications (crianza and reserva among others) to be treated with wood fragments. Therefore, analytical tools must be found in order to distinguish between these two types of treatments and so avoid possible frauds.

The main objective of this study is to find markers that allow us to discriminate between wines aged in barrels and wines fermented or macerated with oak fragments. The aim is to tell the difference between wines that have been made following two quite different enological practices described in the enological CODEX published by the International Organization of Vine and Wine (2006 edition) as “Ageing in small capacity wooden containers (OENO 8/01)” and as “usage of pieces of oak wood in winemaking (OENO 9/01)”.

2. Materials and methods

2.1 Reagents and standards.

The aroma standards were supplied by Aldrich (Gillingham, UK), Fluka (Buchs, Switzerland), Sigma (St. Louis, MO, USA), Lancaster (Strasbourg, France), PolyScience (Niles, USA), Chemservice (West Chester, USA), Interchim (Monluçon, France), International Express Service (Allauch, France) and Firmenich (Geneva, Switzerland). LiChrolut EN resins (styrene-divinylbenzene) and polypropylene cartridges were obtained from Merck (Darmstadt, Germany). Dichloromethane and methanol of LiChrosolv quality were purchased from Merck (Darmstadt, Germany); absolute ethanol, and ammonium sulfate were obtained from Panreac (Barcelona, Spain), all of them of ARG quality. Pure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Semi automated Solid Phase Extraction (SPE) was carried out with a VAC ELUT 20 station supplied by Varian (Walnut Creek, CA, USA).

2.2 Samples.

The grapes and wines used in the assay were of the vintages 2008 and 2009, vinified in 6 experimental centers in 6 different regions of Spain.

The first assay was made at the Centro de Transferencia Agroalimentaria de Aragón (CTA), with wines of the "Garnacha Tinta" variety. The wines of the 2008 vintage were vatted in 12 American oak barrels of 225 liters capacity. Three of them were new and the other 9 semi new from the third, fifth and seventh year of usage, respectively. With the wine from the same batch as mentioned above, 6 tanks of 250 L capacity were filled and 2 different types of oak fragments were added in 6 g/L doses. The wines and fragments were in contact during 60 days, after which the oak fragments were removed by racking and 75 liters were bottled. With the rest of the macerated wine, 6 7-year old American oak barrels were filled. Six and twelve months after vatting the wine in the barrels, corresponding 50 L samples were taken and bottled. In the second year of experiment, with the wine of 2009 vintage, 15 American oak barrels of 225 L capacity were filled, 3 new and 12 semi new from the assay of the previous year, which now were 2, 4, 6 and 8 years old, respectively. In the same way as the first year but with 2009 vintage wine, three 250 L tanks were filled and other American oak fragments were added. In addition, Pyrenean fragments were added to three other 250 L tanks, in both cases in doses of 6 g/L. The assay was repeated in the same way as for the first year, except for the wines macerated with oak fragments that were vatted in 8-year old barrels.

The second assay was made at the Instituto Tecnologico Agrario, Estación enologica de Castilla y León (ITACYL), with wines of the Tinta del Pais (Tempranillo) variety. In the two years of the assay, wines of the 2008 and 2009 vintages were vatted in nine 225 L French oak barrels, 3 new and 6 semi new (3 3-year old and 3 5-year old barrels). Six 250 L tanks were filled with the same wine, to which two different types of French oak fragments were added in 6 g/L doses. The working protocol was the same as that used in the CTA on the first assay.

The third assay was made at the Centro de Investigacion y Desarrollo Agrario de la Rioja (CIDA), with wine of the Tempranillo variety. In the first year, 2 new French oak barrels and 3 American oak barrels, all of 225 L capacity, were filled with wine of the 2008 vintage. Twelve 250 L tanks were filled with wine from the same vinification

batch, to which 2 different types of French oak fragments and 2 different types of American oak were added, all in 6 g/L doses.

During the second year, 3 new French oak barrels and 3 American oak barrels were filled with wine of the 2009 vintage. In addition, twelve 250 L tanks were filled with wine from the same batch to which French, American and Pyrenean oak fragments were added in 6 g/L doses. In 2009, six 250 L tanks containing French and American oak hogshead staves in 0.33 m^3/hl doses were also filled with wine. The wine was macerated with the hogshead staves during 12 months. At six and twelve months samples were taken and bottled.

The fourth assay was made at the Instituto Madrileño de Investigacion y desarrollo Rural Agrario. During the first year, grapes of the Tempranillo variety, 2008 vintage, were fermented in nine 50 L tanks with American oak fragments added in 3, 6 and 9 g/Kg doses. Once the fermentation concluded, 50 L of each treatment were bottled.

Similarly, grapes were fermented without fragments and the wine obtained was placed in three new 225 L American oak barrels and nine 250 L tanks to which American oak fragments were added in 2, 6 and 9 g/L doses. During the second year, the 2008 assay was repeated using wine of the 2009 vintage. The fragments and barrels used in 2009 were made of French oak.

The fifth assay was made at the Estacion Enologica de Navarra (EVENA), with grapes and wines of the Cabernet Sauvignon variety. During the first year, grapes of the 2008 vintage were fermented in nine 250 L tanks with 2 types of American oak fragments and one type of French oak fragment, all in 6 g/Kg doses. Once the fermentation was concluded, 50 L of each treatment were bottled. In addition, grapes of the 2008 vintage were fermented in six 500 L wine tanks without wood fragments. The wines obtained were vatted in 3 new French oak barrels and 3 new American oak barrels, all of 225 L capacity. During the second year, the procedure was repeated with grapes of the 2009 vintage, using different barrels and wood fragments.

The sixth assay took place at the Estacion Enologica de Galicia (EVEGA), with wine of the Mencia variety. During the first year, six new 225 L American oak barrels and three new 225 L new French oak barrels were filled with wine of the 2008 vintage. In addition, twelve 250 L tanks were filled with the same wine, to which American and French fragments and a mixture of 50% of each were added in 6 g/L doses. During the

second year, the same procedure was repeated with wine of the 2009 vintage, using different barrels and fragments.

In all the centers, 3 barrels were prepared for every assay. Two were used for the samples and a subsequent analysis of the wines and the third was used to fill the two first. Also, in all the centers the wines were in contact with the wood fragments during 60 days. Afterwards, the oak fragments were removed by racking and 75 L of wine were bottled. Additionally, in the wines vatted in barrels, 50 L samples were taken at the sixth and twelfth month in order to make the chemical and sensorial analysis

The barrels and fragments used in the assays were provided by wine enterprises located in Spain. In all cases the materials were those commonly used for the vinification of Spanish wines. The barrels were provided by the cooperages MAGREÑAN, QUERCUS, VICTORIA and INTONA. Most of the barrels were made by natural drying of the hogshead staves for between 18 and 36 months, medium toasted with direct fire, at temperatures between 175 and 220 ºC during 40 or 50 minutes. Only 15 barrels were toasted by the TRH system by infrared, at 200 ºC during 35 minutes. The oak fragments were provided by the companies AGROVIN, LAFFORT and SEPSA and the cooperatives MAGREÑAN, QUERCUS and VICTORIA. All were made by natural drying for between 18 and 34 months, toasted at average temperatures of 180 and 230 ºC during 60 and 180 minutes. The toasting was carried out by air convection or by infrared. Moreover, various types of fragments such as hogshead staves or segments were used.

In brief: The study was carried out during two the consecutive years 2008 and 2009. Samples were taken from each wine after 6 and 12 months. Each of the samples taken was prepared twice. 75 volatile compounds were determined in 231 wines; 92 were vatted in oak barrels, 115 were macerated or fermented with fragments and finally 24 were macerated with fragments and later vatted in oak barrels.

2.3. Chemical quantitative analysis

2.3.1. Major Compounds (Liquid-Liquid Microextraction and GC-FID Analysis)

The volatile compounds were analysed using the procedure proposed by Ortega et al. (Ortega, Lopez, Cacho, & Ferreira, 2001) with slight modifications. The 2.7 mL sample to be analysed was transferred into a 10 mL screw-capped centrifuge tube containing 4.05 g ammonium sulphate to which and the following were added: 6.3 mL water, 20

μL standard internal solution (2-butanol, 4-methyl-2-pentanol, 4-hidroxy-4-methyl-2-pentanone, heptanoic acid, ethyl heptanoate and 2-octanol at 140 μg/mL in absolute ethanol) and 0.25 mL dichloromethane. The tube was shaken mechanically for 90 min and later centrifuged at 2500 rpm for 10 min. The dichloromethane phase was recovered with a 0.5 mL syringe, transferred to the autosampler vial, and analysed. Chromatographic analysis was carried out in a GC-3800 supplied by Varian (Walnut Creek, CA, USA) equipped with a DB-Wax column (30 m x 0.32 mm x 0.5 μm) from J&W (Folsom, CA) and a 3 m x 0.32 mm uncoated precolumn (Agilent Technologies, Santa Clara, CA, USA). The column temperature, initially 40 ºC, was raised after 5 min by 4 ºC/min to 102 ºC; 2 ºC/min to 112 ºC; 3 ºC/min to 125 ºC during 5 min; 3 ºC/min to 160 ºC; 6 ºC/min to 200 ºC and 30 min isotherm. The carrier gas was helium at 3 mL/min. The injection was in split mode 1:20 (injection volume 2 μL), with an FID detector. The chromatographic peaks were normalized by one of the internal standards and the relative area was then interpolated in the calibration graphs built by analysing synthetic wines with known concentrations of volatile compounds. Thirty major (mg/L) compounds were determined in this way.

2.3.2. Minor Compounds (SPE and GC-Ion Trap-MS Analysis)

This analysis was carried out using a previously proposed and validated method (Lopez, Aznar, Cacho, & Ferreira, 2002) but with the following changes in the procedure: Standard SPE cartridges (3 mL total volume) filled with 200 mg of LiChrolut EN resins were placed in the vacuum manifold extraction system and the sorbent was conditioned by rinsing the cartridges with 4 mL of dichloromethane, 4 mL of methanol and, finally, 4 mL of a water-ethanol mixture (12%, v/v). The cartridges were then loaded with a 50 mL wine sample and 26 μl of a surrogate standard solution containing 3-octanone, β-damascone and heptanoic acid (all at 200 μg/g of ethanol). This mixture was passed through the SPE cartridges (2 mL/min), followed by a wash step using 5 mL of 40% water-methanol, 1% $NaHCO_3$ solution. The resins were then dried by letting air pass through the resin cartridges (negative pressure of 0.6 bar, 10 min). Analytes were recovered in a 2 mL vial, by elution with 1.6 mL of dichloromethane. Thirty-four microliters of an internal standard solution (300 mg/L of 4-hydroxy-4-methyl-2-pentanone and 2-octanol) were added to the eluted sample. The extract was then analyzed by GC with ion trap MS detection. A GC-450 gas chromatograph fitted to a

Saturn 2200 ion trap MS was used, supplied by Varian. Chromatographic analyses were performed under the conditions described in ref. (Lopez et al., 2002). 45 minor (µg/L) compounds were determined.

2.4. Statistical analysis

Statistical analyses were conducted with an SPSS vs 15.0 system supplied by SPSS Inc. (Chicago, IL). A four factor ANOVA analysis (treatment x vintage x oak origin x production zone) was performed on the analytical data of the wines. The interaction between the treatment factor and the other 3 factors was also evaluated. Moreover, a single factor ANOVA studies were also carried out. The results presented in this article will focus on the treatment factor (use of barrel or oak fragments). Volatile aroma composition data were analyzed by principal component analysis (PCA) using an Unscrambler 9.7 (Camo, Norway) to illustrate the differences between the treatments.

3. Results and discussion

The main aim of this study has been to find out markers that allow us to discriminate wines aged in barrels from those aged with other techniques.

For each of the years on which the study took place (2008 and 2009) and for each zone (6 institutions), 1 factor ANOVAs (wooden fragments or barrel factor) have been carried out to determine the existence or not of significant differences ($p<0.05$) between all the studied samples. The result of these ANOVA studies (data not displayed) indicate that the compounds that show significant differences ($p<0.05$) in all zones and for each of the years are mainly those related to the wood.

Several Principal Component Analysis (PCA) studies have been performed on those compounds which present significant differences over the two years of study (mainly those related to wood), to find out which ones produce the maximum variability among the different samples.

After carrying out these studies, it was found out that out of the 75 analysed compounds, both major and minor, those which best enable discrimination between the samples and explain the higher variance in function of the ageing treatment (barrel or oak fragments) are the following: E-whiskylactone, Z-whiskylactone, vanillin,

acetovanillone, syringaldehyde, furfural, furfuryl alcohol, 5-methylfurfural, 5-hidroxy-methylfurfural, eugenol, methyl vanillate and ethyl vanillate (figure 1a).

As can be observed in Figure 1b, the samples of wines macerated with fragments (C) have been classified in the negative part of component 1, while the wines fermented in barrels (B) can be found in the positive part of the PC1. Among the 115 samples of wines macerated with fragments, 25 have a positive loading.

On the other hand, of the 92 samples that were aged in barrels, 38 have negative PC1. Moreover, of the 24 wines that were first macerated with fragments and then vatted in old barrels, 5 are classified in the barrel zone. The rest of the wines have the same characteristics as those macerated with fragments.

Volatile phenols, lactones and furfural derivatives (Figure 1a) have a positive PC1 while vanillin, acetovanillone and syringaldehyde have a negative PC1. It can thus be said that wines aged in barrels have more volatile phenols, lactones and more furfural derivatives, while wines elaborated with oak fragments have superior concentrations of vanillin, acetovanillone and syringaldehyde.

The high concentrations of the different vanillin compounds found in wines aged with oak fragments can be explained observing the results published by Chatonnet (Chatonnet, 2008)This author found that when small oak fragments are toasted using convection currents, the generation of phenolic aldehydes is increased in comparison with toasting barrels over fire.

As the classification obtained is not completely satisfactory in relation to the selected compound in the PCA and with all the analyzed samples (231), a 4 factor ANOVA has been performed. Factor 1 is the treatment (vatted in oak barrels, macerated with fragments, and macerated with fragments and later vatted in old oak barrels); factor 2 is the year (vintage 2008 or 2009); factor 3 is the origin of the oak (American, French or Pyrenean); and factor 4 is the production zone (Aragón, Castilla y León, Rioja, Madrid, Navarra or Galicia).

The aim of the study is to discover which compounds that present significant differences ($p<0.05$) can be used to discriminate between all the samples in the study depending on whether or not they have been vatted in oak barrels, and the effect of the oak, the zone or the production year. The results are shown in Table 1. As can be seen in this table, the treatment factor introduces significant differences in 11 of the 12 compounds, all except for furfuryl alcohol. Table 2 shows the mean concentrations of

all the compounds with $p < 0.05$ found in the wines according to the way the wines have been elaborated. As we can see, wines elaborated in barrels show higher Z-whiskylactone, eugenol, ethyl vanillate, furfural, 5-hydroxy-methylfurfural and 5-methylfurfural concentrations than wines without wood or wines macerated with fragments. On the other hand, the E-whiskylactone, vanillin, acetovanillone and syringaldehyde concentrations are higher in wines without wood or wines macerated with fragments. Moreover, wines that have been macerated with fragments and vatted in old barrels afterwards show similar concentrations to wines that have only been macerated with fragments. In any case, mean concentrations are similar to wines from new barrels.

The vintage factor introduces significant differences (Table 1) in the extraction of vanillin, ethyl vanillate, acetovanillone, syringaldehyde, 5-methylfurfural and 5-hydroxy-methylfurfural. Moreover, the compounds of the vanillin group present a significant interaction with the treatment. These compounds depend significantly on the method of preparation of the wood even if it comes from the same maker, as Chatonnet has already observed (Chatonnet, 1999).

The compounds that present significant differences (Table 1) depending on the origin of oak used (American, French or Pyrenean) are E-whiskylactone, Z-whiskylactone, eugenol, ethyl vanillate, furfural and 5-methylfurfural. Their mean concentrations and significance are shown in Table 3. The American oak presents higher concentrations of Z-whiskilactone, eugenol, furfural and 5-methylfurfural. Only the ethyl vanillate has a higher concentration in the Pyrenean oak wood. On the other hand, French oak wines present a higher concentration of E-whiskilactone.

Finally, for the area factor (Table 1) all the compounds present significant differences except for vanillin and syringaldehyde. Similarly, there is significant interference for all the compounds except for those of the vanillin group. Table 4 shows the mean values of the compounds with significant differences for each area of the study. It can be seen that Navarra wines have the highest concentrations for all the compounds except for those of the vanillin group and the furfural. Data in Table 1 show that there is interdependence between the area and the given treatment. This interaction can be explained by the experimental design. In every area, the materials that were used are from different suppliers. Therefore the differences are due to the disparity in the materials and not due to the area. (Fernandez de Simon, Muino, & Cadahia, 2010) found high variability in

the composition of the volatile compounds extracted from commercially available fragments. These authors could not clearly relate the composition to either the level of toasting or to the species of oak.

The majority of experimental samples were obtained macerating finished wines with oak fragments or vatting the wines in new barrels. As explained in the Materials and Methods section, in some areas oak fragments were used for alcoholic fermentation while in others wines were put in used barrels after maceration with oak fragments. The experiment has also examined whether using fragments in fermentation or in macerations significantly influences the concentration of the extracted compounds. For this purpose, a one factor ANOVA was carried out (maceration during fermentation or in a finished wine) using just the samples of the wines that were macerated during fermentation and those that were macerated after fermentation had already finished. Table 5 shows that of the 12 studied compounds only the vanillin, syringaldehyde, furfural, 5-methylfurfural and 5-hydroxy-methylfurfural present significant differences for this factor. In Table 6 mean concentrations of compounds with $p<0.05$ are presented. It can be seen that for all compounds, concentration is higher in wines that were macerated with wood fragments after alcoholic fermentation was finished. Only the 5-hydroxy-methylfurfural has similar concentrations in wines macerated during alcoholic fermentation and in finished wines.

Finally in this experiment, the effects of using new barrels were compared with those of using old barrels aged 2, 3, 4, 5, 6, 7, and 8 years. A single factor ANOVA was carried out (the factor being the age of the barrel) in order to determine whether the extraction of the compounds was different between new and old barrels.

In Table 5 it can be appreciated that in relation to this factor all the compounds except for the ethyl vanillate have “p” values lower than 0.05.

Table 7 shows mean values for the 11 compounds that present significant differences according to the age of the barrel. It can be observed that the majority of the compounds are extracted mostly during the first year. From that moment, the extraction of the compounds decreases as the barrel’s age increases. This is particularly marked in the case of the derivatives of furfural. From the second year, these compounds present concentrations that vary between 5 and 15% of the initial concentrations. Concentrations of eugenol, vanillin and syringaldehyde decrease more than 50% from the second year onwards. The Z-whyskilactone decreases about 30% during the second

year and from then on the extraction remains stable throughout the years. Only the concentration of ethyl vanillate increases with the barrel's age. More than twice the amount of this compound was extracted from 8 year-old barrels than from new barrels.

Mean values of acetovanillone oscillate between consecutive years. This result can be explained considering the experimental design: Data from 1-year old barrels correspond to wines of vintages 2008 and 2009 from 2 zones and 2 types of oak. Data obtained from barrels 3, 5 and 7 years old are averages of the 2008 vintage wines from the two zones, while 2, 4 6 and 8 years old barrels were filled with wines of the 2009 vintage from the two zones and in two kinds of oak. Taking this experimental design in account, it can be seen (table 7) that the variation of acetovanillone with barrel age is very small.

If results shown in Tables 6 and 7 are compared with those obtained for wines that were never in contact with wood (control, data not shown), it is observed that all compounds in the latter case exhibit lower concentrations than the lowest values present in the tables.

The ANOVA studies show that various factors have a significant influence on the compounds that are extracted from the wood into the wines. These factors are the age of the barrel, the type of oak, the moment of introducing the wood fragments (fermentation or maceration in finished wine), and the method of preparing wood fragments.

In every sample where the concentrations of the studied compounds are higher than the odor threshold, the wood derived compounds influence the wine aroma.

It was possible to use a criterion to eliminate those wines that did not acquire the organoleptic properties characteristic of wines in contact with wood. As seen in Tables 6 and 7, in compounds that present significant differences depending on the age of the barrel and the fermentation/ maceration with fragments, extraction is reduced with the age of the barrel and is inferior in wines fermented with fragments. Compounds that show $p<0.05$ in two studies (Table 5) are vanillin, syringaldehyde, furfural, 5-methylfurfural and 5-hydroxy-methylfurfural. In the analyzed cases, all the wines that present concentrations of syringaldehyde lower than 100 as well as concentrations lower than 20 mg/L of vanillin or furfural have been kept in old barrels or have fermented with fragments. In these wines we can consider that extraction has been minimal.

Mean concentrations of syringaldehyde and vanillin (**table 2)** are higher in wines macerated with fragments (mean syringaldehyde=2749 µg/L; mean vanillin=640 µg/L) compared with those kept in barrels (mean syringaldehyde=630 µg/L; mean

vanillin=153 µg/L). The concentrations of these compounds in wines aged in barrels are just 22.93 and 23.92% of the concentrations found in the wines macerated with fragments. Moreover, the concentration of acetovanillone of the barrel wines is 38.75% of the value found in macerated wines. These values show that this compound can be used as marker of the maceration of wines with oak fragments. Arapitsas et al. (Arapitsas et al., 2004) suggested that syringaldehyde could be used as a marker for wines aged with oak fragments. In addition, in a study carried out on wines from different price categories, (San Juan, Cacho, Ferreira, & Escudero, 2012) found high concentrations of this compound in low price wines and suggested its possible ageing with wood fragments. On the other hand, Franco et al. (Franco et al., 2006; Ordóñez, Suberviola, Ortega-Heras, & Gómez-Cordovés, 2006) found that vanillin and eugenol were compounds that served to differentiate between wines whose ageing was carried out in barrels and those macerated with wood fragments.

In **Table 2**, we can see that the mean concentration of eugenol is three times higher in wines aged in barrels (X= 34,0 µg/L) compared to those macerated with fragments (X= 9,24 µg/L). Many authors have found similar results (Franco et al., 2006; Garde-Cerdan & Ancin-Azpilicueta, 2006; Guchu, Diaz-Maroto, Perez-Coello, Gonzalez-Vinas, & Ibanez, 2006; Ordóñez et al., 2006).

As all the compounds reveal significant differences depending on the factor under study, only one compound is not enough to distinguish wines aged in barrels or macerated with fragments. Acetovanillone, vanillin, syringaldehyde and eugenol are four compounds that have an important influence on the classification of samples, as seen in Figure 1. Relations between these compounds have been examined, leading to the conclusion that the relationship that best determines whether the wines have been aged in barrels or macerated with fragments is the sum of the concentrations of vanillin and acetovanillone divided by the concentration of eugenol. Taking this into consideration, the following criteria are proposed to discriminate between the wines.

Criterion 1. Wines with concentrations of syringaldehyde lower than 100 µg/L or concentrations lower than 20 µg/L of vanillin or furfural are considered not to have passed the extraction threshold, so they can be regarded as not having had contact with wood.

Criterion 2. A relation (vanillin+acetovanillone)/eugenol < 20, indicates that the wines have been aged in barrels.

Criterion 3. A relation (vanillin+acetovanillone)/eugenol > 20, indicates that the wines have been macerated with wood fragments.

To determine whether these criteria are applicable in all cases, they have been applied to the wines analyzed in this project as well as in others. Extensive research in the bibliography has also been carried out. The majority of authors have not analyzed acetovanillone, so the verification of the criteria could not be done. Nevertheless, the quantification of vanillin, eugenol and acetovanillone has been found in some articles, allowing us to apply the criteria. Garcia Carpintero et al. (Garcia-Carpintero, Gallego, Sanchez-Palomo, & Vinas, 2012) analyzed wines macerated with oak fragments during alcoholic and malolactic fermentation. When applying the criteria, we verified that when oak fragments were used during alcoholic fermentation, the concentrations of vanillin, furfural or syringaldehyde were lower than 20 µg/L. Thus, if we apply criterion 1, it is considered that these wines have had a minimum extraction. For the rest of the wines analyzed in the paper, the relation between acetovanillinone + vanillin/eugenol was higher than 20. According to our criteria, this means that the wines were aged with oak fragments

In addition, Rodriguez Bencomo et al. (Rodriguez-Bencomo, Ortega-Heras, Perez-Magarino, & Gonzalez-Huerta, 2009) analyzed wines macerated with 7 different types of oak fragments. Applying the criteria we found that just one of the analyzed wines was wrongly classified. This wine was macerated with Pyrenean oak fragments. Cerdan et al. (Cerdan, Mozaz, & Azpilicueta, 2002) analyzed wines aged in barrels made of oak of different origins. In all the samples the sum of acetovanillone + vanillin/eugenol was lower than 20. According to our criteria, 100% of the wines presented in the paper were correctly classified. Finally, Castro et al. (Castro-Vazquez et al., 2011) analyzed wines kept in barrels over different periods of time. In all the samples, the concentrations of acetovanillone and vanillin divided by the concentration of eugenol were lower than 20. As in the previously cited study, 100% of the wines were correctly classified.

The results obtained show that using these criteria, more than 90% of wines analyzed have been correctly classified both in the present study and in previous studies carried out by other authors.

4. Conclusions

The compounds that best enable wine samples to be differentiated depending on whether they have been treated in barrels or with wood fragments are E-whiskylactone, Z-whiskylactone, eugenol, vanillin, acetovanillone, methyl vanillate, ethyl vanillate, syringaldehyde, furfural, furfuryl alcohol, 5-methyl furfural, and 5-hydroxy-methyl furfural.

The PC analysis shows that vanillin, acetovanillone and syringaldehyde are the compounds that explain the variance of wines fermented or macerated with wood fragment wines; they are present in higher concentrations than in wines aged in barrels. Eugenol, E-whiskylactone and Z-whiskylactone are the compounds that explain the variance in wines aged in barrels. The concentration of eugenol is significantly high in wines aged in barrels.

The extraction of wood derived compounds is affected by many factors such as the age of the barrel, the application of fermentation or maceration in wines, the dose, etc.

The vanillin + acetovanillone/eugenol ratio is essential for discrimination. It has been observed that when wines have been aged in barrel, the ratio is lower than 20 while, on the other hand, when the wines have fermented or been macerated with wood fragments, the relation is higher than 20.

Acknowledgements.

This study would not have been possible without the funding provided by the Dirección General de Industria y Mercados Alimentarios, Subdirección General de Calidad Diferenciada y Agricultura Ecologica of the Ministerio de Medio Ambiente y Medio Rural y Marino of Spain

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Figure 1. Results of principal component analysis of volatile compound data matrix:

Figure 1a: scores of the 231 wine samples (92 barrel vatted (B), 115 macerated or fermented with oak fragments (C) and 24 macerated with oak fragments and then barrel vatted (BC) in the plane formed by the first two principal components.

Figure 1b: loading of the variables on the first two principal components.

Figure 1 a

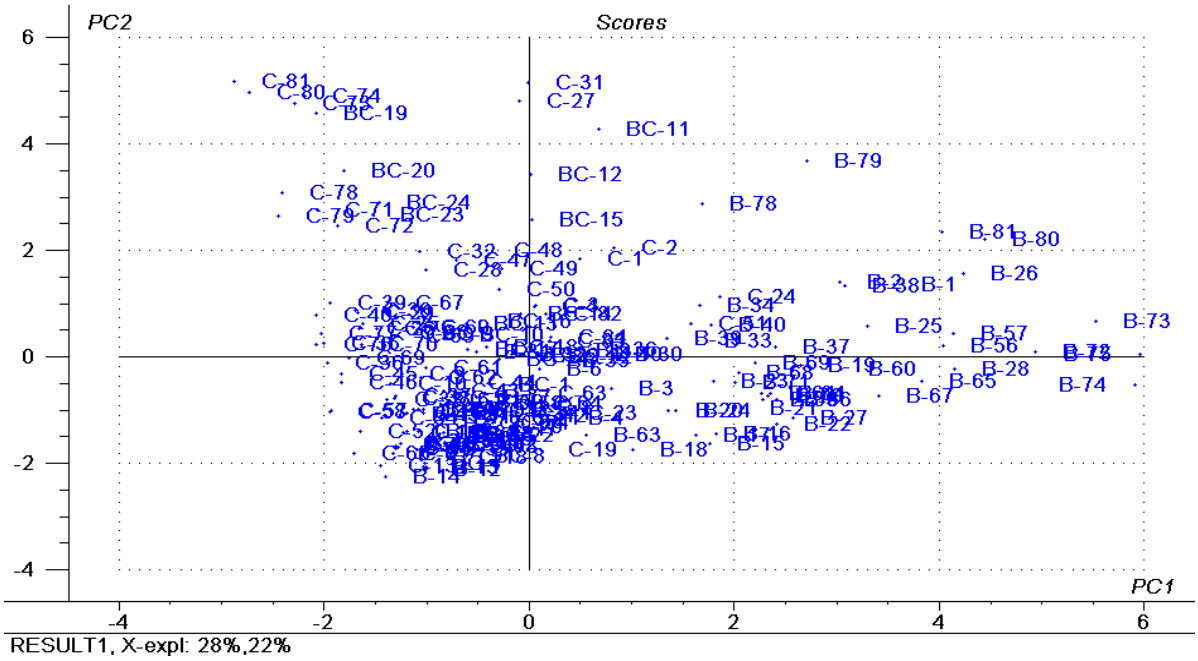

Figure 1b

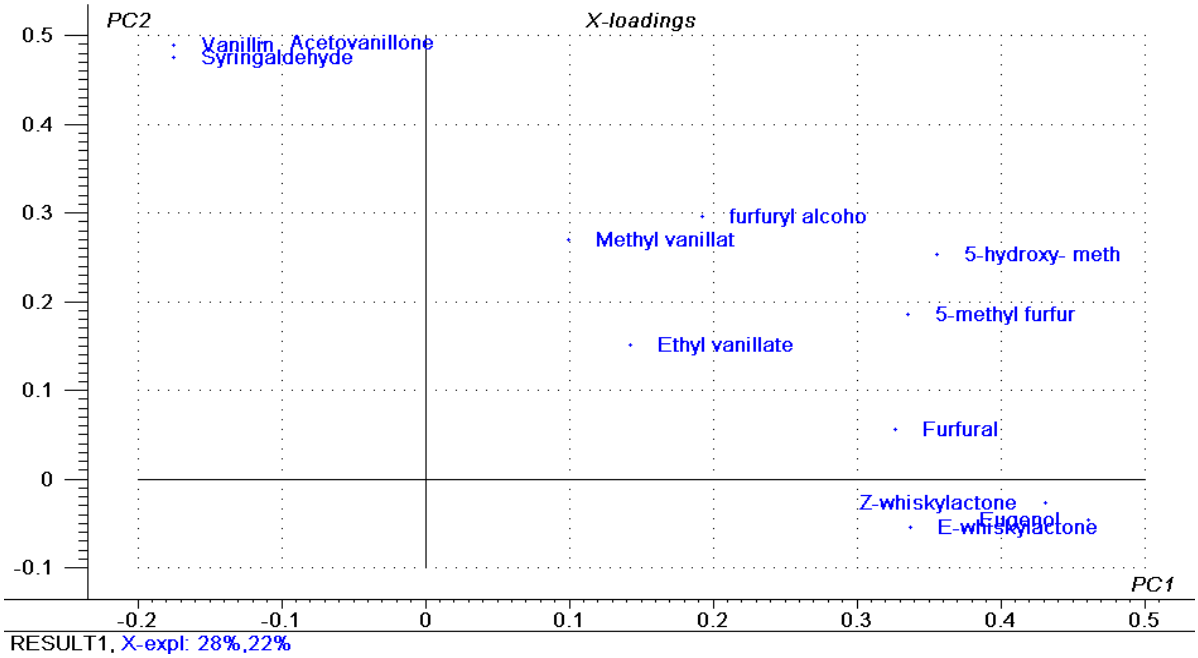

Table 1. Four factor ($p<0.05$) ANOVA study performed with wines macerated with fragments or kept in oak barrels. The study was carried out in 2008 and 2009. Samples were taken after 6 and 12 months of ageing in 6 different zones. Factors: F1: ageing treatment (fragments or barrels); F2: Vintage (2008, 2009); F3: oak origin (French, American or Pyrenean) and F4: production zone (Aragón, Castilla y León, Rioja, Madrid, Navarra and Galicia).

	Probability (p)						
	Treatment	**Vintage**	**Oak origin**	**Production zone**	**Interactions**		
	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**F1/F2**	**F1/F3**	**F1/F4**
E-whiskylactone	0.032	0.305	0.001	0.000	0.028	0.385	0.000
Z-whiskylactone	0.014	0.496	0.000	0.000	0.001	0.120	0.000
Eugenol	0.000	0.551	0.008	0.000	0.587	0.004	0.000
Vanillin	0.000	0.000	0.666	0.301	0.000	0.522	0.426
Methyl vanillate	0.000	0.351	0.077	0.000	0.000	808	0.820
Ethyl vanillate	0.000	0.000	0.000	0.000	0.000	0.356	0.008
Acetovanillone	0.000	0.000	0.567	0.000	0.000	0.631	0.320
Syringaldehyde	0.000	0.002	0,596	0.602	0,019	0.726	0.366
Furfural	0.003	0.235	0.000	0.000	0.852	0.601	0.000
5-methyl furfural	0.006	0,008	0.004	0.005	0.638	0.131	0.000
5-hydroxy- methylfurfural	0.000	0.000	0.204	0.000	0.358	0.000	0.000
Furfuryl alcohol	0.268	0.083	0.100	0.000	0.120	0.009	0.000

Table 2: Mean concentration (µg/L) of compounds related with wood showing significant differences according to the treatment factor (barrel vatting, macerated with oak fragments and macerated with oak fragments and then vatted in barrels)

	Factor treatment		
Concentration (µg/L)	**Barrel**	**Fragment**	**Fragment+barrel**
E-whiskylactone	10,6 a	48,2 c	84.7 b
Z-whiskylactone	404 a	185c	282 b
Eugenol	34,0 a	9.24 c	14.9 b
Vanillin	153 b	640 a	600 a
Methyl vanillate	16.8 b	16.0 b	20.9 a
Ethyl vanillate	479 a	330 b	262 c
Acetovanillone	156 b	250 a	279 a
Syringaldehyde	630 b	2749 a	3318 a
Furfural	174 a	61.7 b	28.9 b
5-methyl furfural	81.4 a	44.0 b	28.2 b
5-hydroxy- methylfurfural	12.2 a	6.10 b	5.12 b

Means with different letters are significantly different according to ANOVA results ($P<0.05$).

Table 3: Mean concentration (μg/L) of compounds related with wood that present significant differences according to oak origin (American, French or Pyrenean).

	Factor Oak origin		
Concentration (μg/L)	**American**	**French**	**Pyrenean**
E-whiskylactone	64.7b	89.8a	56.6b
Z-whiskylactone	430a	193b	236b
Eugenol	25.7a	17.1b	20.1b
Ethyl vanillate	434b	233c	961a
Furfural	130a	99.1ab	44.9b
5-methyl furfural	85.3a	43.9b	16.2b

Means with different letters are significantly different according to ANOVA results ($P<0.05$).

Table 4: Mean concentration (µg/L) of compounds related with wood that present significant differences according to the production zone factor (Aragón, Castilla y León, Rioja, Madrid, Navarra and Galicia).

	Factor production zone					
Concentration (µg/L)	**Aragón**	**Castilla y León**	**Rioja**	**Madrid**	**Navarra**	**Galicia**
E-whiskylactone	51.7 c	83.9 b	65.4 bc	54.7 c	210 a	73.1 bc
Z-whiskylactone	352 b	185 d	290 c	206 cd	593 a	349 b
Eugenol	20.8 b	12.4 c	20.2 b	17.4 bc	56.2 a	21.2 b
Methyl vanillate	40.9 a	7.14 c	8.44 c	8.24 c	21.6 b	18.0 b
Ethyl vanillate	613 b	135 e	169 de	250 d	393 c	1043 a
Acetovanillone	321 a	180 c	171 c	249 b	105 d	183 c
Furfural	27.5 c	31.0 c	132 b	145 b	181 b	259 a
5-methyl furfural	43.2 bc	22.0 c	83.9 ab	50.1 bc	78.3 ab	104 a
5-hydroxy- methylfurfural	8.80 bc	2.53 d	6.72 c	11.7 b	15.8 a	18.8 a
furfuryl alcohol	319 a	173 bc	122 c	131 c	385 a	285 ab

Means with different letters are significantly different according to ANOVA results ($P<0.05$).

Table 5: “p” values obtained in two studies of one factor ANOVA. In the first column the comparison is between wines macerated with oak fragments. In the second column the comparison is between wines aged in barrels of different ages.

	Probability (p)	
	Factor maceration In fermentation or in finished wines	**Factor Age of the barrel**
E-whiskylactone	0.690	0.000
Z-whiskylactone	0.640	0.009
Eugenol	0.795	0.000
Vanillin	0.000	0.000
Methyl vanillate	0.950	0.016
Ethyl vanillate	0.743	0.673
Acetovanillone	0.266	0.000
Syringaldehyde	0.000	0.004
Furfural	0.002	0.004
5-methyl furfural	0.000	0.000
5-hydroxy- methylfurfural	0.040	0.000
Furfuryl alcohol	0.748	0.002

Table 6: mean concentration (µg/L) of compounds related with wood that present significant differences according to the oak fragments maceration factor (in fermentation or in finished wine)

	Factor maceration				
Concentration (µg/L)	**Vanillin**	**Syringaldehyde**	**Furfural**	**5-methyl furfural**	**5-hydroxy-methylfurfural**
Fermentation	18.4	92.7	13.2	0.36	4.35
Finished wine	537	2279	63.6	44.4	6.05

Table 7: Mean concentration (µg/L) of compounds related with wood that present significant differences according to the age of the barrels factor.

	Factor: Age of the barrel (years)							
Concentration (µg/L)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
E-whiskylactone	110	40.8	62.8	56.2	52.8	55.9	30.8	27.8
Z-whiskylactone	436	314	265	300	213	245	303	299
Eugenol	36.6	28.2	17.1	17.5	9.02	13.6	16.5	16.8
Vanillin	196	131	40.7	19.2	36.7	25.9	34.5	0.13
Methyl vanillate	17.1	22.6	24.0	22.3	25.8	23.0	39.3	39.7
Acetovanillone	146	248	116	240	116	242	160	343
Syringaldehyde	636	624	86.2	173	73.5	148	65.5	46.8
Furfural	228	63.8	26.5	10.4	23.2	11.9	22.8	9.52
5-methyl furfural	104	39.3	0.91	1.42	1.15	1.48	0.00	0.86
5-hydroxy- methylfurfural	16.5	7.89	1.63	3.58	1.51	3.61	1,37	5.44
furfuryl alcohol	285	177	109	110	110	109	109	111