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Accelerated aging of wine vinegars with oak chips: evaluation of wood flavour compounds

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Abstract

Assays of accelerated aging in wine vinegars were performed with 2% w/v of oak chips and different pretreatments (toasted at 180 °C, or boiled and toasted at 180 °C). An analytical method for determining volatile compounds from wood (volatile phenols, whisky–lactones, vanillin, etc.) in wine vinegars was set up. This method consisted of a solid phase extraction step followed by gas chromatography. When traditional aging for 180 days (oak barrels) and accelerated aging for 15 days were compared, the results showed that the biggest difference was the rate of extraction for vanillin. Oak-chip aging increased the concentration of this compound 20-fold. Levels of oak-lactone isomers were higher when only toasted oak chips were used. Other factors such as ethanol content or the use of barrels have also been considered. We conclude that oak chips (only toasted) are a valuable alternative to oak barrels for obtaining, in a short period of time (15 days), wine vinegars with the peculiar characteristics given by oak wood.

Keywords: Wine vinegar; Oak chips; Oak barrel; Volatile compounds; SPE; Aging

1. Introduction

During the production of quality wine vinegars, they are usually aged in oak barrels following traditional practices: This is the case in such areas as Jerez–Xérès–Sherry DO, where the oak used is *Quercus alba* (American oak) and the minimum time required for a vinegar to be considered Sherry vinegar is six months (Consejería de Agricultura y Pesca, 2000).

Barrels have been extensively used to store and age wine and other beverages for centuries. (Waterhouse & Towey, 1994). Most casks used to age wine, spirits and another wine derivative products are made of oak (Pérez-Coello, Sanz, & Cabezudo, 1997). The oak wood used in wine-making comes mainly from two sources: American oak (*Q. alba*) and French oak (*Quercus robur* and *Quercus petraea*). Their chemical compositions have been carefully studied and are quantitatively different (Singleton, 1995).

It is widely recognized that oak barrels improve the quality of products. Generally, the sensorial complexity is increased because the wood transfers a series of aromatic substances to the substrate submitted to aging (Gutiérrez, 2002). The aging of wine vinegar involves changes to the colour, the polyphenol profile (Tesfaye, Morales, García-Parrilla, & Troncoso, 2002), and the aroma, which are highly appreciated by consumers (Morales, Tesfaye, García-Parrilla, Casas, & Troncoso, 2002). Vinegar flavour is a complex mixture containing a wide variety of components: carbonyl compounds, ethers, acetals, lactones, acids, volatile phenols and esters (Blanch, Tabera, Sanz, Herraiz, & Reglero, 1992). While vinegars age in barrels, the volatile compounds are enriched as a result of two important processes: they are concentrated because water is lost through the wood and new compounds are formed, such as esters, which have low threshold perceptions (Morales et al., 2002). So far, volatile compounds from wood have not been studied in wine vinegars. The high acetic acid content of vinegars together with their low alcoholic degree will presumably influence the extraction of compounds from wood.

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The extraction of volatile compounds from an oak barrel depends on the quantity of compounds originally present in the unused barrel that are potentially extractable, and on time-related factors such as the contact time between the substrate submitted to aging and the wood (Garde, Rodríguez, & Ancín, 2002a). The factors which affect the pool of oak extractives are the species and geographical origin of the wood (Chatonnet, 1991; Miller, Howell, Michaelis, & Dickmann, 1992), the seasoning of the staves (Sefton, Francis, Pocock, & Williams, 1993; Vivas & Glories, 1993), the toasting of the barrel (Chatonnet, Boidron, & Pons, 1989) and the age of the barrel (Chatonnet, 1991).

Although hundreds of volatile compounds have been identified in untoasted oak wood (Chatonnet, 1991; Maga, 1984; Nishimura, Ohnishi, Masuda, Koga, & Matsuyama, 1983), relatively few volatile compounds can be effectively extracted in significant amounts (Chatonnet, 1991; Sefton et al., 1993). Among these are vanillin, eugenol, guaiacyl derivatives and *cis*- and *trans*-β-methyl-γ-octalactone, commonly known as the oak or whisky–lactones (Kepner, Web, & Muller, 1972). Other volatile compounds are originated by the thermal degradation of lignin, cellulose and hemicellulose during toasting. Toasting can also increase the levels of those compounds found in untoasted oak (Chatonnet et al., 1989).

Eugenol and vanillin extracted from oakwood are products of lignin degradation or even by-products of lignin biosynthesis. Eugenol has a clove-like aroma and its concentration can be increased by medium or heavy toast levels when barrels are heated. Vanillin, the principal aroma component of natural vanilla (Clark, 1990), is generally considered to be an important contributor to the quality of barrel-aged wines and spirits (Sefton et al., 1993). The concentration of vanillin in the wood can be increased by heating (Nishimura et al., 1983; Chatonnet et al., 1989).

Oak lactones are among the most important of the oak volatile compounds that contribute to the flavour of barrel-aged beverages (Chatonnet, Boidron, & Pons, 1990; Dubois, 1989). Large differences have been reported in the total concentrations of these lactones and in the ratio of the isomers in oak wood from different origins. The concentration of these compounds can be also increased by toasting (Chatonnet et al., 1989; Maga, 1989a). Heating and charring the wood does not seem to affect the ratio between the two oak lactone isomers and consequently this ratio has been used as an index for identifying woods of different species and different geographical origins (Pérez-Coello et al., 1997; Waterhouse & Towey, 1994).

Largely for economic reasons, alternatives to oak barrels are being used in wine production. This is the case of oak chips (Gutiérrez, 2002). Destillates have been aged by steeping with oak chips and the results are similar to those obtained by barrel aging for several years (Giménez-Martínez, García-Serrana, Villalón-Mir, & López-Martínez, 1997). Quality vinegars can be obtained at lower prices and in less time if this practice is used to age these products.

The objectives of this study are to determine the volatile compounds from wood in aged vinegars and to test oak chips as an alternative to oak barrels in the maturation of wine vinegars. Oak chips were conditioned by two methods: boiling in water and then toasting, and just toasting. Both treatment (boiling plus toasting and just toasting) have been tested in accelerated aging of Oloroso Sherry wine (Monedero, Olalla, Quesada, López-Ga, & López-Martínez, 1998). The first one tries to resemble traditional barrel making, using hot water and heating. The reused of barrels for vinegar aging is also considered.

Because of the low concentrations expected, compounds from wood such as volatile phenols, oak lactone and vanillin need a powerful isolation preconcentration step before gas chromatography (GC). These compounds have been extensively studied in wine and distillates, and several extraction methods have been proposed. Most authors have applied liquid–liquid extraction (LLE) techniques with different organic solvents. Waterhouse and Towey studied oak lactones in wine fermented in American and French oak-wood barrels. They used LLE with dichloromethane (Towey & Waterhouse, 1996a, 1996b; Waterhouse & Towey, 1994). Several modifications were made to this method by Díaz-Plaza, Reyero, Pardo, and Salinas (2002). To analyse the compounds from wood in white wines fermented in the presence of wood chips made from American and French oaks, a continuous LLE process with pentane and dichloromethane was carried out (Pérez-Coello, González-Viñas, García-Romero, Cabezudo, & Sanz, 2000a). In another study, the effect of the origin, volume, and age of the barrel on the wine's volatile composition was evaluated, with pentane–ether (2:1) as the organic solvent (Pérez-Prieto, López-Roca, Martínez-Cutilla, Pardo Mínguez, & Gómez-Plaza, 2002).

Garde, Torrea, and Ancín (2002b) used a mixed method to study the extraction of volatile compounds from wood in a wine maturated in a reused barrel. The method consists of LLE with dichloromethane–pentane (2:3,v/v) before solid phase extraction (SPE).

Solid phase extraction methods have been used very little in this context even though it is a less polluting practice and the last concentration step, mandatory in most LLE methodologies, is not required.

Three extraction methods are tested in this study. One of these was based on LLE with dichloromethane, adapted from Diaz-Plaza et al. (2002). The other two consisted of a SPE technique using different cartridges: LiChrolut EN from Merck and Bond Etut ENV from Varian, both of which had a polymeric adsorbent of

200 mg and a total volume of 3 ml (Ferreira, Jarauta, Ortega, & Cacho, 2004; López, Aznar, Cacho, & Ferreira, 2002).

2. Materials and methods

2.1. Reagents and standards

Pure standards for guaiacol, eugenol, whisky–lactones, 2-furfural and 3,4-dimethylphenol (internal standard to extraction process) were obtained from Sigma-Aldrich (Steinheim, Germany); vanillin and 2-octanol (internal standard to GC analysis) were supplied by Merck (Darmstadt, Barcelona); 5-methylfurfural and furfuryl alcohol were from Fluka (Steinheim, Germany). The reagents dichloromethane (p.a. for instrumental analysis) and methanol (p.a. for liquid chromatography) were obtained from Merck.

2.2. Samples

A commercial (non-aged) vinegar (6 acetic degrees) was used to select an extraction method and also in the recovery studies.

A total of 27 samples was studied, nine of which were aged by oak chips and 18 by the traditional oak barrel method (Tables 3 and 4).

2.3. Aging conditions

2.3.1. Accelerated aging with oak chips

Medium-sized wood chips (5–10 mm) were obtained from American oak (*Q. alba*) and supplied by Jose y Miguel Martin S.L. (Bollullos, Spain).

The oak chips were prepared by two methods: (i) chips were boiled in water for 2 h and then heated in an oven at 180 °C for 3 h. (B–T); (ii) chips were directly heated in the same conditions (T), but without previous boiling.

A wine vinegar (10 acetic degrees, 1 alcoholic degree) was used for the experiments. Half of it was enriched with distilled alcohol from wine so that its final alcoholic degree was 2 (%,v/v). The original vinegar and the alcoholic enriched vinegar were submitted to both experiments, (i) and (ii). In every case, 4 l of wine vinegar were placed in a glass container and oak chips were added at a dose of 2% w/v. Samples were taken at 15 and 90 days.

2.3.2. Aging in oak barrels

Three wine vinegars (BD_0, BE_0, and BF_0) obtained by submerged fermentation were submitted to static aging in unused, 16 l, *Q. alba* oakwood barrels purchased from Jose y Miguel Martin S.L. (Bollullo, Spain) and previously conditioned with Sherry wine. The barrels were filled up to 3/4 of total capacity. Successive samples were taken at 90 days and six months. After two years, the barrels were emptied and refilled with three different wine vinegars (BG_0, BH_0 and BI_0) to study the extraction of volatiles from wood in re-used barrels. Vinegars accounted for different ethanol residual contents (Table 4). Sampling took place at 90 days and six months.

2.4. GC determination of volatile compounds

2.4.1. Sample pretreatment methods

2.4.1.1. Liquid–liquid extraction. Ten microlitres of a solution containing 3,4-dimethylphenol (1 mg/ml in acetic acid–water 1:5 v/v) and 3 g of ammonium sulfate were added to 10 ml of vinegar. 2 ml of dichloromethane was added to this solution, stirred for 2 min and centrifuged (3 min at 2000 rpm). The organic phase was separated and filtered through sodium sulfate and the filtrate was vacuum concentrated and dichloromethane added up to 0.490 ml. 10 μl of 2-octanol as GC internal standard (2.624 mg/ml in dichloromethane) was added.

2.4.1.2. Solid phase extraction. The SPE experiments were performed in a Visipred SPE vacuum manifold from Supelco (Bellefonte, PA). Two cartridges were tested:

(A) LiChrolut EN cartridge extraction (López et al., 2002): The adsorbent was first conditioned with 2 ml of pure methanol and 6 ml of water. 10 ml of vinegar plus 10 μl of internal standard (3,4-dimethylphenol, 1 mg/ml) were passed through the previously conditioned cartridge at around 2 ml/min. The column was rinsed with 5 ml of water and dried by letting air pass through it (−0.6 Bar, 10 min). Finally, the compounds studied were recovered by elution with 1 ml of dichloromethane.

(B) Bond Elut ENV cartridge extraction (Ferreira et al., 2004): The adsorbent was conditioned in the same way as described above. 10 ml of vinegar plus 10 μl of internal standard were loaded into the cartridge, which was then rinsed with 10 ml of a solution containing 40% v/v pure methanol and 1% w/v sodium bicarbonate followed by 2 ml of water. A drying step was carried out before the analytes were eluted with 1 ml of dichloromethane.

In both cases, after SPE, the eluted samples were centrifugated (3 min at 2000 rpm) and the final recovered volume was 0.490 ml. Then 10 μl of 2-octanol solution (IS) was added. Finally, a 20-fold concentration is obtained.

2.4.2. GC analytical conditions

A Hewlett-Packard 6890 gas chromatograph equipped with a flame ionization detector (FID) was used. 1 μl of extract was injected in the splitless mode into a CP-Wax 57 CB, 50 m × 0.25 mm D.I. × 0.2 μm film thickness (Chrompack, Middelburg Holanda). The carrier gas was H_2 at 1.5 ml/min. The program temperature

Table 1
Calibration graphs data for the 8 volatile compounds under study and the IS of extraction process

Volatile compound	Concentration levels (*n*)	Range (mg/l)	Intercept	Slope	r^2
2-Furfural	5	5.72–102.1	−0.0156	0.0116	0.9991
5-Methylfurfural	5	5.69–101.7	−0.0191	0.0139	0.9994
Furfuryl	5	5.83–104.1	−0.0063	0.0114	0.9997
Guaiacol	5	5.72–102.1	−0.0143	0.0144	0.9995
trans-β-Methyl-γ-octalactone	5	6.18–110.4	−0.0116	0.0164	0.9995
cis-β-Methyl-γ-octalactone	5	5.55–99.1	−0.0141	0.0165	0.9995
Eugenol	5	5.75–102.7	−0.0151	0.0166	0.9990
3,4-Dimethylphenol[a]	7	8.0–200	−0.0199	0.0206	0.9994
Vanillin	5	5.6–100	−0.0148	0.0103	0.9888

[a] Internal standard of extraction.

Table 2
Results for recovery assays obtained with Lichrolut EN cartridges

Volatile compounds	Added (mg/l)	Recovery (%)	Mean recovery ± SD
2-furfural	0.267	59.0	60.1 ± 2.4
	0.545	62.8	
	0.812	58.4	
5-Methylfurfural	0.254	87.4	88.1 ± 3.4
	0.519	91.8	
	0.774	85.0	
Furfuryl	0.260	62.6	63.1 ± 3.4
	0.532	66.7	
	0.792	60.0	
Guaiacol	0.255	90.1	87.8 ± 3.0
	0.522	89.0	
	0.777	84.4	
trans-β-Methyl-γ-octalactone	0.207	105.7	101.7 ± 3.9
	0.423	101.4	
	0.630	98.0	
cis-β-Methyl-γ-octalactone	0.231	84.2	81.4 ± 2.6
	0.472	80.9	
	0.702	79.0	
Eugenol	0.246	91.9	89.1 ± 2.9
	0.503	89.4	
	0.749	86.0	
Vanillin	0.230	79.2	79.1 ± 7.4
	0.470	86.4	
	0.700	71.7	

was: 100 °C, 2.5 °C/min, 180 °C (2 min), 1 °C/min, 200 °C (Diaz-Plaza et al., 2002). The injector was set to 200 °C and the detector to 220 °C. Data acquisition software was HPChemstation data processing system (Hewlett-Packard).

2.4.3. Calibration and quantification

Calibrations were carried out by the GC analysis of dichloromethane solutions that contained every volatile phenol, isomers of oak-lactone and 3,4-dimethylphenol (IS of extraction process). Five different concentration levels were used, and each level was injected in triplicate.

The calibration graphs were built representing the area relative to the internal standard (2-octanol) against the concentrations. Equations and regression coefficients are shown in Table 1. The identities of the two isomers were assigned in the way described by Chatonnet (1991), who showed that the *trans* isomer eluted first in this kind of column. It was assumed that the FID detector response was equal for the two isomers (Waterhouse & Towey, 1994).

The quantification was carried out by the internal standard method. The concentrations were corrected by the corresponding mean recovery percentages of the SPE procedure shown in Table 2.

2.5. Statistical analysis

Multivariate analysis of data including Principal Components Analysis (PCA) was performed by means of Statistica® software.

3. Results and discussion

3.1. Extraction of odorants: Selection of the method

A wine vinegar was spiked with standards ($n = 9$) and submitted to three different extraction methods: LLE and two SPE. Liquid–liquid extraction gave a higher recovery than the SPE methods only for furfuryl alcohol; in contrast, it gave lower recovery for whisky–lactones (half that of SPE recovery). Hence, LLE was rejected as an extraction method. When SPE Bond Elut ENV cartridges were used, furanic compounds such as 2-furfural, 5-methylfurfural and furfuryl alcohol, as well as vanillin were not detected in the vinegar extract. Therefore, SPE with LiChrolut EN cartridges successfully covers a wider range of volatile compounds from wood in aged vinegar and it was selected as the extraction method.

3.2. Recovery values

An average recovery value must be estimated for every compound studied if quantification is to be done properly. A commercial (not aged) wine vinegar was

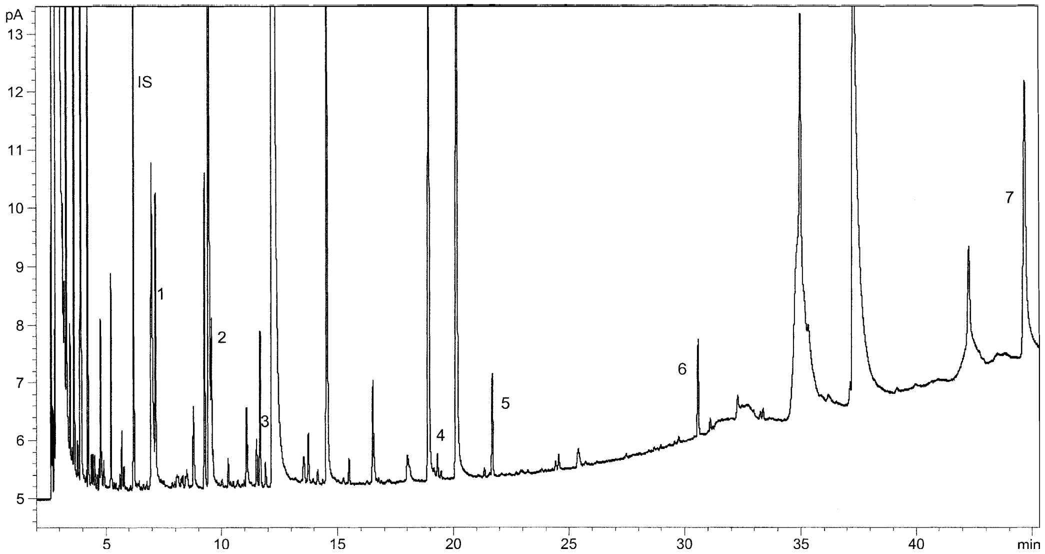

Fig. 1. Chromatogram of a wine vinegar steeped for 15 days with oak chips. Peaks: IS – Internal Standard; (1) 2-furfural; (2) 5-methylfurfural; (3) furfuryl alcohol; (4) *trans*-β-methyl-γ-octalactone; (5) *cis*-β-methyl-γ-octalactone; (6) 3,4-dimethylphenol; (7) vanillin.

spiked with nine volatile standard compounds at three different levels of concentration (ranging between 0.2 and 0.8 mg/l) so that recovery values could be calculated. Since these compounds have not been determined in wine vinegar until now, there is no indication about what the adequate levels for standard addition are. So we decided to select volatile compound levels that were similar to those previously reported for wine samples (Chatonnet et al., 1990; Garde et al., 2002a; Pérez-Coello et al., 2000b; Towey & Waterhouse, 1996a, 1996b). The amounts added and the recovery values obtained are shown in Table 2. 2-Furfural and furfuryl alcohol were the compounds that gave lowest recovery values (around 60%) and the remaining compounds showed recoveries of around 80%.

3.3. Evolution of volatile compounds with aging

3.3.1. The advantages of oak chips over barrels

Volatile compounds will be affected by whether barrels or oak chips are used to age vinegars. On one hand, the small size of the chips (5–10 mm) means that the vinegar is absorbed quickly. The liquid penetrates and

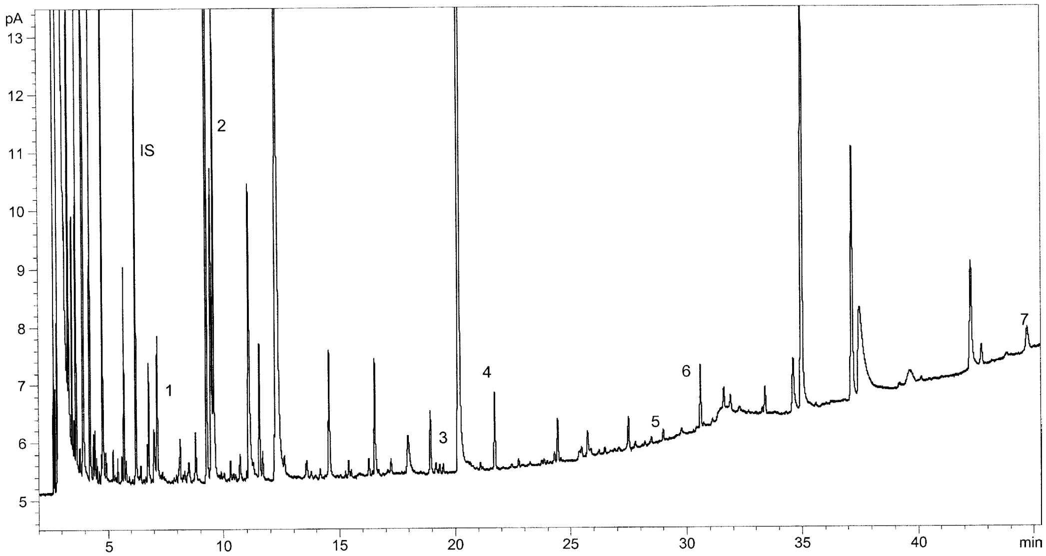

Fig. 2. Chromatogram of a wine vinegar aged in the barrel for 180 days. Peaks: IS – Internal Standard; (1) 2-furfural; (2) 5-methylfurfural; (3) *trans*-β-methyl-γ-octalactone; (4) *cis*-β-methyl-γ-octalactone; (5) eugenol; (6) 3,4-dimethylphenol; (7) vanillin.

soaks the chip totally, which makes the process of compounds diffusing from the wood to the liquid in contact with it easier. On the other hand, in the barrel only the layer closest to the liquid is soaked.

The content of volatile compounds from wood in wine vinegars steeped for 15 days with oak chips (accelerated aging, Fig. 1) was compared with that in wine vinegars that had been in the barrel for 180 days (traditional aging, Fig. 2) to test whether the use of chips could improve the extraction process in vinegar. This was because Sherry wine vinegar requires a minimum of six months (180 days) in the barrel before it is marketed.

When the selected extraction methods were applied, the most marked difference between the two aging methods was found for the extraction of vanillin. The initial values of vanillin ranged from non-detectable amounts to 0.661 mg/l in wine vinegars subject to traditional aging and 0.663 mg/l in the wine vinegar submitted to the accelerated aging assays. Vinegars aged with chips reached values of around 12.4–15.4 mg/l whereas barrel-aged vinegars achieved final contents ranging between 1.4 and 3.4 mg/l (Tables 3 and 4). As mentioned above, vanillin concentration can be increased by heating. In fact, several authors have pointed out that barrel toasting can result in up to 50-fold increases in vanillin in oakwood (Chatonnet et al., 1989; Maga, 1989b; Nishimura et al., 1983). Spillman, Pollnit, Liacopoulos, Skouroumounis, and Sefton (1997) found that the content of vanillin in barrel wood can depend on differences in heat penetration, rather than the intensity of the toasting. When barrels are toasted only the most superficial layer is affected, whereas when chips are heated, the heat penetrates quite substantially and leads to an enhanced formation of vanillin.

For some samples, 2-furfural values were similar after 15 days of accelerated aging and 180 days in the barrel.

Eugenol was only present in vinegars aged in barrels and furfuryl alcohol, formed by enzymatic reduction of the analogous furan aldehyde (Towey & Waterhouse, 1996b), was only found in oak chip vinegars because it was present in the initial vinegar (CA0). Anyway, this compound does not increase during accelerated aging (Table 3). 5-Methylfurfural, formed during the toasting process, showed higher concentrations in vinegars aged in barrels and it was not detected in any of the initial samples (time zero). On the other hand, guaiacol was not detected in any of the vinegars studied.

The extraction of oak-lactone isomers showed different trends. *Trans* -β-methyl-γ-octalactone was found in higher concentrations in oak chip samples and *cis*-β-methyl-γ-octalactone in barrels. The *cis*/*trans* ratios were 2–5 and 9–12, respectively. Some authors consider that the *cis*/*trans* ratio is a characteristic of the wood's source (Guichard, Fournier, Masson, & Puech, 1995; Masson, Guichard, Fournier, & Puech, 1995; Waterhouse &

Table 3
Volatile compounds from wood contents in a wine vinegar (A) submitted to accelerated aging with oak chips

Samples	Characteristics of samples				Volatile compounds (mg/l)						
	Days	Aging method	Oak chips pretreatment[a]	Alcohol degree[b]	2-Furfural	5-Methyl-furfural	Furfuryl	*trans*-β-Methyl-γ-octalactone	*cis*-β-Methyl-γ-octalactone	Eugenol	Vanillin
CA0	0	–	–	1	1.46 ± 0.02	n.d.	0.227 ± 0.004	0.102 ± 0.003	0.207 ± 0.012	n.d.	0.663 ± 0.008
CA1	15	Chips	B–T	1	4.73 ± 0.09	0.364 ± 0.004	0.300 ± 0.002	0.128 ± 0.000	0.325 ± 0.008	n.d.	12.0 ± 0.1
CA2	90	Chips	B–T	1	8.67 ± 0.03	0.324 ± 0.010	0.192 ± 0.003	0.128 ± 0.007	0.309 ± 0.002	n.d.	12.6 ± 0.2
CA3	15	Chips	T	1	2.68 ± 0.01	0.297 ± 0.023	0.273 ± 0.014	0.271 ± 0.012	1.38 ± 0.03	n.d.	11.8 ± 0.4
CA4	90	Chips	T	1	5.85 ± 0.05	0.219 ± 0.014	n.d.	0.256 ± 0.005	1.35 ± 0.04	n.d.	12.0 ± 0.1
CA5	15	Chips	B–T	2	2.15 ± 0.01	0.274 ± 0.004	0.594 ± 0.006	0.115 ± 0.002	0.280 ± 0.008	n.d.	14.5 ± 0.7
CA6	90	Chips	B–T	2	8.39 ± 0.01	0.525 ± 0.033	0.285 ± 0.008	0.125 ± 0.003	0.288 ± 0.004	n.d.	15.4 ± 0.2
CA7	15	Chips	T	2	5.42 ± 0.02	0.434 ± 0.002	0.571 ± 0.008	0.279 ± 0.008	1.43 ± 0.01	n.d.	13.0 ± 0.1
CA8	90	Chips	T	2	10.7 ± 0.06	0.438 ± 0.009	0.275 ± 0.012	0.285 ± 0.005	1.47 ± 0.00	n.d.	14.7 ± 0.1

n.d. = non detected.
[a] T = toasted, B = boiled.
[b] Alcohol degree: %v/v.

Table 4
Volatile compounds from wood contents in different wine vinegars (D, E, F, G, H and I) submitted to aging in oak barrels

Samples	Characteristics of samples				Volatile compounds (mg/l)						
	Days	Aging method	Times used barrel	Alcohol degree[a]	2-Furfural	5-Methyl furfural	Furfuryl	*trans*-β-Methyl-γ-octalactone	*cis*-β-Methyl-γ-octalactone	Eugenol	Vanillin
BD0	0	Barrel	0	1	1.89 ± 0.01	n.d.	n.d.	n.d.	0.253 ± 0.001	n.d.	0.661 ± 0.002
BD1	90	Barrel	0	1	4.54 ± 0.01	0.497 ± 0.011	n.d.	0.167 ± 0.001	1.35 ± 0.00	0.174 ± 0.010	1.60 ± 0.01
BD2	180	Barrel	0	1	5.73 ± 0.15	0.730 ± 0.003	n.d.	0.213 ± 0.004	2.14 ± 0.01	0.220 ± 0.007	1.90 ± 0.08
BE0	0	Barrel	0	2	0.945 ± 0.029	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BE1	90	Barrel	0	2	6.88 ± 0.07	0.663 ± 0.001	n.d.	n.d.	0.577 ± 0.015	n.d.	1.42 ± 0.04
BE2	180	Barrel	0	2	8.45 ± 0.03	0.939 ± 0.019	n.d.	0.129 ± 0.004	0.990 ± 0.005	n.d.	1.75 ± 0.08
BF0	0	Barrel	0	2	0.761 ± 0.028	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BF1	90	Barrel	0	2	6.22 ± 0.01	0.598 ± 0.005	n.d.	0.110 ± 0.002	0.864 ± 0.014	n.d.	1.40 ± 0.17
BF2	180	Barrel	0	2	8.94 ± 0.07	0.845 ± 0.009	n.d.	0.137 ± 0.005	1.36 0.01	0.173 ± 0.004	1.41 ± 0.03
BG0	0	Barrel	1	0.6	0.820 ± 0.012	n.d.	n.d.	n.d.	0.158 ± 0.000	n.d.	0.352 ± 0.002
BG1	90	Barrel	1	0.6	8.08 ± 0.01	0.609 ± 0.03	n.d.	0.172 ± 0.002	1.49 ± 0.00	0.160 ± 0.011	2.97 0.14
BG2	180	Barrel	1	0.6	9.01 ± 0.00	0.786 ± 0.021	n.d.	0.233 ± 0.006	2.16 ± 0.01	0.183 ± 0.008	3.50 ± 0.08
BH0	0	Barrel	1	2	0.988 ± 0.008	n.d.	n.d.	n.d.	0.197 ± 0.003	n.d.	0.372 ± 0.004
BH1	90	Barrel	1	2	6.10 ± 0.07	0.366 ± 0.019	n.d.	0.170 ± 0.009	1.42 ± 0.02	n.d.	2.10 ± 0.12
BH2	180	Barrel	1	2	5.19 ± 0.04	0.447 ± 0.004	n.d.	0.213 ± 0.004	2.33 ± 0.03	0.192 ± 0.011	3.45 ± 0.14
BI0	0	Barrel	1	2	0.789 ± 0.004	n.d.	n.d.	n.d.	0.149 ± 0.002	n.d.	n.d.
BI1	90	Barrel	1	2	2.86 ± 0.02	0.260 ± 0.016	n.d.	0.183 ± 0.006	2.15 0.01	0.182 ± 0.005	3.28 ± 0.04
BI2	180	Barrel	1	2	1.63 ± 0.01	0.259 ± 0.014	n.d.	0.216 ± 0.003	2.64 ± 0.01	0.223 ± 0.011	3.34 ± 0.00

n.d. = non detected.

[a] Alcohol degree: %v/v.

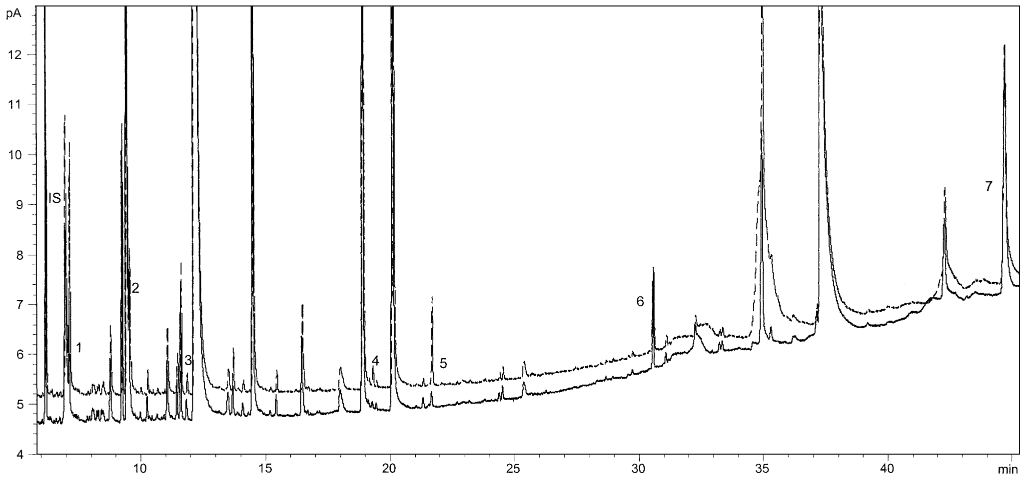

Fig. 3. Overlaped chromatograms of vinegars steeped for 15 days with oak chips pretreated by two methods: ---- Toasted (T); —— Boiled and Toasted (BT). Peaks: IS – Internal Standard; (1) 2-furfural; (2) 5-methylfurfural; (3) furfuryl alcohol; (4) *trans*-β-methyl-γ-octalactone; (5) *cis*-β-methyl-γ-octalactone; (6) 3,4-dimethylphenol; (7) vanillin.

Towey, 1994). The *cis/trans* ratios of wine aged in American oak barrels are always greater than 5, while the ratios in European oak-aged wines are always 2 or less (Towey & Waterhouse, 1996b). However, our results do not reflect this trend.

Waterhouse and Towey (1994) pointed out that the extraction of wood compounds follows a curve in which the rate of extraction is initially high, because of the considerable concentration difference between the wood and the substrate, but decreases when wood and substrate concentrations are equal. In our case, during the first 15 days of steeping with oak chips, most of the volatile compounds from wood were released and their concentrations, except that of furfural, did not increase greatly at 90 days (Fig. 3). Furfural's kinetic diffusion is slower and its concentration increases more gradually. At 90 days, in most cases, furfural values had doubled.

3.3.2. Oak chip pretreatment

Oak-lactone concentration was seen to depend to a considerable extent on the pretreatment applied to oak chips (Table 3). Vinegars steeped with oak chips that had only been heated showed increases in their concentrations of around 60% and 85% for *trans* and *cis* isomers, respectively, at 15 days. These concentrations remained practically constant in assays with boiled and toasted chips (Fig. 4). We found a high correlation between the kind of pretreatment that oak chips were subject to and the *trans* and *cis* isomer content of vinegars. The *cis/trans* isomer ratio was also different: 5 for toasted chips and 2 for boiled and toasted chips, which supported the Waterhouse and Towey (1994) hypothesis that dehydratation will increase the oak lactone levels, specially *cis* isomer. The remaining compounds behaved in a similar way regardless of how the oak chips had been pretreated.

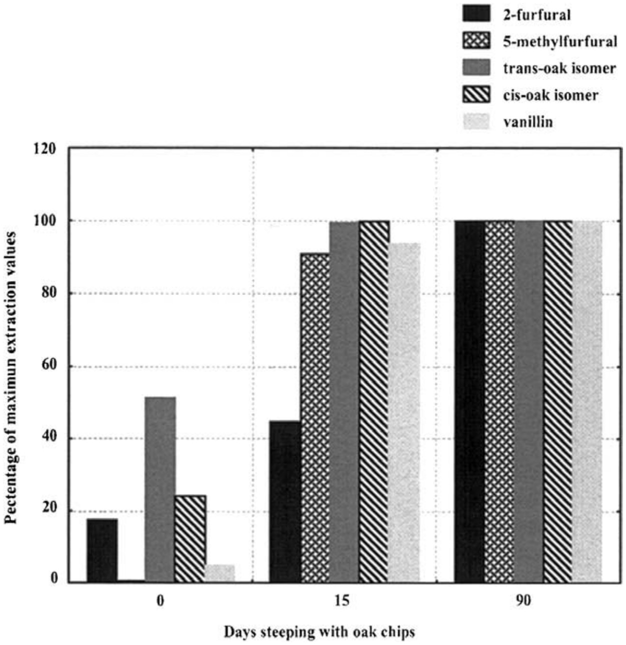

Fig. 4. Rate of extraction of different compounds in accelerated aging. The concentrations are expressed as an average percentage. To calculate these figures the high concentration of the compound found in each sample has been assigned the value of 100%.

3.3.3. New and re-used barrels

The degree of extraction was also compared in new and re-used barrels. We observed that the migration rate was higher for vanillin (Fig. 5) and oak isomers with re-used barrels, as was reported for white wines aged in oak barrels (Towey & Waterhouse, 1996b). Probably, in the second year, the vinegar penetrates as far as the most

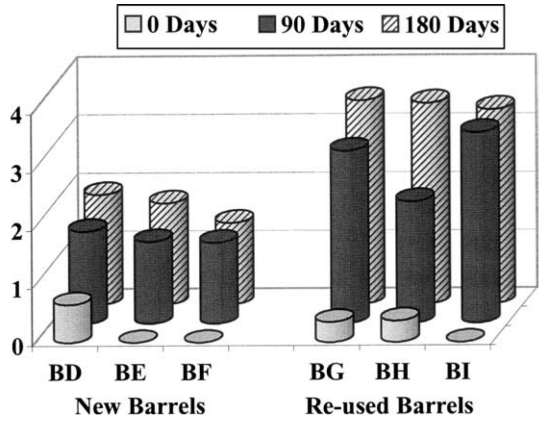

Fig. 5. Evolution of vanillin contents in wine vinegars aged in new and re-used barrels (axis *X*: concentration of vanillin in mg/l).

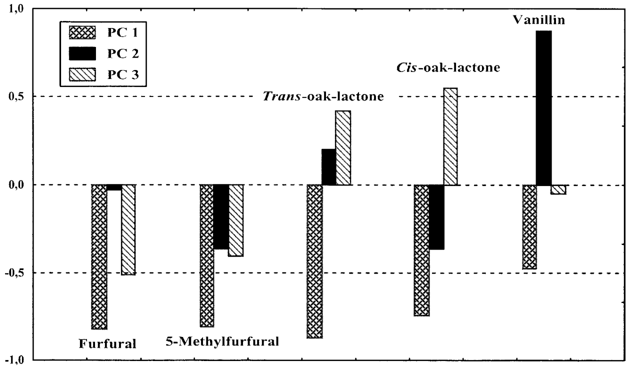

Fig. 6. Loading of volatile compounds on principal component 1–3.

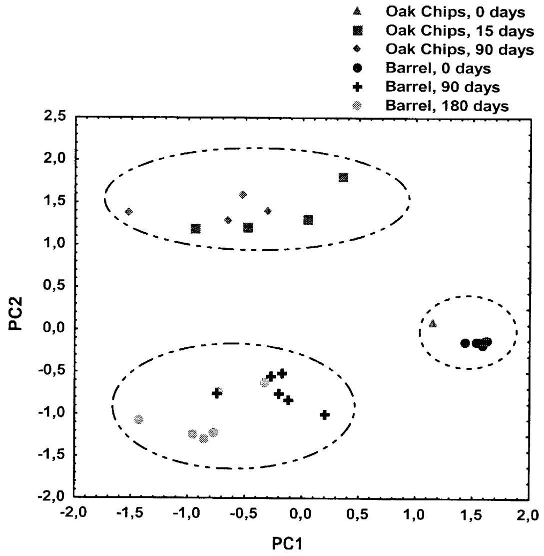

Fig. 7. Distribution of vinegar samples on the plane of the first two principal components.

internal layer of the wood barrel, which leads to a higher extraction level for these compounds.

On the other hand, slightly higher amounts of 5-methylfurfural were detected in vinegars aged in a new barrel (Table 4).

3.3.4. Effect of residual alcohol content

The residual alcohol degree of vinegars clearly influences their final content of volatile compounds, mainly ethyl acetate (Morales et al., 2002). However, this factor may not be involved in the release of volatile compounds from wood. In this respect, our experiment revealed no significant effects except for vanillin. When the correlation matrix is built with all the variables, the correlation between ethanol residual content and vanillin concentration in vinegars aged with chips was high ($r = 0.87$).

3.4. Principal components analysis

The PCA considered five variables and left out those that were absent in most of the samples studied (eugenol and furfuryl alcohol). Three PCs that accounted for 96.4% of the variance were chosen. Although factor number three has an eigenvalue of 0.89, we selected it so that the variable communalities would be higher than 0.5 (Table 5). The loadings of the first three PCs are shown in Fig. 6. The first component is mainly influenced by the *trans*-oak lactone isomer, furfural, 5-methylfurfural and the *cis*-oak-lactone isomer, and the second component is mainly influenced by vanillin. The score plot obtained by selecting the first two PCs as axes (Fig. 7) shows that the first component (PC1) makes it possible to differentiate between aged and non-aged vinegars. The second component (PC2) distinguishes between vinegars aged with oak chips and in barrels (accelerated versus traditional). Notice that PC2 is highly influenced by vanillin, the main marker of oak-chip aging.

Table 5
Eigenvalues, percentage of explained and accumulated variance of three factors obtained in PCA

Principal components	Eigenvalues	Explained variance (%)	Acumulated variance (%)
PC1	2.869	57.38	57.38
PC2	1.056	21.12	78.50
PC3	0.894	17.88	96.38

4. Conclusions

In general, we found more volatile compounds from wood in our vinegars (accelerated and traditional aging) than those reported in wines aged or fermented in barrels (white or red). The numbers of furfural, vanillin and oak-isomers were particularly high. In contrast, wines had a higher fufuryl alcohol concentration (Diaz-Plaza et al., 2002; Ferreras, Fernández, & Farqué, 2002; Garde et al., 2002a, 2002b; Pérez-Coello et al., 2000a; Pérez-Prieto et al., 2002; Salinas, Alonso, Pardo, & Bayonove, 1998; Towey & Waterhouse, 1996b). Thus, ethanol content of wine does not enhance migration volatile compounds of wood. Moreover the high acetic acid content of vinegar does not seem an obstacle to wood flavour compounds extraction.

Vanillin seems to be the main marker for oak-chip aging.

Oak chips are a valuable alternative to oak barrels to obtain, in a short period of time (15 days), wine vinegars with the peculiar characteristics given by oak wood.

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