

Concurrent Phenomena Contributing to the Formation of the Aroma of Wine during Aging in Oak Wood: An Analytical Study

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Red wine was stored in different oak barrels or in stainless steel, and samples were taken for two years to determine 79 aroma compounds. Aging in oak affects 41 compounds. The type of wood affects 11 compounds. At least seven different processes seem to take place concurrently in aroma evolution, and five such processes, affecting 37 compounds, are linked to the oak cask. These are extraction from the wood, oxidation of wine alcohols and amino acids, microbiological formation of ethyl phenols, sorption processes, and condensation of acetaldehyde with polyphenols. The wood can release linear γ - and δ -lactones, β -damascenone, and ionones. Some compounds are released very fast from wood, which suggests they lie in the external part of the wood. Some extraction profiles are too complex to be explained by physical processes. Finally, the levels of 2,5-dimethyl-4-hydroxy-3(2H)-furanone and 2-ethyl-5-methyl-4-hydroxy-3(2H)-furanone increase even in the reference wine, which suggests the presence of a precursor.

KEYWORDS: Wine; aroma; flavor; aging; aroma evolution; oak; GC-MS; furaneol; sotolon; homofuraneol; maltol; phenylacetaldehyde; gamma-lactones

INTRODUCTION

Aging in oak barrels is a very old tradition used to improve the sensory characteristics of wines and spirits. When aging in oak barrels, wine undergoes a series of processes that cause important improvements in wine aroma, color, taste, and astringency. The process of aging in oak barrels is very expensive and is not exempt from several risks, such as microbiological contamination with *Brettanomyces/Dekkera* (1, 2) or with bacteria (2–4) or the existence of irreproducible results (3, 5). Despite its importance, the process of aging wine in oak barrels is not yet fully understood, and in the wineries a part of the process is still considered as an art in which experience is more important than knowledge.

The effect of aging of wine in oak casks in the aroma formation and improvement has been the subject of numerous scientific studies (6–20). However, it must be acknowledged that most such scientific studies have focused almost exclusively on the role of oak wood as a source of extractable aromas. Furthermore, most studies control just 10 or a dozen compounds, all of them well-known wood odorants, and neglect the possible existence of other changes that could be also important from an aromatic point of view. Of course, the extraction of important odorants, particularly whisky lactones, volatile phenols, and vanillin, plays an outstanding role in the aroma of wood-aged wine, as has been repeatedly demonstrated (6–9, 21), but oak cask is an active recipient from physical, chemical, and

biochemical points of view, and the existence of numerous concurrent phenomena other than simple extraction acting on the aroma should be considered. A very good example has been recently shown by Ramírez et al. (22), who demonstrated that the wood is able to retain a significant part of wine aroma compounds.

The goals of the present paper are, first, to describe the changes in the aroma profile that take place during the maturation of a red wine in oak casks; second, to determine the existence of patterns in such changes, and third to link such patterns with the different physical, chemical, and microbiological processes concurring during the aging of wine in wood barrels.

MATERIAL AND METHODS

Wine. The red wine used in this study was made in year 2000 with grapes from the varieties Tempranillo (63%), Cabernet Sauvignon (17%), and Merlot (17%). The wine was made following standard winemaking practices. After fermentation, its pH was 3.55 and its alcoholic degree was 13.0% (v/v). The total volume of homogeneous wine used in the experiment was 15 000 L.

Oak Barrels. The 225 L barrels used in the experiment were made with American oak (*Quercus Alba* from Missouri) or French oak (*Quercus Sessilis* from Allier). The casks were made by Toneleria Intona, owned by Caja Rural de Navarra, one of the organizers of the experiment. The species of oak were determined by the suppliers but were not independently confirmed by us. The seasoning of the wood was carried out by storing the wood outdoors for 3 years. The barrels were toasted to a medium intensity (190–200 °C for 10 min).

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Wine Aging Experiment. Twelve new 225 L barrels of American oak and another 12 of French oak were filled with the wine in April 2001 and stored in a conditioned cellar. Each 12 barrel set was divided into 4 subsets. The wine from the first subsets was taken after 3 months of aging; the wine from the second subset was taken after 6 months; that from the third subset was taken after 12 months; and the fourth set was kept for 24 months in the barrel. For analysis, three 1.5 L samples were taken from each subset, one from each one of the barrels, and analyzed separately except in the cases of compounds determined following methods C and D. A large volume of the same wine was similarly stored in a 10 000 L stainless steel vat. Samples from this vat were taken at months 0, 3, 6, and 12. In all cases, immediately after sample extraction, samples were transported to the laboratory and analyzed. The three subsamples from each subset were analyzed immediately and separately for major and trace volatiles (analytical methods A and B). After these analyses, the remaining volumes of each of the three subsamples from each subset were collected together in amber flasks and kept frozen ($-25\text{ }^{\circ}\text{C}$) under nitrogen atmosphere for the rest of the analysis. Therefore, in the cases of compounds determined following methods C and D (see **Table 1**), the analysis was carried out on the composite sample obtained by mixing the three equivalent samples from three different barrels. These analyses were carried out simultaneously at the end of the experiment (April–June 2003).

Analytical Reagents and Chemical Standards. The chemical standards were supplied by Aldrich (Gillingham, UK), Fluka (Buchs, Switzerland), Sigma (St. Louis, USA), Lancaster (Strasbourg, France), PolyScience (Niles, USA), Chemservice (West Chester, USA), Interchim (Monlucon, France), International Express Service (Allauch, France), and Firmenich (Geneva, Switzerland), as is shown in **Table 1**.

LiChrolut EN resins, prepacked in 200 mg cartridges (6 mL total volume) or in bulk, were obtained from Merck (Darmstadt, Germany). Varian Bond Elut ENV resins prepacked in 200 mg cartridges were purchased from Varian (Walnut Creek, USA).

Dichloromethane, HPLC quality was obtained from Fisher Scientific (Loughborough, UK); methanol, LiChrosolv quality, was from Merck (Darmstadt, Germany), absolute ethanol (ACS quality), pentane, potassium hydrogen phthalate, sodium hydrogen carbonate, and ammonium sulfate were from Panreac (Barcelona, Spain) and were ARG; pure water was obtained from a Milli-Q purification system (Millipore, USA).

The BHA (3-*tert*-butyl-4-hydroxyanisole) solution contained 10 mg of this compound per gram of ethanol. Semiautomated solid-phase extraction was carried out with a VAC ELUT 20 station from Varian (Walnut Creek, USA).

Analysis. Four different analytical methods were used. All of them have been developed and validated in our laboratory and have undergone extensive testing before their use. Such testing included the definition of acceptance criteria for the system suitability and for the determination of the validity of the analysis. The list of compounds analyzed and the analytical methods used for their determination can be seen in **Table 1**.

(A) Major Compounds (Microextraction and GC-FID Analysis). Quantitative analysis of major compounds was carried out using the method proposed and validated by Ortega et al. (23). In accordance with this method, 3 mL of wine and 7 mL of water were salted with 4.5 g of ammonium sulfate and extracted with 0.2 mL of dichloromethane. The extract was then analyzed by GC (Hewlett-Packard 5890 series II) with FID detection using the conditions described elsewhere. Quantitative data were obtained by interpolation of relative peak areas in the calibration graphs built by the analysis of synthetic wines containing known amounts of the analytes. 2-Butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, and 2-octanol were used as internal standards and for quality control purposes. For quality control, acceptance regions for both absolute areas of each one of the internal standards and for relative areas to 4-methyl-2-pentanol were predetermined.

(B) Minor Compounds (SPE and GC–Ion Trap–MS Analysis). This analysis was carried out using the method proposed and validated by López et al. (24). In accordance with the method, 50 mL of wine,

containing 25 μL of BHA solution and 75 μL of a surrogate standards solution (surrogates were isopropyl propanoate, 3-octanone, heptanoic acid, and β -damascone), was passed through a 200 mg LiChrolut EN cartridge at about 2 mL/min. The sorbent was dried by letting air pass through (-0.6 Bar , 10 min). Analytes were recovered by elution with 1.3 mL of dichloromethane. An internal standard solution (2-octanol and 4-hydroxy-4-methyl-2-pentanone in dichloromethane) was added to the eluted sample. The extract was then analyzed by GC (Star 3400CX from Varian) with Ion Trap MS detection (Saturn 4 from Varian) under the conditions described in reference. Quality control: acceptance regions for absolute areas of the internal standards and for the relative areas of the surrogates to 2-octanol were predefined.

(C) Furanol (2,5-Dimethyl-4-hydroxy-3(2H)-furanone), Homofuranol (2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone) Maltol (3-Hydroxy-2-methyl-4-pyrone), and sotolon (4,5-dimethyl-3-hydroxy-2(5H)-furanone). (SPE and GC–Ion Trap–MS analysis). This analysis was carried out using the method proposed and validated in ref 25. In accordance with the method, 50 mL of wine (to which 7.5 g of ammonium sulfate had been previously added) were loaded into a SPE bed formed by 800 mg of LiChrolut EN resins packed in a 6 mL filtration tube from Supelco (Madrid, Spain). The bed was washed with 5 mL of water first, then dried, and finally washed with 15 mL of a mixture pentane/dichloromethane (20/1). Analytes were eluted with 6 mL of dichloromethane, and this volume was spiked with 50 μL of the internal standard solution (67 mg L^{-1} of 2-octanol in dichloromethane). This volume was concentrated to 100 μL by evaporation in a centrifuge tube heated at $47\text{ }^{\circ}\text{C}$ and analyzed by GC–Ion Trap MS under the conditions described in reference. Quality control: one of the wine samples per batch (composed usually of six wine samples) was spiked with an ethanolic solution containing the analytes and analyzed following the procedure. The increment of the signal (MS area normalized to that of 2-octanol) obtained in the analysis of the spiked sample was compared with that obtained in the analysis of a dichloromethane solution containing known amounts of analytes. Acceptance regions for such increments were predefined.

(D) Minor Aliphatic γ - and δ -Lactones γ -Octa, Nona, Deca, Undeca, and Dodecalactones and δ -Decalactone (SPE and GC–Ion Trap–MS Analysis). This analysis was carried out following the procedure indicated in ref 26. Following such procedure, prepacked cartridges (3 mL total volume) filled with 200 mg of Bond Elut ENV resins were placed in the extraction system and conditioned by rinsing with 2 mL of methanol and 4 mL of water. Fifty milliliters of wine were passed through the SPE cartridge at 2 mL/min. The bed was then washed with 5 mL of water, and the interferences were removed with 20 mL of a mixture of methanol/water 40% (v/v) enriched with 1% (w/v) NaHCO_3 . The cleaned cartridge was dried by letting air pass through (30 min). Analytes were recovered by elution with 1.8 mL of dichloromethane. This volume, placed in a centrifuge test tube, was spiked with 100 μL of the internal standard solution (49 mg L^{-1} of 2-octanol in dichloromethane) and was further concentrated to 0.15 mL in a water bath at $47\text{ }^{\circ}\text{C}$. The volume was finally transferred to a microvial, sealed, and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Calibration graphs were prepared by the GC-MS analysis of dichloromethane solutions containing known amounts of the standards and of the internal standard. Quality control was carried out by the analysis of a spiked sample per batch (six wine samples), as indicated in method C.

Data Treatment. To determine if the evolution with time in the oak barrel follows a significant trend and to evaluate the influence of the type of wood a two-way analysis of variance was carried out for each of the 70 aroma compounds determined, by methods A and B. The factors were aging time (with levels 3, 6, 12, or 24) and type of wood (French or American oak). This study was carried out with the statistical package SPSS for windows, vs 10.0.

RESULTS AND DISCUSSION

The main goal of the present paper is to describe the different phenomena that affect wine aroma composition concurrently during the aging of red wine in oak barrels. To do this, the aroma composition of a red wine aged in two different types of oak barrels was monitored during two years and compared with

Table 1. Compounds Analyzed in the Present Work, Suppliers, and Data about Their Analysis^a

	analyte	chemical standard	internal standard	GC-FID	GC-MS (m/z)	method ^b	
aldehyde ketone	acetaldehyde	Aldrich	2B	+		1	
	3-hydroxy-2-butanone (acetoin)	Aldrich	4M2P	+		1	
	2,3-butanedione (diacetyl)	Aldrich	2B	+		1	
	2-furaldehyde (furfural)	Chemservice	2O		95	2	
	phenylacetaldehyde	Aldrich	4O4M2P		91	2	
	5-methyl-2-furaldehyde (5-methylfurfural)	Fluka	2O		97	2	
	5-hydroxymethylfuraldehyde (5-hydroxymethylfurfural)	Fluka	4O4M2P		109	2	
	vanillin	Polyscience	2O		151+152	2	
	acetovanillone	Aldrich	2O		151+166	2	
	syringaldehyde	Aldrich	2O		182	2	
	β -damascenone	Firmenich	2O		121	2	
	α -ionone	Sigma	2O		121	2	
	β -ionone	Sigma	2O		177	2	
acids	butyric acid	Polyscience	4H4M2P	+		1	
	isovaleric acid	Aldrich	4H4M2P	+		1	
	hexanoic acid	Polyscience	2O	+		1	
	octanoic acid	Fluka	2O	+		1	
	decanoic acid	Polyscience	2O	+		1	
	2-methylbutyric acid	Aldrich	4O4M2P		74	2	
	phenylacetic acid	Aldrich	2O		91	2	
	benzoic acid	Aldrich	2O		105 + 122	2	
	ethyl isobutyrate	Aldrich	4M2P		115	2	
	ethyl 3-methylbutyrate	Aldrich	4M2P		85+88+87	2	
ester	ethyl 3-hydroxybutyrate	Aldrich	4H4M2P	+		1	
	butyl acetate	Polyscience	2O		56+61	2	
	ethyl acetate	Polyscience	4M2P	+		1	
	isobutyl acetate	Chemservice	4M2P		56+61	2	
	isoamyl acetate	Chemservice	4M2P2	+		1	
	phenylethyl acetate	Chemservice	2O		104	2	
	ethyl butyrate	Aldrich	4M2P	+		1	
	ethyl hexanoate	Polyscience	2O	+		1	
	ethyl octanoate	Polyscience	2O	+		1	
	ethyl decanoate	Polyscience	2O		RIC	2	
	ethyl lactate	Aldrich	4H4M2P	+		1	
	diethyl succinate	Fluka	2O	+		1	
	ethyl furoate	Chemservice	2O		95	2	
	ethyl dihydrocinnamate	Fluka	2O		104	2	
	ethyl cinnamate	Fluka	2O		131	2	
	ethyl vanillate	Lancaster	2O		151+196	2	
	methyl vanillate	Lancaster	2O		151+182	2	
	ethyl 2-methylbutyrate	Fluka	2O		102	2	
	terpenes	linalool	Aldrich	4O4M2P		93+121+136	2
		β -citronellol	Aldrich	2O		123	2
α -terpineol		Fluka	2O		121+136	2	
lactones	Z-whiskylactone	Aldrich	2O		99	2	
	E-whiskylactone	Aldrich	2O		99	2	
	δ -octalactone	Aldrich	2O		99	4	
	γ -octalactone	Aldrich	2O		85	4	
	γ -nonalactone	Aldrich	2O		85	4	
	δ -decalactone	Lancaster	2O		99	4	
	γ -decalactone	Fluka	2O		85	4	
	γ -undecalactone	Fluka	2O		85	4	
	γ -dodecalactone	Aldrich	2O		85	4	
	4,5-dimethyl-3-hydroxy-2-(5h)-furanone (sotolon)	Aldrich	2O		83	3	
	3-hydroxy-2-methyl-4-pyrone (maltol)	Fluka	2O		126	3	
	2,5-dimethyl-4-hydroxy-3(2h)-furanone (furaneol)	Aldrich	2O		81	3	
	2-ethyl-5-methyl-4-hydroxy-3(2h)-furanone (homofuraneol)	Aldrich	2O		142	3	
phenols	γ -butyrolactone	Aldrich	4H4M2P	+		1	
	2-methoxyphenol (guaiacol)	Aldrich	4O4M2P		109+124	2	
	4-allyl-2-methoxyphenol (eugenol)	Aldrich	2O		164	2	
	E-isoeugenol	Aldrich	2O		164	2	
	4-ethylphenol	Aldrich	2O		107+122	2	
	4-ethylguaiacol	Lancaster	4O4M2P		137	2	
	4-vinylphenol	Lancaster	4O4M2P		120	2	
	4-vinylguaiacol	Lancaster	2O		135+150	2	
	4-propylguaiacol	Lancaster	2O		137+166	2	
	2,6-dimethoxyphenol	Aldrich	2O		154	2	
	4-allyl-2,6-dimethoxyphenol	Aldrich	2O		194	2	
	m-cresol	Aldrich	2O		107	2	
	o-cresol	Aldrich	2O		108	2	

Table 1. Continued

	analyte	chemical standard	internal standard	GC-MS		method ^b
				GC-FID	(m/z)	
alcohols	furfuryl alcohol	Fluka	4O4M2P		98	2
	3-methylthio-1-propanol (methionol)	Aldrich	2O		105+106	2
	2-methyl-1-propanol	Merck	2B	+		1
	isoamyl alcohol	Aldrich	2B	+		1
	β -phenylethanol	Fluka	2O	+		1
	1-hexanol	Sigma	4M2P	+		1
	z-3-hexen-1-ol	Aldrich	4M2P	+		1
	benzyl alcohol	Aldrich	4H4M2P	+		1

^a Internal standard. 4M2P, 4-methyl-2-pentanol (Polyscience). 2O, 2-octanol (Polyscience). 4H4M2P, 4-hydroxy-4-methyl-2-pentanone (Aldrich). 2B, 2-butanol (Merck).
^b (1) Method for major compounds, (2) method for minor compounds, (3) method for enolons, (4) method for minor aliphatic lactones.

a reference of the same wine stored in a stainless steel vat. Seventy-nine aroma compounds were determined, although a complete set of replicates existed only for 70 compounds, as detailed in Materials and Methods. The 70 (compounds) \times 3 (barrels) \times 4 (time points) \times 2 (types of barrel) = 1680 data points were first processed by two-way ANOVA to determine which changes were statistically significant and whether the type of barrel had any influence. The results of such a study are given in **Table 2** and, as can be seen, 30 out of the 70 aroma compounds undergo significant changes during the maturation in oak barrels. Changes were dependent on the type of barrel (American or French oak) in 11 cases, and the interaction between factors was significant in 7 cases. In addition, there are some compounds not appearing in **Table 2** but for which important differences between the wine stored in wood and the reference wine stored in stainless steel can be observed. In this group, furfural, guaiacol, some γ and δ C8–C12 lactones, and polar compounds such as furaneol, homofuraneol, maltol, and sotolon, can be found. As a result, the compounds affected by the aging in wood are the 41 compounds whose concentrations can be seen in **Table 3** and their corresponding standard deviations in **Table 4**. Changes affected not only the chemical compounds usually classified as wood-extractable but many other wine aroma compounds with other origins, which confirm the existence of different concurrent phenomena other than simple extraction deeply affecting wine aroma composition.

A detailed study of the different trends followed by such 41 compounds during the experiment, together with the basic knowledge of wine chemistry, and the specific scientific literature on this topic, allows us to classify the compounds whose concentration changes during the aging process into the following general categories:

(i) Genuine wood-extractable compounds. These are the compounds whose level after the maturation is always highest in the wine stored in cask, as a likely consequence of the extraction of the compounds from the wood into the wine. To this category belong the compounds usually considered wood-extractable.

(ii) Compounds likely extracted from the surface of wood. These are the compounds whose level after three months of storage is always highest in any of the wines stored in wood, but from this point there are not additional increments.

(iii) Compounds extracted from wood but also released or formed from precursors in the wine. These are the compounds that partly come from the wood, but a significant part is also formed in the wine, without having any contact with wood.

(iv) Compounds released or formed by precursors in wine, which are the compounds formed by the slow hydrolysis (or other reaction) of nonvolatile precursors (glycosides and probably others) in processes not influenced by the wood.

Table 2. Two-Way ANOVA Carried out on Quantitative Data to Analyze the Effect of the Aging and of the Type of Wood (American or French Oak)

	Factor 1 ^a		Factor 2 ^b		interaction ^c	
	F-value	P-value ^d	F-value	P-value	F-value	P-value ^d
aldehydes and ketones						
acetaldehyde	17.1	0*	3.451	0.082	4.585	0.017*
phenyl-acetaldehyde	106	0*	0.671	0.425	0.481	0.631
5-methylfurfural	12.5	0*	0.330	0.574	0.203	0.893
furfural	1.66	0.214	2.716	0.119	0.711	0.559
syringaldehyde	50.9	0*	4.663	0.046*	1.378	0.286
vanillin	70.9	0*	0.061	0.808	1.105	0*
acetovanillone	140	0*	5.106	0.038*	0.273	0.844
β -damascenone	532	0*	5.375	0.034*	1.656	0.216
β -ionone	20.0	0*	0.485	0.496	1.852	0.178
lactones						
E-whiskylactone	17.29	0*	54.446	0*	5.427	0.009*
Z-whiskylactone	103	0*	48.393	0*	14.17	0*
γ -butyrolactone	3.41	0.043*	0.035	0.854	0.154	0.925
phenols						
4-propylguaiacol	15.8	0*	1.769	0.202	0.608	0.619
2,6-dimethoxyphenol	5.28	0.010*	8.849	0.009*	0.139	0.935
eugenol	94.2	0*	1.547	0.232	3.319	0.047*
4-allyl-2,6-dimethoxyphenol	28.5	0*	5.407	0.034*	0.625	0.609
m-cresol	16.8	0*	44.095	0.000*	2.786	0.074
4-ethylguaiacol	793	0*	0.015	0.903	2.293	0.117
4-ethylphenol	692	0*	0.000	1.000	3.901	0.029*
alcohols						
1-hexanol	52.6	0*	6.381	0.022*	1.150	0.359
β -phenylethanol	8.13	0.002*	0.514	0.484	1.302	0.308
methionol	6.07	0.006*	1.257	0.279	1.095	0.380
acids						
hexanoic acid	9.87	0.001*	0.084	0.775	1.029	0.406
butyric acid	6.95	0.003*	0.050	0.825	0.725	0.552
isovaleric acid	3.64	0.035*	2.157	0.161	1.275	0.316
octanoic acid	48.2	0*	5.961	0.027*	0.159	0.923
esters						
methyl vanillate	279	0*	7.140	0.017*	2.223	0.125
ethyl vanillate	1028	0*	0.038	0.848	6.390	0.005*
ethyl 3-methylbutyrate	419	0*	1.803	0.198	1.759	0.195
isoamyl acetate	39.2	0*	0.767	0.394	1.719	0.203
terpenes						
linalool	179	0*	2.103	0.166	0.275	0.842

^a Factor 1, aging time (with levels 3, 6, 12, and 24 months). ^b Factor 2, type of wood (French or American oak). ^c Interaction, interaction between time of maturation \times type of oak. ^d An asterisk (*) indicates that $p < 0.05$, significant change.

(v) Compounds formed by microbiological action on wine precursors.

Table 3. Quantitative Data of the Compounds Whose Concentration Was Found to Change during the Aging or Because of the Presence of Wood

(i) Genuine Extractable Compounds									
Compound ($\mu\text{g/L}$)	3m	6m	12m	24m	Compound ($\mu\text{g/L}$)	3m	6m	12m	24m
furfural ($\mu\text{g/L}$)					5-methylfurfural ($\mu\text{g/L}$)				
American oak	239	150	232	74.6	American oak	446	878	488	319
French oak	101	145	127	62.2	French oak	325	864	439	345
stainless steel	12.0	19.6	8.76		stainless steel	0.7	1.76	4.18	
Z-whiskylactone ($\mu\text{g/L}$)					E-whiskylactone ($\mu\text{g/L}$)				
American oak	113	473	845	1122	American oak	51.4	102	152	119
French oak	95.8	373	629	548	French oak	91.8	308	412	232
stainless steel	3.1	0.65	n.d.		stainless steel	0.4	1.73	n.d.	
eugenol ($\mu\text{g/L}$)					4-propylguaiaicol ($\mu\text{g/L}$)				
American oak	17.2	65.5	81.5	128	American oak	4.87	31.2	50.6	51.9
French oak	17.4	60.2	91.8	101	French oak	4.55	31.0	35.1	42.6
stainless steel	2.6	4.92	4.73		stainless steel	n.d.	0.49	0.45	
2,6-dimethoxyphenol ($\mu\text{g/L}$)					4-allyl-2,6-dimethoxyphenol ($\mu\text{g/L}$)				
American oak	53.4	65.4	89.6	86.8	American oak	13.6	25.2	48.8	69.4
French oak	36.7	40.5	62.4	69.7	French oak	9.68	20.8	36.7	52.2
stainless steel	41.9	20.1	24.1		stainless steel	2.5	2.95	4.69	
m-cresol ($\mu\text{g/L}$)					α -cresol ($\mu\text{g/L}$)				
American oak	2.02	2.02	2.52	3.56	American oak	1.72	3.06	4.63	5.23
French oak	1.54	1.35	1.76	2.11	French oak	1.72	2.58	3.72	3.65
stainless steel	1.8	1.40	1.29		stainless steel	1.70	2.15	2.85	
syraldehyde ($\mu\text{g/L}$)					vanillin ($\mu\text{g/L}$)				
American oak	3.69	233	66.3	399	American oak	32.6	291	165	453
French oak	3.68	138	60.5	311	French oak	37.9	240	214	430
stainless steel	3.7	5.91	1.33		stainless steel	2.4	7.8	3.7	
γ -octalactone ($\mu\text{g/L}$)					maltol ($\mu\text{g/L}$)				
American oak	0.63	3.04	3.00	1.79	American oak	78.0	119	135	185
French oak	n.d.	1.91	1.48	1.61	French oak	76.1	126	119	137
stainless steel	0.85	n.d.	n.d.		stainless steel	43.1	36.9	22.2	
γ -nonalactone ($\mu\text{g/L}$)					γ -decalactone ($\mu\text{g/L}$)				
American oak	10.1	19.1	22.1	15.6	American oak	0.61	2.20	1.70	2.49
French oak	16.1	16.6	20.4	16.7	French oak	0.90	1.04	1.17	1.02
stainless steel	19.6	19.2	8.9		stainless steel	1.13	0.90	0.50	
guaiaicol ($\mu\text{g/L}$)					δ -decalactone ($\mu\text{g/L}$)				
American oak	22.9	17.7	22.4	23.3	American oak	7.66	14.1	18.3	12.1
French oak	15.8	10.7	11.8	15.7	French oak	35.8	16.3	19.8	19.5
stainless steel	8.10	2.67	2.40		stainless steel	23.2	19.9	8.71	
(ii) Compounds Likely Extracted from the Surface of the Wood									
butyric acid (mg/L)					hexanoic acid (mg/L)				
American oak	2.49	1.74	0.66	1.18	American oak	3.02	2.65	1.99	2.30
French oak	2.06	1.35	0.57	1.05	French oak	2.70	2.56	2.04	2.53
stainless steel	0.6	1.69	1.21		stainless steel	2.2	2.28	2.37	
octanoic acid (mg/L)					isovaleric acid (mg/L)				
American oak	2.24	2.35	1.59	1.38	American oak	2.02	2.05	1.35	1.41
French oak	2.40	2.56	1.81	1.48	French oak	3.70	2.15	1.69	1.35
stainless steel	2.0	2.32	2.68		stainless steel	2.5	2.23	2.27	
methionol (mg/L)					β -phenylethanol (mg/L)				
American oak	8.08	7.81	6.33	3.49	American oak	75.9	71.0	55.6	57.7
French oak	13.3	8.02	6.51	3.37	French oak	70.0	73.1	58.0	67.1
stainless steel	8.1	8.64	8.21		stainless steel	54.5	67.0	65.5	
γ -butyrolactone (mg/L)									
American oak	31.9	33.9	28.8	19.8					
French oak	37.1	33.2	28.6	18.4					
stainless steel	6.4	37.1	30.7						
(iii) Compound Extracted from Wood and Released from Precursors									
β -damascenone ($\mu\text{g/L}$)					β -ionone ($\mu\text{g/L}$)				
American oak	2.06	4.83	4.76	2.85	American oak	0.13	0.29	0.44	0.28
French oak	1.99	4.47	4.73	2.77	French oak	0.13	0.33	0.43	0.24
stainless steel	2.2	4.13	3.99		stainless steel	0.1	0.32	0.30	
ethyl vanillate ($\mu\text{g/L}$)					methyl vanillate ($\mu\text{g/L}$)				
American oak	28.1	86.2	126	134	American oak	6.63	18.1	24.9	24.5
French oak	27.3	84.6	136	125	French oak	6.35	16.7	24.7	21.1
stainless steel	34.0	92.9	105.2		stainless steel	7.2	18.0	19.8	
acetovanillone ($\mu\text{g/L}$)					furaneol ($\mu\text{g/L}$)				
American oak	30.1	95.9	125	120	American oak	78.9	141	143	105
French oak	26.1	83.3	119	110	French oak	68.9	136	146	124
stainless steel	26.1	66.3	74.2		stainless steel	86.3	92.8	123	
(iv) Compounds Formed from Precursors in Wine									
linalool ($\mu\text{g/L}$)					homofuraneol ($\mu\text{g/L}$)				
American oak	4.45	10.6	10.8	8.65	American oak	36.5	41.8	44.2	51.3
French oak	4.39	10.4	10.3	8.24	French oak	36.9	43.9	47.3	41.8
stainless steel	5.3	10.5	10.5		stainless steel	40.3	38.2	45.5	

Table 3. Continued

(v) Compounds Formed by Microbiological Action on Wine Precursors									
4-ethylphenol ($\mu\text{g/L}$)					4-ethyl-guaiacol ($\mu\text{g/L}$)				
American oak	32.9	406	240	471	American oak	8.83	80.7	95.1	96.2
French oak	55.3	372	263	459.8	French oak	11.1	74.1	98.3	96.74
stainless steel	1.1	233	267		stainless steel	2.9	36.2	45.0	
(vi) Compounds Formed by the Oxidation Taking Place Exclusively in the Oak Cask									
sotolon ($\mu\text{g/L}$)					phenylacetaldehyde ($\mu\text{g/L}$)				
American oak	3.15	2.97	3.88	6.61	American oak	n.d.	3.28	7.15	6.64
French oak	2.97	4.94	4.23	4.37	French oak	n.d.	n.d.	6.84	6.84
stainless steel	2.70	1.37	1.76		stainless steel	n.d.	n.d.	1.93	
(vii) Compounds Disappearing Due to the Oxidation Taking Place Exclusively in the Oak Cask ^a									
1-hexanol (mg/L)									
American oak			3.54		3.52	2.69		1.85	
French oak			3.96		3.59	3.26		1.95	
stainless steel			3.7		3.47	3.72			
See also β -phenylethanol and methionol in category ii									
(viii) Compounds Disappearing Due to Condensation with Compounds Extracted from Wood									
acetaldehyde (mg/L)									
American oak			114		49.3	36.5		2.44	
French oak			49.2		56.6	33.1		4.87	
stainless steel			56.0		46.6	57.2			
(ix) Compounds Disappearing Due to Sorption Processes									
See butyric, hexanoic, octanoic and isovaleric acid in category ii									
(x) Compounds Whose Concentration Changes Due to Acid+Alcohol/Ester Equilibria									
ethyl-2-methylbutyrate ($\mu\text{g/L}$)					isoamyl acetate (mg/L)				
American oak	3.9	11.9	22.5	18.4	American oak	0.79	1.02	0.31	0.33
French oak	3.9	12.5	21.1	17.1	French oak	1.05	0.93	0.35	0.32
stainless steel	4.2	12.1	18.4		stainless steel	1.1	0.98	0.68	

^a Also see β -phenylethanol and methionol in category ii.

(vi) Compounds formed by the oxidation taking place exclusively in the oak cask (and not at all or in smaller proportion in the stainless steel vat).

(vii) Compounds disappearing due, most likely, to the oxidation taking place exclusively in the oak cask.

(viii) Compounds disappearing due to condensation with compounds extracted from wood.

(ix) Compounds disappearing in the wines stored in wood as a likely consequence of sorption processes (on the wood or induced by the storage in wood).

(x) Compounds whose concentration changes due to acid + alcohol/ester equilibria.

Of course, it must be understood that the previous categories are not totally exclusive and that in some cases the same compound has to be included in two or even three different categories. This is particularly true in the case of some fatty acids that follow complex trends and may be classified in categories ii, ix, and x.

Quantitative data in Table 3 are organized following the previous categories. The discussion will also follow such order and will focus exclusively on aspects not previously reported in the scientific literature.

(i) Genuine Wood-Extractable Compounds. Here well-known wood compounds are found. Among them, 5-methylfurfural, furfural, *E*- and *Z*-whiskylactones, eugenol, guaiacol, 4-propylguaiacol, 2,6-dimethoxyphenol, 4-allyl-2,6-dimethoxyphenol, *m*-cresol, *o*-cresol, maltol, vanillin, and syringaldehyde. These are the compounds that are usually considered in the different studies on wood aging (6–8, 10, 12, 13, 15–20, 27–29).

However, as shown in Table 3, several aliphatic lactones (γ -octa, nona, and decalactones and δ -decalactone) also seem to be wood-extractable compounds. In general, their levels steadily decay in the wine stored in stainless steel, while they slightly increase in the wines stored in oak casks, never reaching very high levels. The behavior can be exemplified by γ -octalactone, shown in Figure 1a. This behavior is consistent with a recent report in which these compounds were found to be at highest concentration in aged red wines, in comparison with young reds or whites (26). The origin of these components in wine has not been studied in depth. In malt whisky, it has been shown that they derive from the sequential action of lactic acid bacteria and Brewer's yeast on unsaturated fatty acids (30). This origin can explain the levels found in young wines, but the role of the oak cask in their formation remains unclear. A compound following a similar trend is maltol, whose presence in dry wine is usually attributed to the wood. However, as is shown in Table 3, it is naturally found in relatively high amounts in the reference wine.

In most cases, the extraction of the compounds of this group with time follows trends consistent with those reported in the literature, although there are some remarkable differences. First, it has been found that the levels of *E*-whiskylactone in both kinds of wood and of *Z*-whiskylactone in French oak barrels tend to decrease in the second year of aging in wood (Figure 1b and Table 3), which indicates that these molecules are degraded, as observed also for the other aliphatic γ -lactones. A recent report supports this observation (14). Second, the *Z/E* ratio changes with time, as has been previously observed (7). The pattern of variation is, however, different from that

Table 4. Standard Deviations of the Compounds Whose Concentration Was Found to Change during the Aging or Because of the Presence of Wood

(i) Genuine Extractable Compounds									
	3m	6m	12m	24m		3m	6m	12m	24m
furfural ($\mu\text{g/L}$)					5-methylfurfural ($\mu\text{g/L}$)				
American oak	229	68	62	15	American oak	96	245	33	153
French oak	56	49	82	21	French oak	71	297	125	159
Z-whiskylactone ($\mu\text{g/L}$)					E-whiskylactone ($\mu\text{g/L}$)				
American oak	24	84	72	120	American oak	8.6	24	17	11
French oak	7.9	47	53	136	French oak	20	44	119	58
eugenol ($\mu\text{g/L}$)					4-propylguaiacol ($\mu\text{g/L}$)				
American oak	2.3	13	5.1	14	American oak	2.8	9.8	2.2	24
French oak	1.5	2.1	3.7	21	French oak	0.19	9.0	16	10
2,6-dimethoxyphenol ($\mu\text{g/L}$)					4-allyl-2,6-dimethoxyphenol ($\mu\text{g/L}$)				
American oak	7.4	26	18	27	American oak	3.5	6.3	3.4	20
French oak	2.6	7.5	21	14	French oak	1.0	4.6	14	9.9
m-cresol ($\mu\text{g/L}$)					o-cresol ($\mu\text{g/L}$)				
American oak	0.12	0.13	0.20	0.26	American oak	0.59	0.21	0.48	0.1
French oak	0.49	0.16	0.32	0.51	French oak	0.10	0.29	0.45	0.78
syringaldehyde ($\mu\text{g/L}$)					vanillin ($\mu\text{g/L}$)				
American oak	0.01	92	31	97	American oak	3.7	50	40	31
French oak	0.01	14	55	25	French oak	19	24	110	30
γ -octalactone ($\mu\text{g/L}$)					maltol ($\mu\text{g/L}$)				
American oak					American oak				
French oak					French oak				
γ -nonalactone ($\mu\text{g/L}$)					γ -decalactone ($\mu\text{g/L}$)				
American oak					American oak				
French oak					French oak				
guaiacol ($\mu\text{g/L}$)					δ -decalactone ($\mu\text{g/L}$)				
American oak	8.3	6.0	3.3	5.2	American oak				
French oak	0.33	2.3	4.5	3.2	French oak				
(ii) Compounds Likely Extracted from the Surface of the Wood									
butyric acid (mg/L)					hexanoic acid (mg/L)				
American oak	1.6	0.37	0.28	0.68	American oak	0.43	0.52	0.15	0.24
French oak	0.72	0.02	0.21	0.49	French oak	0.21	0.03	0.17	0.14
octanoic acid (mg/L)					isovaleric acid (mg/L)				
American oak	0.27	0.21	0.18	0.13	American oak	0.25	0.21	0.23	0.06
French oak	0.18	0.10	0.17	0.01	French oak	2.4	0.03	0.03	0.03
methionol (mg/L)					β -phenylethanol (mg/L)				
American oak	1.01	1.2	0.60	0.03	American oak	13	6.5	6.9	4.2
French oak	8.3	0.42	0.12	0.30	French oak	3.9	1.13	6.6	4.7
γ -butyrolactone (mg/L)									
American oak	13	3.8	2.2	1.1					
French oak	22	2.9	1.1	2.6					
(iii) Compounds Extracted from Wood and Released from Precursors									
β -damascenone ($\mu\text{g/L}$)					β -ionone ($\mu\text{g/L}$)				
American oak	0.07	0.33	0.08	0.11	American oak	0.01	0.04	0.03	0.08
French oak	0.01	0.14	0.09	0.08	French oak	0.01	0.02	0.03	0.02
ethyl vanillate ($\mu\text{g/L}$)					methyl vanillate ($\mu\text{g/L}$)				
American oak	0.24	2.0	1.5	2.5	American oak	0.09	0.80	0.72	0.72
French oak	0.41	1.8	0.39	9.7	French oak	0.13	0.74	1.5	2.6
acetovanillone ($\mu\text{g/L}$)					furaneol ($\mu\text{g/L}$)				
American oak	1.3	9.2	7.2	9.6	American oak				
French oak	0.68	6.0	17	8.9	French oak				
(iv) Compounds Formed from Precursors in Wine									
linaol ($\mu\text{g/L}$)					homofuraneol ($\mu\text{g/L}$)				
American oak	0.12	0.84	1.19	0.35	American oak				
French oak	0.12	0.25	0.32	1.1	French oak				
(v) Compounds Formed by Microbiological Action on Wine Precursors									
4-ethylphenol ($\mu\text{g/L}$)					4-ethyl-guaiacol ($\mu\text{g/L}$)				
American oak	27	15	8.0	23	American oak	4.2	4.3	3.4	3.2
French oak	11	3.9	24	9.9	French oak	1.4	1.4	4.8	4.1
(vi) Compounds Formed by the Oxidation Taking Place Exclusively in the Oak Cask									
sotolon ($\mu\text{g/L}$)					phenylacetaldehyde ($\mu\text{g/L}$)				
American oak					American oak	1.9	0.96	0.29	
French oak					French oak		0.92	1.0	
(vii) Compounds Disappearing Due to the Oxidation Taking Place Exclusively in the Oak Cask ^a									
1-hexanol (mg/L)									
American oak			0.13				0.31		0.10
French oak			0.14				0.12		0.03
See also β -phenylethanol and methionol in category ii									

Table 4. Continued

(viii) Compounds Disappearing Due to Condensation with Compounds Extracted from Wood									
acetaldehyde (mg/L)									
American oak	52			5.5		8.0			0.39
French oak	12			3.3		3.8			1.0
(ix) Compounds Disappearing Due to Sorption Processes See butyric, hexanoic, octanoic and isovaleric acid in category ii									
(x) Compounds Whose Concentration Changes Due to Acid+Alcohol/Ester Equilibria									
ethyl-2-methylbutyrate ($\mu\text{g/L}$)									
American oak	0.36	2.0	0.21	1.1	isoamyl acetate (mg/L)				
French oak	0.10	0.80	0.57	0.76	American oak	0.33	0.06	0.03	0.03
					French oak	0.03	0.18	0.11	0.02

^a Also see β -phenylethanol and methionol in category ii.

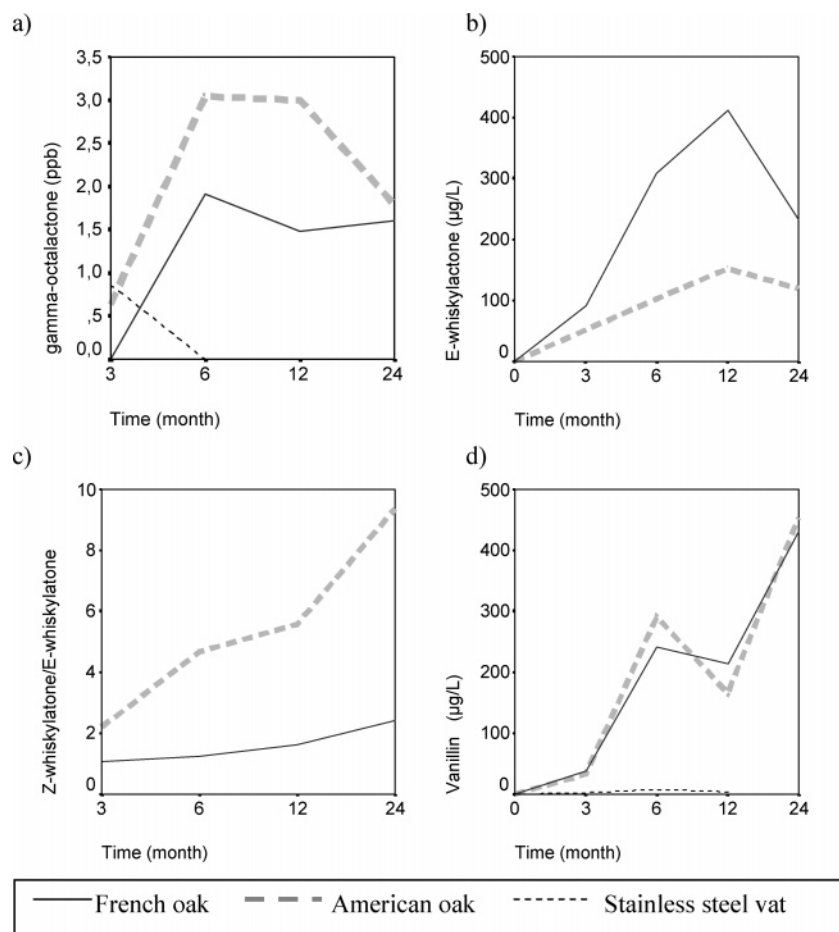


Figure 1. Concentration vs time plots in three different aging conditions (American oak, French oak, or stainless steel vat) for some wood-extractable compounds: (a) γ -octalactone; (b) E-whiskylactone; (c) Z/E isomeric ratio; (d) vanillin.

previously reported, as can be seen in **Figure 1c**. The Z/E ratio in the case of French oak ranges from 1.1 to 2.2, while in the case of American oak it ranges from 2.2 to 9.5. Third, the evolution with time of vanillin and syringaldehyde is quite complex, as can be seen in **Figure 1d** and **Table 3**. Other authors have also shown complex extraction profiles for these compounds (14). In our study, a relative minimum at 12 months, coincident with the end of winter, and a relative maximum at 6 months, coincident with the end of summer, are observed. This result suggests that the levels of these compounds are the result of a complex extraction, whose kinetics are probably related to the microbiological activity in wood and a degradation that is also related to microbiological reduction (11).

(ii) Compounds Likely Extracted from the Surface of Wood. A wide range of compounds seems to be released very fast by the wood into the wine. All of them are well-known byproducts of important metabolic pathways, which suggest that, during the process of natural seasoning of wood, the surface of the wood is partly colonized by different microorganisms able to produce volatile compounds that are finally released into the wine stored in such cask. In all cases, compounds are released in the first months, which mean that they lie in the most external part of the wood. Compounds belonging to this category are fatty acid metabolism byproducts, such as butyric, hexanoic, and octanoic acids, and some amino acid metabolism byproducts, such as isovaleric acid, methionol, β -phenylethanol, and

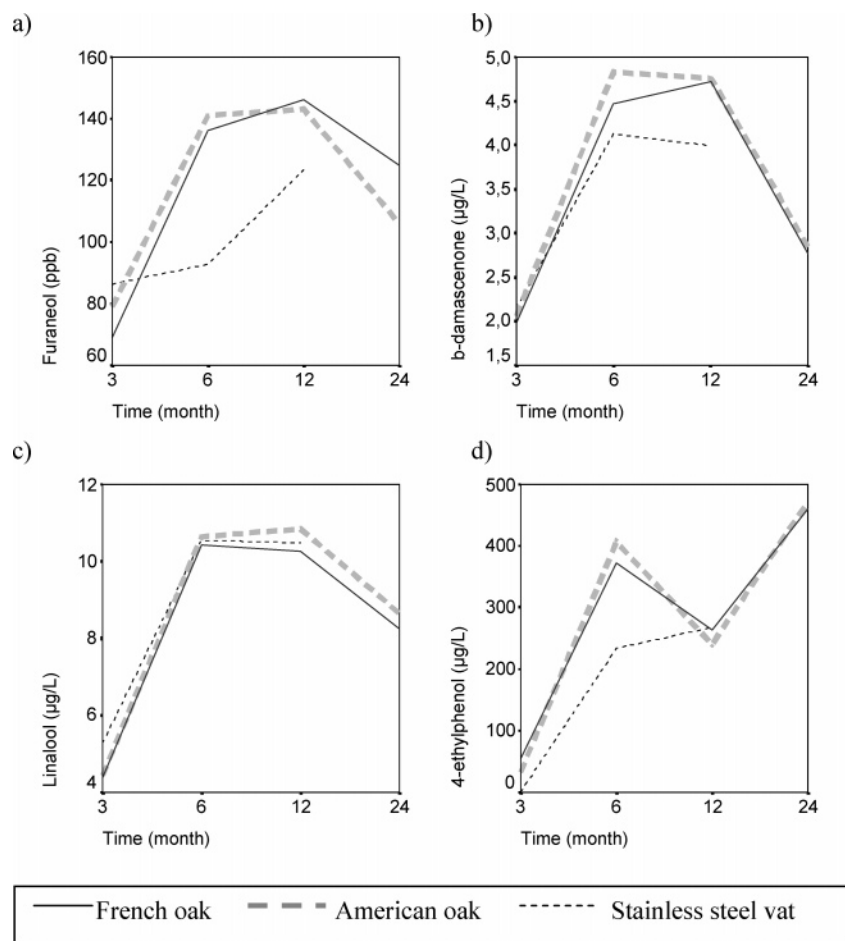


Figure 2. Concentration vs time plots in three different aging conditions (American oak, French oak or Stainless Steel vat) for compounds formed from precursors or by microbiological action: (a) furaneol; (b) β -damascenone; (c) linalool; (d) 4-ethylphenol.

γ -butyrolactone. There are some literature antecedents supporting these observations and hypothesis, although the existence of these processes is very often neglected. Reazin described years ago similar results on whisky maturation (31), and the trend recently described for the extraction of γ -butyrolactone in a model wine is consistent with a fast extraction from the surface (20).

(iii) Compounds Extracted from Wood and Released from Precursors. In this group, the following compounds can be found: acetovanillone, methylvanillate, ethylvanillate, furaneol, β -damascenone, β -ionone, and α -ionone. The first four compounds are usually considered to be wood extractable; however, as data in the **Table 3** show, not only are they found in wine, but their levels also increase in the wine stored in stainless steel. In the case of acetovanillone, methylvanillate, and ethylvanillate, such increase is probably due to the hydrolysis of glycosidic precursors. In the case of furaneol, shown in **Figure 2a**, the result is more surprising since the existence of a precursor of this molecule in wine has not been reported. It should be remarked that the contribution of wood is not the most important one from a quantitative point of view, except for acetovanillone. As to β -damascenone and α - and β -ionones, the case is the opposite since these compounds are not usually considered to be wood-extractable. However, as data in the **Table 3** and **Figure 2b** show, and in accordance with some previous reports (32), the wood may be able to produce small amounts of these compounds, which would confirm that carotenoids from wood are also an active source of odorants. In this category, the type of wood does not seem to exert any clear effect.

(iv) Compounds Formed from Precursors in Wine. This category groups those compounds following an increasing trend in all the wines, as a likely consequence of a spontaneous synthesis from a precursor naturally occurring in the wine. Linalool and homofuraneol follow such kind of behavior. As can be seen in **Table 3** and in **Figure 2c**, there is no difference in the evolution of linalool between the wines stored in stainless steel and those aged in oak. Linalool is formed from glycosidic precursors or may also be formed from other monoterpenes, as it has been recently demonstrated (33). The same behavior is observed in the case of homofuraneol, albeit, for this compound, no precursor has been reported.

(v) Compounds Formed by Microbiological Action on Wine Precursors. This is the case of 4-ethylphenol and 4-ethylguaiacol, whose formation from wine *p*-coumaric and ferulic acids by *Brettanomyces/Dekkera* yeast is well-known (1, 2). In both cases, the amount formed in the wines stored in wood is higher than that found in the reference wine, which indicates that the microbiological activity is at its highest in wood. It should be remarked, however, that the behavior of 4-ethylphenol is not as simple as one would have expected. **Figure 2d** shows that the level of this compound decreases between the 6th and 12th month of aging in wood, irrespectively of the type of wood, following a behavior parallel to that of vanillin. As a result, for these six samples the ratio of 4-ethylphenol/4-ethylguaiacol is different from the rest of the wines analyzed, and the level of 4-ethylphenol is maximum for the wines stored in the stainless steel vat. We have no

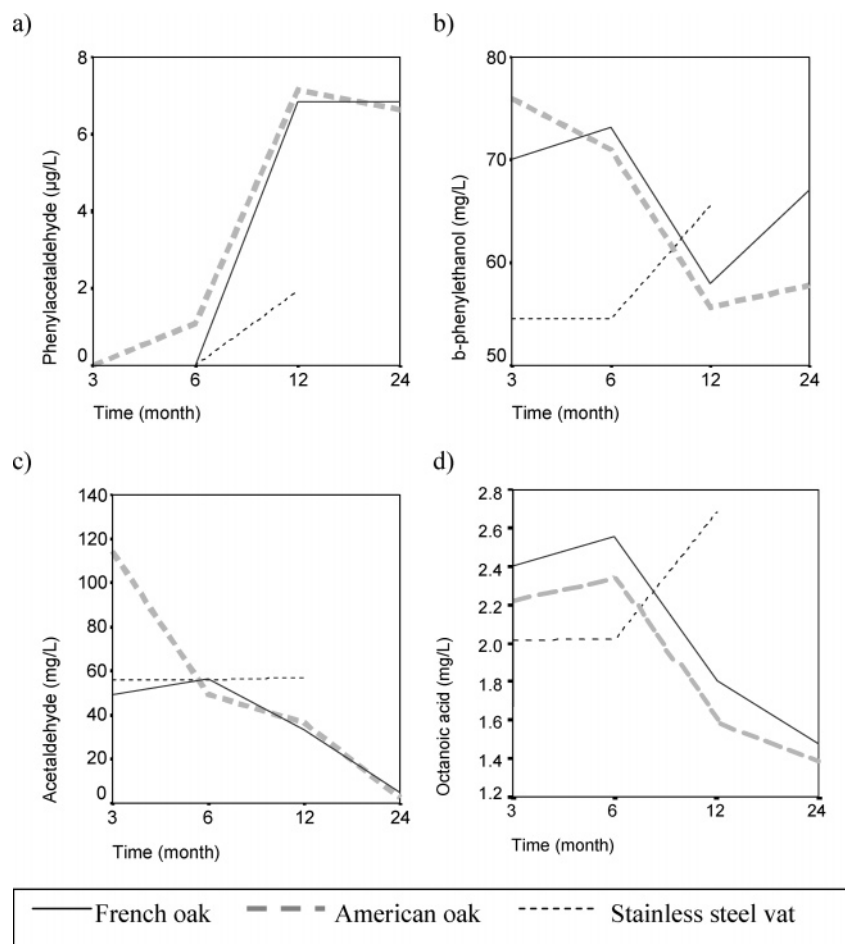


Figure 3. Concentration vs time plots in three different aging conditions (American oak, French oak, or stainless steel vat) for compounds affected by oxidation, condensation with wood phenols, or absorption: (a) phenylacetaldehyde; (b) β -phenylethanol; (c) acetaldehyde; (d) octanoic acid.

satisfactory explanation for this observation, although a complex evolution for 4-ethyl phenol has also been recently reported (14).

(vi) Compounds Formed by the Oxidation Taking Place Exclusively in the Oak Cask. In this category, two very important odorants are included: sotolon and phenylacetaldehyde. In the case of sotolon, the wine contains small amounts just after fermentation, but while the level of this compound decays in the wine stored in stainless steel, it increases steadily in the wines stored in any type of wood. The formation of this compound seems to be related to the oxidative degradation of threonine (34). On the contrary, phenylacetaldehyde is not at all present in the freshly fermented wine and is formed during the aging process. As can be seen in the **Figure 3a**, it is formed faster and in bigger amounts in the wines stored in oak casks. This compound is related to the oxidative degradation of phenylalanine but can also be formed by direct oxidation of β -phenylethanol. Both odorants reach concentrations above their corresponding odor thresholds ($5 \mu\text{g L}^{-1}$ for sotolon and $2 \mu\text{g L}^{-1}$ for phenylacetaldehyde). It should be noted that in a previous paper, phenylacetaldehyde levels were found to be closely related to the old-wood-oxidation character of red wines (35).

(vii) Compounds Disappearing Due, Most Likely, to the Oxidation Taking Place Exclusively in the Oak Cask. This is the case of important wine alcohols, such as hexanol, β -phenylethanol, and methionol. The simplest behavior is observed for hexanol, whose level strongly decreases only in the wines stored in oak casks. The same is true of methionol, although in this case the barrels made with French oak released

a significant amount of this compound at the beginning of the aging process (commented on ii). A similar behavior can be observed in both types of wood for β -phenylethanol (**Figure 3b**), although, in this case, a slight increase is observed at the end of the aging process, regardless of the type of recipient in which the wine is stored. This increment could be explained by the release of this molecule from glycosidic precursors. Although hexanal and methionol were not determined in the present experiment, the close inverse relationship observed between the trends of β -phenylethanol and phenylacetaldehyde suggests that the three alcohols are oxidized to their corresponding aldehydes. As aldehydes have smaller odor thresholds than alcohols, this oxidation may have a deep sensory effect, not because of the diminution of the alcohols but because of the presence of aldehydes.

(viii) Compounds Disappearing Due to Condensation with Compounds Extracted from Wood. This category is formed by a single compound: acetaldehyde. Its evolution along the aging process can be seen in **Figure 3c**, which shows that the level of this compound does not change in the wine stored in stainless steel, while there is a very strong decrease in the wines stored in oak casks. The highest level of this compound found in the wine stored in American oak barrels may be due to the presence of the molecule at the surface of the new wood, although it also could be due to a higher level of oxidation of wine in the American oak. The standard deviation of these data in **Table 4** reveals an anomalous behavior.

The diminution of the level of this compound may be attributed to its condensation with polyphenols extracted from

wood. This process has an outstanding importance in the stabilization of wine color and in the fining of wine flavor, and our data indirectly demonstrate the importance of aging in oak casks in such processes. It should be taken into account that, most likely and judging from what was observed for alcohols and aldehydes, acetaldehyde is being continuously formed by oxidation of ethanol in the wines stored in oak, but the kinetics of the condensation processes are higher than those of the oxidation. This means that the number of moles of acetaldehyde consumed by the wine stored in oak is, probably, very big.

(ix) Compounds Disappearing in the Wines Stored in Wood as a Likely Consequence of Sorption Processes. The clearest case in this category is that of octanoic acid, but hexanoic, butyric, and isovaleric acids also behave similarly. These compounds are not as easily oxidized in wine as are the alcohols, and therefore, the decrease in the cases of the wines stored in wood suggests the existence of a sorption process. Such sorption could take place directly in the wood, as suggested by Ramírez et al. (22), but could also occur on the solid deposits formed as a consequence of the natural wine settling. These deposits are higher in the barrels, as a consequence of the higher levels of tannins and oxygen. The trend followed by octanoic acid can be seen in the **Figure 3d**, which clearly shows that this compound strongly decreases in the wines stored in oak casks, while a slight increase is seen in the wine stored in stainless steel. This increment is partly due to the partial hydrolysis of ethyl octanoate (its level decreases about 0.05 ppm). The slight increment observed in the first months of maturation in the barrels must be attributed to the presence of small amounts of this compound in the new wood as discussed earlier. The trends observed in the other acids are quite similar, although the diminution is less important, and an increase is even noted at the end of the maturation process in the cases of butyric and hexanoic acids. This is coherent with the fact that octanoic acid is the most nonpolar compound of these four acids.

(x) Compounds Whose Concentration Changes Due to Acid+Alcohol/Ester Equilibria. These processes are concomitant with any kind of aging and have been previously discussed in the scientific literature (36). Therefore, they will not be further commented on in the present discussion.

The previous behaviors need to consider at least seven different concurrent processes:

1. Extraction from wood (categories i, ii, and iii)
2. Synthesis from precursors (categories iii and iv)
3. Microbiological formation (category v)
4. Oxidation (categories vi and vii)
5. Condensation with phenols and some other molecules (viii)
6. Sorption in the wood or in the solid sediments (ix)
7. Esterification equilibria (x)

Five out of these seven processes are concomitantly linked to the aging in wood, or are at least more intense when the aging is carried out in an oak cask. These five processes (extraction from wood, microbiological formation, oxidation, condensation, and sorption) are responsible for changes in the concentrations of 37 different compounds, as shown in **Table 3**.

All these observations demonstrate that the aging of wine in oak casks is a complex process in which different processes take place simultaneously. The wood is able to release into the wine not only the compounds usually considered to be wood extractable but also some common fermentation byproducts and some other aroma compounds that are normal constituents of wine, some of which are also released by nonvolatile precursors. A second and very important characteristic of aging in oak

barrels is related to the ability of the oak barrel to induce the oxidation of some wine components. Such oxidation manifests itself in two ways in the aroma profile of wine. On one hand, important odorants such as sotolon, phenylacetaldehyde, and some other aldehydes are formed, and on the other hand there is a significant reduction in the amount of some alcohols in wine. A third aspect of aging in oak is the higher activity of *Brettanomyces/Dekkera* yeast able to form ethylphenols. A fourth characteristic of the aging in oak is the sorption of significant amounts of nonpolar volatile compounds. The fifth and very important characteristic of aging in oak is the fact that a large number of molecules of acetaldehyde are fixed by bridging different wine and wood polyphenols, which contributes to the stability of wine color and to the fining of wine flavor.

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LITERATURE CITED

- (1) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N.; Pons, M. The origin of ethylphenols in wines. *J. Sci. Food Agric.* **1992**, *60*, 165–178.
- (2) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N. The influence of *Brettanomyces dekkera* sp. yeasts and lactic acid bacteria on the ethylphenol content of red wines. *Am. J. Enol. Vitic.* **1995**, *46*, 463–468.
- (3) Castino, M.; Cravero, M. C.; Ponzetto, L. Some considerations on the use of barrels for ageing of red wines. *Enotecnico* **1993**, *29*, 49–53.
- (4) Chatonnet, P.; Viala, C.; Dubourdieu, D. Influence of polyphenolic components of red wines on the microbial synthesis of volatile phenols. *Am. J. Enol. Vitic.* **1997**, *48*, 443–447.
- (5) Swaffield, C. H.; Scott, J. A.; Jarvis, B. Observations on the microbial ecology of traditional alcoholic cider storage vats. *Food Microbiol.* **1997**, *14*, 353–361.
- (6) Boidron, J. N.; Chatonnet, P.; Pons, M. Influence du bois sur certaines substances odorantes des vins. *Conn. Vigne Vin.* **1988**, *22*, 275–293.
- (7) Chatonnet, P.; Boidron, J. N.; Pons, M. Maturation of red wines in oak barrels: evolution of some volatile compounds and their aromatic impact. *Sci. Aliments* **1990**, *10*, 565–587.
- (8) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N. Incidence des conditions de fermentation et d'élevage des vins blancs secs en barriques sur leur composition en substances cédées par le bois de chêne. *Sci. Aliments* **1992**, *12*, 665–685.
- (9) Spillman, P. J.; Pollnitz, A. P.; Liacopoulos, D.; Skouroumounis, G. K.; Sefton, M. A. Accumulation of Vanillin During Barrel-Aging of White, Red, and Model Wines. *J. Agric. Food Chem.* **1997**, *45*, 2584–2589.
- (10) Towey, J. P.; Waterhouse, A. L. The Extraction of Volatile Compounds From French and American Oak Barrels in Chardonnay During Three Successive Vintages. *Am. J. Enol. Vitic.* **1996**, *47*, 163–172.
- (11) Spillman, P. J.; Pollnitz, A. P.; Liacopoulos, D.; Pardon, K. H.; Sefton, M. A. Formation and degradation of furfuryl alcohol, 5-methylfurfuryl alcohol, vanillyl alcohol, and their ethyl ethers in barrel-aged wines. *J. Agric. Food Chem.* **1998**, *46*, 657–663.
- (12) Spillman, P. J.; Iland, P. G.; Sefton, M. A. Accumulation of volatile oak compounds in a model wine stored in American and Limousin barrels. *Aust. J. Grape Wine Res.* **1998**, *4*, 67–73.
- (13) Garde, T.; Rodriguez Mozaz, S.; Ancin Azpilicueta, C. Volatile composition of aged wine in used barrels of French oak and of American oak. *Food Res. Int.* **2002**, *35*, 603–610.
- (14) Garde, T.; Torrea, D.; Ancin, C. Accumulation of volatile compounds during ageing of two red wines with different composition. *J. Food Eng.* **2004**, *65*, 349–356.

- (15) Perez-Prieto, L. J.; Lopez-Roca, J. M.; Gomez-Plaza, E. Differences in major volatile compounds of red wines according to storage length and storage conditions. *J. Food Comp. Anal.* **2003**, *16*, 697–705.
- (16) Perez-Prieto, L. J.; Lopez-Roca, J. M.; Martinez-Cutillas, A.; Pardo-Minguez, F.; Gomez-Plaza, E. Extraction and formation dynamic of oak-related volatile compounds from different volume barrels to wine and their behavior during bottle storage. *J. Agric. Food Chem.* **2003**, *51*, 5444–5449.
- (17) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N. Effects of fermentation and maturation in oak barrels of the composition and quality of white wines. *Wine Ind. J.* **1991**, *Feb.*, 73–84.
- (18) Diaz-Plaza, E. M.; Reyero, J. R.; Pardo, F.; Alonso, G. L.; Salinas, M. R. Influence of oak wood on the aromatic composition and quality of wines with different tannin contents. *J. Agric. Food Chem.* **2002**, *50*, 2622–2626.
- (19) Perez-Prieto, L. J.; Lopez-Roca, J. M.; Martinez-Cutillas, A.; Pardo Minguez, F.; Gomez-Plaza, E. Maturing wines in oak barrels. Effects of origin, volume, and age of the barrel on the wine volatile composition. *J. Agric. Food Chem.* **2002**, *50*, 3272–3276.
- (20) Ancín, C.; Garde, T.; Torrea, D.; Jimenez, N. Extraction of volatile compounds in model wine from different oak woods: effect of SO₂. *Food Res. Int.* **2004**, *37*, 375–383.
- (21) Pocock, K. F.; Sefton, M. A.; Williams, P. J. Taste thresholds of phenolic extracts of French and American oakwood: the influence of oak phenols on wine flavor. *Am. J. Enol. Vitic.* **1994**, *45*, 429–434.
- (22) Ramirez, G.; Lubbers, S.; Charpentier, C.; Feuillat, M.; Voilley, A.; Chassagne, D. Aroma compound sorption by oak wood in a model wine. *J. Agric. Food Chem.* **2001**, *49*, 3893–3897.
- (23) Ortega, C.; Lopez, R.; Cacho, J.; Ferreira, V. Fast analysis of important wine volatile compounds: Development and validation of a new method based on gas chromatographic-flame ionisation detection analysis of dichloromethane microextracts. *J. Chromatogr. A* **2001**, *923*, 205–214.
- (24) López, R.; Aznar, M.; Cacho, J.; Ferreira, V. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *J. Chromatogr. A* **2002**, *966*, 167–177.
- (25) Ferreira, V.; Jarauta, I.; Lopez, R.; Cacho, J. Quantitative determination of sotolon, maltol and free furaneol in wine by solid-phase extraction and gas chromatography-ion-trap mass spectrometry. *J. Chromatogr. A* **2003**, *1010*, 95–103.
- (26) Ferreira, V.; Jarauta, I.; Ortega, L.; Cacho, J. Simple strategy for the optimization of solid-phase extraction procedures through the use of solid–liquid distribution coefficients: Application to the determination of aliphatic lactones in wine. *J. Chromatogr. A* **2004**, *1025*, 147–156.
- (27) PerezCoello, M. S.; Sanchez, M. A.; Garcia, E.; GonzalezVinas, M. A.; Sanz, J.; Cabezudo, M. D. Fermentation of white wines in the presence of wood chips of American and French oak. *J. Agric. Food Chem.* **2000**, *48*, 885–889.
- (28) Díaz-Maroto, M. C.; Sánchez-Palomo, E.; Pérez-Coello, M. S. Fast screening method for volatile compounds of oak wood used for aging wines by headspace SPME-GC-MS (SIM). *J. Agric. Food Chem.* **2004**, *52*, 6857–6861.
- (29) Arapitsas, P.; Antonopoulos, A.; Stefanou, E.; Dourtoglou, V. G. Artificial aging of wines using oak chips. *Food Chem.* **2004**, *86*, 563–570.
- (30) Wanikawa, A.; Hosoi, K.; Kato, T. Conversion of unsaturated fatty acids to precursors of gamma-lactones by lactic acid bacteria during the production of malt whisky. *J. Am. Soc. Brewing Chem.* **2000**, *58*, 51–56.
- (31) Reazin, G. Chemical mechanisms of whiskey maturation. *Am. J. Enol. Vitic.* **1981**, *32*, 283–289.
- (32) Sefton, M. A.; Francis, I. L.; Williams, P. J. Volatile norisoprenoid compounds as constituents of oak woods used in wine and spirit maturation. *J. Agric. Food Chem.* **1990**, *38*, 2045–2049.
- (33) Pedersen, D. S.; Capone, D. L.; Skouroumounis, G. K.; Pollnitz, A. P.; Sefton, M. A. Quantitative analysis of geraniol, nerol, linalool and alpha-terpineol in wine. *Anal. Bioanal. Chem.* **2003**, *375*, 517–522.
- (34) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J. Agric. Food Chem.* **1999**, *47*, 2837–2846.
- (35) Aznar, M.; Lopez, R.; Cacho, J.; Ferreira, V. Prediction of aged red wine aroma properties from aroma chemical composition. Partial least squares regression models. *J. Agric. Food Chem.* **2003**, *51*, 2700–2707.
- (36) Ramey, D. D.; Ough, C. S. Volatile ester hydrolysis or formation during storage of model solutions and wines. *J. Agric. Food Chem.* **1980**, *28*, 928–934.

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