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ORIGINAL PAPER

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Changes in the polyphenolic and volatile content of "Fino" Sherry wine exposed to high temperature and ultraviolet and visible radiation

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Abstract Experiments of accelerated oxidation of "Fino" Sherry wines have been conducted at different temperatures (25 and 45 °C) and under the influence of UV–Vis radiation (a xenon lamp of 1500 W). Two types of glass bottle were employed: topaz bottles (with low values of transmittance in the UV–Vis range) and transparent bottles. To identify significant differences between the wine before and after being subjected to the influence of these factors, the values of absorbance at 420 nm and the concentrations of various polyphenolic and volatile compounds were submitted to a multivariate variance analysis. The three factors "temperature", "radiation", and "time" had a statistically significant effect on the values of absorbance at 420 nm and on the concentration of most of the polyphenolic and volatile compounds, while the "bottle" factor was only significant for polyphenol content. All the wines showed losses in several polyphenolic compounds, which were more severe for the wines bottled in transparent glass. The combined application of high temperature and UV–Vis radiation provoked significant decreases in most of the volatile compounds monitored.

Keywords Sherry wine · Oxidation · Polyphenol · Volatile compound · Ultraviolet–visible radiation · Temperature

Abbreviations UV: Ultraviolet · Vis: Visible · DAD: Diode array detector · GRP: 2-*S*-glutathionyl caftaric acid · MANOVA: Multivariate analysis of variance · PCA: Principal component analysis · PC: Principal component · GC: Gas chromatography

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Introduction

The phenomenon known as browning is notable among those that produce undesirable changes in the organoleptic characteristics of white wine. It has been known for many years that this deterioration is due to the oxidation of the polyphenolic compounds present in the wine that are transformed into quinonic compounds. The appearance of these compounds is the direct cause of the visual changes observed [1, 2] in the wine. Wine oxidation also involves the appearance of new odorants and the disappearance of several original odorants [3]. There are several aroma compounds related to the effect of oxygen in wines [4]. Some of these are products of the oxidative degradation of unsaturated fatty acids [4], while others have a miscellaneous origin.

These alterations in the organoleptic properties often lead to the rejection of the wine affected, which causes not only financial loss but also loss of consumer confidence in wine.

In addition to the main factors involved in the phenomenon of browning (polyphenols and oxygen), there are many other factors that intervene in the browning of a bottled white wine. These include the environmental conditions of conservation (temperature, humidity, illumination, etc.) and the conditions under which the wine has been bottled (type of bottle, type of stopper, introduction of inert gas [5, 6], etc.). It is known that high temperatures [7] and exposure to light accelerate the process of browning of wine. However, it has been demonstrated that the reactions due to a high temperature are different from those produced during natural browning [8].

In the case of UV–Vis radiation, there is very little literature to be found into the bibliography.

The "Fino" Sherry wines, typical of the Jerez-Xérès-Sherry and Manzanilla de Sanlúcar Denomination of Origin region (in the SW of Spain) [9], along with the other types of white wine, are affected by browning that occurs after they have been bottled.

Previous experiments of accelerated oxidation of "Fino" Sherry wines conducted at 25 °C and under the influence of UV–Vis radiation showed that an excess

of UV–Vis radiation provoked significant decreases in several polyphenolic compounds. Regarding volatile compounds, significant changes were observed during that study [10].

The objective of this study is to determine the degree of participation of both a high temperature and UV–Vis radiation in the changes in the polyphenolic and volatile compounds of this particular type of white wine bottled in transparent and topaz glass. The latter type of glass presents low transmittances in the UV–Vis range.

Material and methods

Wine samples

A "Fino" Sherry wine (ethanol content 15% v/v) from the Jerez-Xérès-Sherry and Manzanilla de Sanlúcar Denomination of Origin region (SW Spain) was bottled in glass bottles of two colors, topaz and transparent. Nine bottles of each type were placed in a controlled-climate chamber for 30 days at two temperatures, 25 and 45 °C and under the influence of solar-type radiation provided by a xenon lamp of 1500 W (emission of UV and visible radiation). The trial was repeated on wine from the same batch and under the same temperature conditions (25 and 45 °C), but without illumination.

The bottles were initially arranged randomly inside the chamber, and then each day, the bottles placed furthest from the light source were interchanged with those placed closest, to try to ensure that all bottles received the same degree of illumination. Three bottles of each type were removed after being kept for periods of 10, 20, and 30 days, respectively, in the climate chamber. During the entire period of the trial, the bottles were left without a stopper, and covered only with a piece of cotton, in order to accelerate the process of browning of the wine contained. The volume of wine lost by evaporation (approximately 4% for 25 °C and 20% for 45 °C) was measured to allow correction of the results obtained.

Determination of the polyphenolic compounds

Phenolic acids

Volumes of 80 µl of "Fino" Sherry wine after filtration (0.45 µm pore size) were analyzed by HPLC (Waters Cromatografia, S.A., Barcelona, Spain) in duplicate. The elution phases used were: solvent A (95% water, 5% methanol) and solvent B (95% methanol, 5% water) at pH 2.5 (extra pure sulfuric acid). The elution gradient was: from 100 to 85% solvent A in 5 min; from 85 to 50% solvent A in 40 min; and isocratic elution for 35 min. The analyses were carried out using a C_{18} column (Lichrospher 100 RP-18, 250 mm × 3 mm, 5 µm particle size) at a flow rate of 0.5 ml/min and detection at 280 and 320 nm.

The various polyphenolic compounds present were identified by comparison with a library of DAD spectra and retention times of standards. Commercial standards of several polyphenols (gallic acid, protocatechuic acid, protocatechualdehyde, *p*-hydroxybenzaldeyde, vanillic acid, catechin, caffeic, epicatechin, ferulic acid, and i-ferulic acid) were purchased from Fluka (Buchs, Switzerland). Other polyphenolic standards (tyrosol, syringic acid, and *p*-coumaric acid) were supplied by Eastman Kodak (Rochester, NY). Caftaric and coutaric acids were isolated by the method described by Singleton et al. [11].

In order to obtain the UV–Vis spectra and retention times of *cis* isomers of *p*-coutaric and *p*-coumaric acids, we employed a climatic chamber with solar-type radiation (xenon lamp of 1500 W). For this, individual solutions of *trans* isomers were placed in the controlled-climate chamber for 10 days at 25 °C and under the influence of illumination.

Each compound was quantified by comparison with a calibration curve (absorbances at 320 nm for caftaric acid, *cis* and *trans p*-coutaric acids, fertaric acid, GRP, caffeic acid, *cis* and *trans p*-coumaric acids, i-ferulic acid, and ferulic acid; absorbances at 280 nm for the rest polyphenols) obtained with the corresponding standard. For *cis p*-coutaric and *p*-coumaric acids, the quantification has been carried out considering that their molar absorption coefficients are equal to those of *trans* isomers.

2-*S*-glutathionyl caftaric acid (GRP) was quantified as caftaric acid, and fertaric acid as ferulic acid. The identification of these polyphenols was done by analogy of UV–Vis spectra and retention times from the literature [12].

Flavan-3-ols

The flavanols present were identified by comparison with a library of DAD spectra and retention times of standards. Commercial standards were purchased from Extrasynthese (Barcelona, Spain). Each compound was quantified by comparison with a calibration curve (fluorescence signal) obtained with the corresponding standard.

Extraction

A volume of 50 ml of each sample was concentrated under vacuum at 40 °C in order to eliminate the alcohol content. Then, each sample was submitted, in duplicate, to SPE, under the conditions detailed in Table 1. This process consists of two stages, a prior stage of cleaning and preconcentration, and a fractionation stage. For the first stage, a volume of 10 ml of concentrated sample, after dilution to a final volume of 20 ml with a saturated NaCl solution acidificated at pH 2 with HCl, was passed through a C_{18} cartridge (1 g, DSC-18, Supelco, Barcelona, Spain). The polyphenolic extract was eluted with methanol:phosphate buffer (1:1) at pH 6.5. This extract was passed through a SAX cartridge (500 mg, Bond Elut, Scharlau, Barcelona, Spain) after adjusting its pH value to 6.5. Acidic polyphenols were retained on the SPE-SAX while non-acidic polyphenols (flavanols) were eluted with 1 ml of phosphate buffer (pH

Table 1 Solid-phase extraction for the determination of procyanidins

Adsorbent	Operation	Solvent
Cleaning and preconcentration stage. C_{18} (1000 mg, DCS-18, Supelco)	Solvation	10 ml methanol
	Conditioning	10 ml saturated NaCl at pH 2
	Sample	10 ml wine with 10 ml saturated NaCl at pH 2
	Washing	1° 2 ml saturated NaCl at pH 2
		2° 2 ml 0.01 M HCl
	Elution of Polyphenolic extract	2 ml Methanol/buffered phosphate solution at pH 6.5 (1/1)
Fractionation stage. SAX (500 mg, Bond Elut, Scharlau)	Conditioning	10 ml Milli-Q water
		1° Polyphenolic extract
	Sample	2° 1 ml buffered phosphate solution at pH 6.5
	Washing	2 ml Milli-Q water

6.5, molarity 0.05 M) and Milli-Q water until a final volume of 5 ml. This extract was used for the determination of the flavan-3-ols.

The precision of this method was calculated using five extractions of a sample of wine. Coefficients of variation between 2.5 and 4.3% were obtained. The technique of standard additions was used in order to check the accuracy of this analytical method. A representative sample of wine was taken as matrix and known quantities of a global standard solution containing all the analytes were added at five levels and in duplicate. Recoveries close to 100% were obtained for all the flavanols.

HPLC analysis

Catechin, epicatechin, and procyanidins B1 and B2 were quantified in this fraction. Eighty microliters of each extract was analyzed by HPLC (Waters Cromatografía, S.A., Barcelona, Spain). The elution phases used were: solvent A (95% water, 5% methanol) and solvent B (95% methanol, 5% water) at pH 2.5 (extra pure sulfuric acid). The elution gradient was: from 100 to 75% solvent A in 5 min; and from 75 to 50% solvent A in 40 min. The analyses were carried out using a C_{18} column (Lichrospher 100 RP-18, 250 mm × 3 mm, 5 μm particle size) at a flow rate of 0.5 ml/min. Two detectors were used, a DAD (absorbance at 280 nm) and a fluorescence detector (excitation at 276 m and emission at 316 nm).

Determination of the volatile profile

SPME

SPME methodology was previously optimized in our laboratory [13].

Briefly, for each SPME analysis, a volume of 25 ml of sample was pipetted and placed into a 50-ml glass vial with 3.0 g of NaCl. Each sample was spiked with 75 μl of a solution of 4-methyl-2-pentanol (2.516 g/l in Milli-Q water containing 15% v/v of ethanol) and equilibrated for 15 min at sampling temperature (40 °C). After this, the SPME fibre (CAR/PDMS, 85 μm) was inserted into the headspace. During the sampling time (45 min), the sample was stirred at constant speed. Each sample was analyzed in duplicate.

Gas chromatography

The samples were analyzed using a GC 8000 chromatograph with a FID detector (Fisons Instruments, Milan, Italy). The injection was made in the splitless mode for 2 min. For the desorption of the analytes inside the GC injection port, the temperature was 280 °C.

The GC was equipped with a DB-Wax capillary column (J&W Scientific, Folsom, CA, USA), 60 m × 0.25 mm I.D., with a 0.25 μm coating. The carrier gas was helium at a flow rate of 1.1 ml/min. The detector temperature was 250 °C. The GC oven was programmed as follows: held at 35 °C for 10 min, then ramped at 5 °C/min to 100 °C. Then, it was raised to 210 °C at 3 °C/min and held for 40 min.

The compounds were identified by mass spectrometric analysis. In these analyses, the same GC coupled to a MD 800 mass detector (Fisons Instruments, Milan, Italy) was used. The mass detector operated in EI+ mode at 70 eV in a range of 30–450 amu. GC analytical conditions were the same as described above.

The signal was recorded and processed with Masslab software supplied with the Wiley 6.0 MS library. Peak identification was carried out by analogy of mass spectra and confirmed by retention indices of standards. All standards used in this study were supplied by Sigma–Aldrich (St. Louis, Mo, USA). Each compound was quantified by comparison with a calibration curve, obtained using the relative peak area in relation to that of 4-methyl-2-pentanol, the internal standard.

Spectrophotometric measurements

A Unicam Model PU8730 Spectrophotometer was used to determine the absorbances at 420 nm of the wines during the course of the study; this is the wavelength typically

used when measuring the degree of browning undergone by a wine.

Statistical treatment

A multifactor analysis of the variance (MANOVA) was carried out on the replicated samples for each compound, in relation to temperature, UV–Vis radiation, time, and type of bottle. The compounds with a high dependence ($p<0.01$) on some of the factors considered were subjected to a principal components analysis (PCA) on the replicated samples. The computer program used was the Statgraphics Statistical Computer Package "Statgraphics Plus 5.0" for Windows 98.

Results and discussion

This paper focuses on the chemical changes that take place in the composition of polyphenolic and volatile compounds in a "Fino" Sherry wine during oxidative storage under UV–Vis radiation and high temperature, in order to establish the general pattern of changes and to study the influence of both parameters.

With a view to finding significant differences between the initial wine and that subjected to varying periods of influence of UV–Vis radiation and/or high temperature, the values of absorbance at 420 nm and the concentrations of various polyphenolic and volatile compounds were submitted to a multifactor variance analysis. The factors considered were time, temperature, UV–Vis radiation, and type of bottle used. Results are given in Tables 2 and 3. Only significant two-factor interactions are included in these tables.

Evolution of the color

Table 2 gives the absorbances at 420 nm and the concentrations found for the identified polyphenolic compounds both in the initial wine and in the wines after 30 days under the influence of high temperature and UV–Vis radiation.

In relation to the evolution of the color (absorbances at 420 nm), all factors had a significant influence on this.

Table 2 Means (mg/l) and standard deviations of polyphenols after 30 days under influence of UV–Vis radiation and high temperature (45 °C). Multifactor analysis of variance applied to all samples

Polyphenolic compounds	Initial	Mean ± SD		*p*-value						
		Transp	Topaz	T^a	*t*	Radiation	Bottle	T^a –*t*	T^a -radiat	*t*-radiat
Gallic acid	7.73±0.101	3.36±0.238	6.66±0.819	0.2740	0.5897	0.0000[a]	0.0000[a]	0.5377	0.0002[a]	0.1229
Protocatechuic acid	7.49±0.008	3.45±0.716	4.17±0.804	0.3886	0.0000[a]	0.0001[a]	0.0001[a]	0.0000[a]	0.0007[a]	0.1175
Tyrosol	68.0±2.33	39.0±1.26	58.3±2.17	0.0000[a]	0.0000[a]	0.0006[a]	0.0006[a]	0.0079[a]	0.5109	0.0000[a]
p-Hydroxybenzoic acid	11.6±0.02	11.7±0.86	14.1±0.37	0.0337	0.0613	0.0000[a]	0.0000[a]	0.0128	0.1355	0.0000[a]
Vanillic acid	5.30±.0.412	4.18±0.735	7.37±1.556	0.5737	0.0000[a]	0.0000[a]	0.0236	0.0000[a]	0.0000[a]	0.0000[a]
Syringic acid	1.98±0.078	1.42±0.357	1.92±0.227	0.3676	0.0048[a]	0.0012[a]	0.1128	0.1269	0.9414	0.0237
Caftaric acid	9.79±0.786	2.17±0.288	6.76±0.448	0.0000[a]	0.0000[a]	0.0000[a]	0.0000[a]	0.0007[a]	0.0193	0.0000[a]
GRP	2.42±0.122	2.25±0.296	2.81±0.202	0.0000[a]	0.0004[a]	0.3257	0.0000[a]	0.3137	0.0001[a]	0.0008[a]
cis p-Coutaric acid	3.58±0.098	1.83±0.090	2.59±0.004	0.0758	0.0000[a]	0.0000[a]	0.0000[a]	0.0276	0.0568	0.0012[a]
trans p-Coutaric acid	3.49±0.023	2.82±0.317	4.86±0.047	0.0076[a]	0.5989	0.0001[a]	0.0000[a]	0.5670	0.0004[a]	0.0000[a]
Fertaric acid	5.50±0.102	2.57±0.095	4.07±0.082	0.0000[a]	0.8946	0.0000[a]	0.0000[a]	0.2901	0.0097[a]	0.0000[a]
Caffeic acid	0.85±0.004	0.74±0.199	1.44±0.091	0.0000[a]	0.0000[a]	0.0000[a]	0.7023	0.0004[a]	0.0000[a]	0.0000[a]
Vanillin	1.32±0.010	1.11±0.042	1.10±0.093	0.0033[a]	0.1131	0.8398	0.5551	0.0141	0.0003[a]	0.0002[a]
Syringialdehyde	0.39±0.002	0.69±0.045	1.05±0.069	0.0709	0.0000[a]	0.6067	0.0003[a]	0.0002[a]	0.0000[a]	0.0004[a]
trans p-Coumaric acid	0.77±0.004	1.63±0.069	1.70±0.052	0.0000[a]	0.0000[a]	0.0000[a]	0.0873	0.0000[a]	0.0702	0.2835
Ferulic acid	0.53±0.023	0.90±0.022	1.25±0.224	0.0000[a]	0.0000[a]	0.3561	0.0002[a]	0.1358	0.7298	0.0012[a]
i-Ferulic acid	0.64±0.011	0.53±0.066	0.70±0.038	0.0634	0.0276	0.0000[a]	0.0026[a]	0.0000[a]	0.9356	0.0000[a]
Catechin	21.8±1.23	22.1±1.11	24.6±0.02	0.0018[a]	0.0006[a]	0.0000[a]	0.0270	0.0000[a]	0.0943	0.0034[a]
Procyanidin B1	5.54±0.782	9.12±0.727	10.8±0.053	0.0004[a]	0.0159	0.0000[a]	0.0010[a]	0.0000[a]	0.8199	0.4380
Procyanidin B2	4.42±0.232	3.16±0.197	4.45±0.326	0.5330	0.0000[a]	0.0768	0.0000[a]	0.0000[a]	0.0000[a]	0.7199
Epicatechin	17.2±0.22	1.97±0.119	8.97±0.477	0.0000[a]	0.0000[a]	0.0000[a]	0.0000[a]	0.0000[a]	0.0059[a]	0.0117
Color (absorbance at 420 nm)	0.13±0.010	0.61±0.002	0.45±0.051	0.0000[a]	0.0000[a]	0.0000[a]	0.0091[a]	0.0000[a]	0.0000[a]	0.0000[a]

[a]Values are significant at $p<0.01$

GRP: 2-*S*-glutathionyl caftaric acid

Table 3 Means (relative peak area) and standard deviations of volatile compounds after 30 days under influence of UV–Vis radiation and high temperature (45 °C) Multifactor analysis of variance applied to all samples

Volatile compounds	Initial	Mean ± SD		*p*-value						
		Transp	Topaz	T^a	*t*	Radiation	Bottle	T^a –*t*	T^a -radiat	*t*-radiat
Acid and esters										
Ethyl acetate	7.26±0.123	0.07±0.010	0.10±0.012	0.0000[a]	0.0000[a]	0.0000[a]	0.8303	0.0001[a]	0.0935	0.0001[a]
Ethyl butanoate	0.55±0.033	0.06±0.014	0.04±0.003	0.4153	0.3379	0.1445	0.0040[a]	0.0000[a]	0.9129	0.0066[a]
Ethyl pentanoate	0.14±0.09	nd	nd	0.0000[a]	0.0000[a]	0.0015[a]	0.0074[a]	0.0000[a]	0.0015[a]	0.0001[a]
Isoamyl acetate	0.56±0.120	0.01±0.003	0.01±0.000	0.0000[a]	0.0000[a]	0.0000[a]	0.0253	0.0000[a]	0.0090[a]	0.0004[a]
n-Hexyl acetate	0.58±0.071	0.01±0.001	0.01±0.002	0.0000[a]	0.0000[a]	0.0000[a]	0.0378	0.3525	0.0322	0.0009[a]
Ethyl lactate	1.11±0.005	0.36±0.022	0.38±0.019	0.0030[a]	0.0000[a]	0.0000[a]	0.4058	0.0010[a]	0.0023[a]	0.0123
Ethyl octanoate	7.13±0.302	0.03±0.024	0.04±0.005	0.0000[a]	0.0000[a]	0.0000[a]	0.1146	0.0000[a]	0.0001[a]	0.0000[a]
Methyl octanoate	0.04±0.000	0.01±0.001	0.01±0.000	0.0074[a]	0.0834	0.2782	0.6417	0.0009[a]	0.2296	0.0039[a]
Methyl decanoate	0.23±0.012	0.01±0.000	0.01±0.002	0.0262	0.0000[a]	0.0049[a]	0.1417	0.0005[a]	0.0000[a]	0.0001[a]
Ethyl 2-furoate	0.02±0.009	0.04±0.001	0.02±0.004	0.5308	0.3630	0.3190	0.0000[a]	0.0000[a]	0.5674	0.0223
Ethyl decanoate	0.09±0.003	0.03±0.005	0.05±0.001	0.0018[a]	0.0319	0.2318	0.0409	0.0004[a]	0.0576	0.0201
Ethyl succinate	5.24±0.104	1.01±0.116	1.65±0.19	0.0000[a]	0.0000[a]	0.0000[a]	0.7827	0.0000[a]	0.0231	0.0000[a]
Ethyl-2-phenyl acetate	0.21±0.011	0.01±0.001	0.01±0.001	0.0000[a]	0.0000[a]	0.0002[a]	0.1098	0.0000[a]	0.0008[a]	0.0000[a]
Phenylethyl acetate	0.26±0.014	0.01±0.001	0.01±0.002	0.0000[a]	0.0000[a]	0.0001[a]	0.8696	0.0000[a]	0.0000[a]	0.0000[a]
Butanoic acid	2.35±0.022	0.04±0.005	0.07±0.006	0.0000[a]	0.0000[a]	0.0000[a]	0.7138	0.0000[a]	0.0000[a]	0.0000[a]
Acetic acid	1.59±0.321	0.09±0.022	0.16±0.020	0.0732	0.0000[a]	0.6257	0.0000[a]	0.0000[a]	0.0150	0.0000[a]
Hexanoic acid	0.37±0.038	0.12±0.014	0.16±0.019	0.0000[a]	0.0000[a]	0.0058[a]	0.2711	0.0000[a]	0.0058[a]	0.0068[a]
Octanoic acid	1.99±0.115	0.15±0.029	0.26±0.018	0.0000[a]	0.0000[a]	0.0000[a]	0.5923	0.0000[a]	0.0001[a]	0.0000[a]
Decanoic acid	0.35±0.004	0.00±0.000	0.00±0.003	0.0000[a]	0.0000[a]	0.7780	0.0000[a]	0.0000[a]	0.0000[a]	0.0000[a]
Alcohols										
2-Methyl-1-butanol	15.10±0.455	0.63±0.065	0.63±0.013	0.0000[a]	0.0000[a]	0.0000[a]	0.8168	0.0000[a]	0.0000[a]	0.3160
Isoamyl alcohol	2.66±0.023	0.04±0.007	0.05±0.004	0.0000[a]	0.0000[a]	0.0000[a]	0.4183	0.0001[a]	0.0000[a]	0.0000[a]
1-Hexanol	0.66±0.022	0.07±0.005	0.07±0.003	0.0000[a]	0.0000[a]	0.0000[a]	0.4872	0.0000[a]	0.0000[a]	0.0000[a]
cis-3-Hexen-1-ol	0.06±0.006	0.01±0.002	0.01±0.003	0.0004[a]	0.2273	0.2741	0.2145	0.0837	0.9454	0.0002[a]
2,3-Butanediol	0.29±0.112	0.03±0.009	0.02±0.004	0.0003[a]	0.0001[a]	0.0619	0.0010[a]	0.0001[a]	0.0307	0.0001[a]
Benzyl alcohol	0.11±0.005	0.03±0.004	0.04±0.003	0.0000[a]	0.0000[a]	0.0002[a]	0.5744	0.0000[a]	0.0004[a]	0.0000[a]
Phenylethanol	10.81±0.112	1.71±0.303	2.65±0.254	0.0000[a]	0.0000[a]	0.0000[a]	0.8416	0.0000[a]	0.0011[a]	0.0000[a]
4-Ethylguaiacol	0.19±0.161	0.01±0.001	0.02±0.001	0.0000[a]	0.0000[a]	0.0000[a]	0.7321	0.0000[a]	0.0000[a]	0.0000[a]
4-Ethylphenol	0.14±0.041	0.02±0.002	0.04±0.001	0.0000[a]	0.0000[a]	0.0000[a]	0.8281	0.0000[a]	0.0342	0.0000[a]
Aldehydes and ketones										
Furancarboxaldehyde	0.33±0.001	0.20±0.026	0.30±0.023	0.0000[a]	0.0020[a]	0.3849	0.9327	0.0039	0.7977	0.0000[a]
Benzaldehyde	0.82±0.104	0.45±0.039	0.15±0.006	0.0001[a]	0.0016[a]	0.0191	0.0000[a]	0.0003[a]	0.6345	0.0000[a]

[a]Values are significant at $p<0.01$

Wines submitted to high temperature (45 °C) and UV–Vis radiation exhibited the highest degree of visual browning (abs. 420 nm) after 30 days. For these, the wines contained in transparent bottles showed a higher absorbance at 420 nm than those contained in topaz bottles, in line with the increasing time. At 25 °C and under the influence of UV–Vis radiation, wines in both types of bottle suffered a similar browning after 30 days (Fig. 1).

In a previous study of accelerated oxidation of "Fino" Sherry wines conducted at 25 °C and under the influence of UV–Vis radiation [10], wines bottled in transparent glass exhibited, after 45 days, a lower degree of visual browning than those bottled in topaz glass.

The shorter storage time could explain the similarity found for both wines in this study.

Polyphenolic compounds

It can be observed that all individual factors have a statistically significant effect on most polyphenolic compounds (Table 2). In relation to two factor interactions,

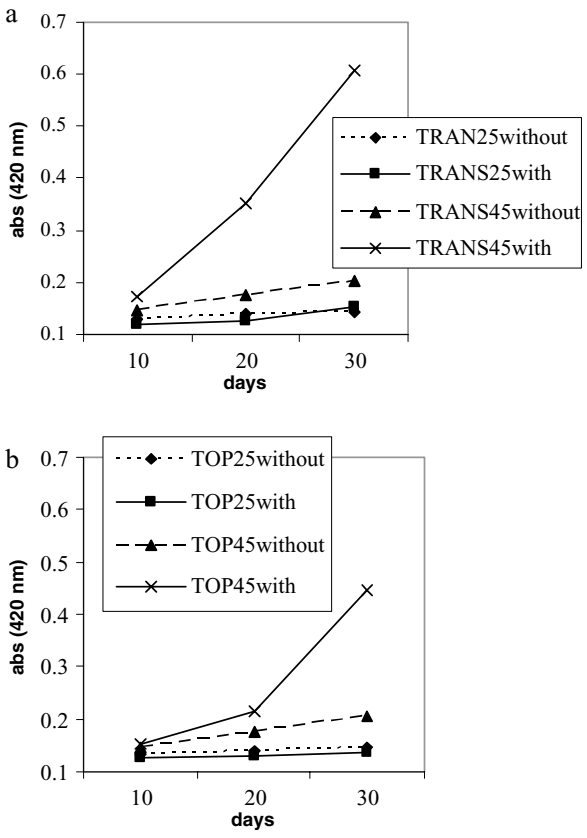

Fig. 1 Evolution of color found for the samples studied. **a** transparent bottles; **b** topaz bottles. TRAN: transparent; TOP: topaz; 25 and 45: temperatures of storage; with: with UV–Vis radiation; without: without UV–Vis radiation

only temperature–time, temperature–radiation, and time–radiation interactions have a significant effect on several polyphenols.

All wines showed losses in several polyphenolic compounds (gallic acid, vanillic acid, caftaric acid, *cis* and *trans* *p*-coutaric acids, fertaric acid, epicatechin, protocatechuic acid, etc.), which were more severe for the wines submitted to UV–Vis radiation, high temperature, and bottled in transparent glass (Table 2). For these wines, significant increases were found for compounds such as caffeic acid, syringialdehyde, *trans p*-coumaric acid, ferulic acid, catechin, procyanidin B1, etc. These increases were higher for wines submitted only to high temperature.

Most of these compounds cited have been previously shown to present a marked tendency towards oxidation [1, 8, 14]. Mayén et al. [8] observed that, in white wine subjected to a temperature of 50 °C for a prolonged period, no significant decreases in monomeric and dimeric flavan-3-ols took place in spite of the browning observed in the wines. They explained this finding as due to hydrolysis reactions of oligomeric derivates, facilitated by this high temperature.

In a previous study carried out on this type of white wine subjected to the influence of UV–Vis radiation, several increases in its polyphenolic content were observed [10].

From these results, it could be deduced that both factors, UV–Vis radiation and high temperature, are facilitating reactions that are different from those that characterize the visual browning. Singleton [14] observed a reduced production of CO_2 per unit of O_2 consumed in oxidation reactions occurring in the presence of light. This fact would suggest that under the influence of light, these reactions compete with others also stimulated by the incidence of this agent.

Others reactions, such as acid hydrolysis of esters to their respective acids [15, 16] and *cis*/*trans* conversion [17] facilitated by UV–Vis radiation and a high temperature, should also be taken into account. These could explain the increase or stabilization found for certain polyphenols, such as caffeic acid, *trans p*-coumaric acid, and ferulic acid.

All of these ways would counteract the losses of polyphenolic content produced as a result of the oxidation reactions.

Taking into account the results obtained at 45 °C, the existence of synergism could be postulated for temperature and UV–Vis radiation. It would appear that, for the UV–Vis radiation to act clearly as a catalyst for the process of browning, a high temperature is also required.

In relation to type of bottle, this factor has a statistically significant effect on most polyphenolic compounds (Table 2), with lower values for transparent glasses, which present high transmittances in the UV–Vis range.

In respect to time–temperature and time–UV–Vis radiation interactions, both of these appear to produce significant effects on the color of the wines and on several phenolic compounds, with larger variations occurring as the time factor increases. This could be evidence that, in order for UV–Vis radiation and/or temperature to act as effective catalyzers of browning, a certain period of time is needed.

Volatile compounds

Table 3 shows the relative areas (compound area/internal standard area) found for the volatile compounds studied and the statistical significance of each factor on them. In relation to two factor interactions, only those more significant have been included.

As can be seen, the great majority of compounds were affected by temperature, UV–Vis radiation, and time. Only a few compounds were affected by the bottle factor.

Esters and acids

Wines submitted to UV–Vis radiation and high temperature showed losses in most of these volatile compounds (Table 3).

In the previous study carried out at 25 °C and under the influence of UV–Vis radiation [10], several increases in the wine's volatile content were observed.

In wines stored in anaerobic conditions at 45 °C for 20 days, Zoecklein et al. [18] found significant decreases in the concentrations of most esters. Marais and Rapp [19] noted that acetates decreased in concentration with time

and high temperature. Fatty acids and esters may increase or decrease during storage due to esterification or hydrolysis reactions [20]. Rapp and Mandery [21] found that ethyl esters hydrolyze more slowly than acetate esters. Significant decreases in these types of volatile compound were found by various authors [22, 23] for white wines stored at high temperature.

In this case, the application of a high temperature seems to counteract the increases facilitated by UV–Vis radiation.

Alcohols

Wines submitted to high temperature and UV–Vis radiation after 30 days presented a low content in this type of compounds.

Previously, in the study of the influence of UV–Vis radiation [10], no clear relationship between this parameter and these volatile compounds was obtained. In the present study, this factor appears to provoke decreases in most of these compounds.

In the case of high temperature, 2-methyl-1-butanol, isoamyl alcohol, and 1-hexanol decrease, whereas *cis*-3-hexen-1-ol, 2,3-butanediol, benzyl alcohol, phenylethanol, 4-ethylguaiacol, and 4-ethylphenol show significant increases.

Marais et al. [24] observed more significant decreases in the concentration of some acetates and alcohols in wines stored at 30 °C than those stored at 15 °C.

Ferreira et al. [4] explained the increases in the concentrations of some alcohols in wines stored under oxygen on the basis of the degradation of some of the precursors present in the wine, following a process similar to that of oxidative aging in wood.

Aldehydes and ketones

Furancarboxaldehyde and benzaldehyde increased in wines submitted to high temperature and UV–Vis radiation. Marais et al. [25] found that appreciable amounts of these aldehydes were produced under oxidative conditions. The first is obtained from carbohydrate dehydration followed by cyclation in Maillard-type systems [26] whereas benzaldehyde is obtained from the oxidation of phenylalanine [27]. However, in this study, their concentrations decreased as the time factor increased. This finding could be due to the extreme environmental conditions applied.

Principal component analysis

PCA is a good statistical tool for investigating associations between variables, and moreover, it is also useful for detecting natural groups among samples. When the data matrix was subjected to PCA, nine significant PCs emerged according to Kraiser's criterion (eigenvalues > 1). With these 9 factors, 89.37% of total variance is explained. The first PC, component 1, which explains 34.32% of total variance, mainly contains ethyl-2-phenyl acetate, ethyl acetate, 2-phenylethanol, decanoic acid, butanoic acid, ethyl octanoate, and acetic acid. For the second factor, component 2, hexanol, 2-methyl-1-butanol, furancarboxaldehyde, *n*-hexyl acetate, *trans p*-coumaric acid, and color were the main constituents. As can be seen, these two PCs are most highly correlated with volatile compounds.

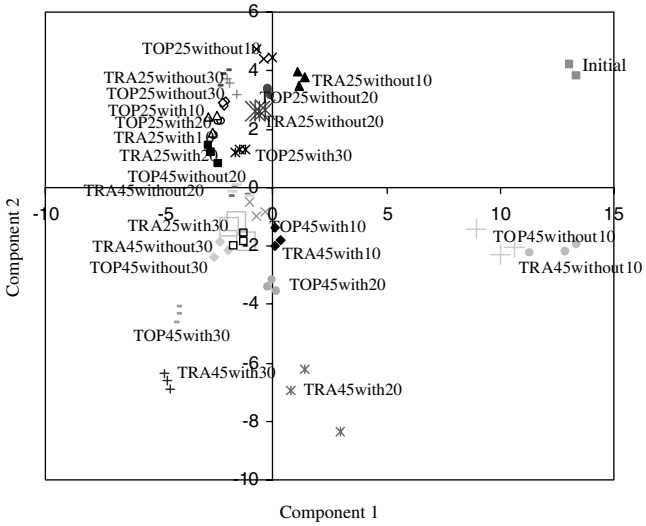

Fig. 2 Score plot on the first and second principal components, for the samples studied. TRA: transparent; TOP: topaz; 25 and 45: temperature of storage; with: with UV–Vis radiation; without: without UV–Vis radiation; 10, 20, and 30: days of storage

Figure 2 shows the score plot for the studied samples obtained by selecting the first two principal components as axes. As can be seen, the second component (component 2), which explains 19.03% of total variance, allows us to differentiate between samples submitted to high (45 °C) and low (25 °C) temperature. Samples submitted to high temperature show negative values for this component whereas those submitted to low temperature present positive values. For this component, some of its main constituents, such as hexanol, 2-methyl-1-butanol, and *n*-hexyl acetate, decrease as the factor temperature increases.

Component 1 could be correlated with time of storage. Samples submitted to 30 days of storage show negative values for this component whereas those submitted to 10 and 20 days of storage present positive values. This distribution would corroborate the conclusion that, during the storage of this type of white wine, the main changes in its polyphenolic and volatile content are mainly due to high temperature. This factor would therefore be the main parameter to control during the storage of these wines.From the results obtained, it can be seen that "Fino" Sherry wine oxidation facilitated by UV–Visible radiation and high temperature involves significant changes in its volatile and polyphenolic content. For polyphenols, both factors are facilitating reactions in which some of these compounds are recovered. Regarding volatile compounds, significant decreases have been observed during this study. A high temperature appears to counteract the increases in esters and acids promoted by the application of UV–Vis radiation. The type

of glass employed does not have a statistically significant effect on volatile compounds.

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