Phenolic Compounds and Browning in Sherry Wines Subjected to Oxidative and Biological Aging

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The composition in hydroxybenzoic and hydroxycinnamic acids, hydroxycinnamic esters, tyrosol, syringaldehyde, and flavan-3-ol derivatives of three different types of sherry wine obtained by aging of the same starting wine under different conditions was studied. So-called "fino" wine was obtained by biological aging under flor yeasts, "oloroso" wine by oxidative aging, and "amontillado" wine by a first stage of biological aging followed by a second oxidative step. On the basis of the results, the wines subjected to oxidative aging exhibited higher phenol contents, in addition to scarcely polar compounds absorbing at 420 nm that were absent in the wines obtained by biological aging. Taking into account that flavan-3-ol derivatives play an important role in wine browning, a model catechin solution was inoculated with flor yeast which, contrary to the findings of other authors in the absence of yeasts, formed no colored compounds. This different behavior may account for the resistance to browning of pale sherry wines in the presence of flor yeasts.

Keywords: *Wine browning; biological aging; phenolic compounds*

INTRODUCTION

An important different feature of pale sherry wine ("fino") in relation to white table wines is its special resistance to browning during biological aging, a property that disappears as soon as the flor yeasts growing on its surface are removed. Traditionally, the absence of browning in this type of wine has been ascribed to a protective effect on the action of atmospheric oxygen exerted by these yeasts, which are typically responsible of the biological aging (Casas, 1985). However, this hypothesis is at least partly questionable because this aging process involves the periodic transfer of a fraction of the volume in each cask from a younger wine to an older one ("rocio"). During these transfers, the wine is saturated by oxygen, so it should also brown to some extent, which is not the case in practice.

In addition to the fino type, two other kinds of sherry wines are obtained by subjecting the same starting wine to different types of aging. "Oloroso" wines, dark brown in color, are exclusively produced through oxidative aging by increasing the ethanol content in the starting wine to avoid the spontaneous growth of flor yeasts. On the other hand, "amontillado" wines, also dark in color, very old, and of a high quality, are obtained by combining two aging stages: a first biological aging followed by a second oxidative aging. All three types of sherry wine are obtained from the same grape variety under identical must fermentation conditions. More detailed information about these wine-making processes can be found in Casas (1985).

Phenols are known to be responsible for wine browning. Changes in their composition during the aging process are very complex because of the wide variety of factors involved. Thus, cask wood acts as an extraction support for various phenolic compounds, so the wines are enriched with them over time (Rous and Alderson, 1983; Chatonnet et al., 1992; Miller et al., 1992; Pocock et al., 1994; Fernandez de Simon et al., 1996; Monedero et al., 1998). Also, some hydrolysis reactions affecting the flavan-3-ol oligomer fraction, increasing the contents in their monomer constituent, have been reported (Timberlake and Bridle, 1976; Dallas et al., 1995). In addition, browning decreases the contents of the compounds that cause it and simultaneously gives rise to condensed forms of oxidized phenols that result in the typical darkening of wines under oxidative aging. Finally, if volume losses in the wood barrels resulting from the slow evaporation of water and/or ethanol are not compensated, then the concentrations of all species present in the wine rise, in a variable degree dependent on the environmental conditions of the cellar.

The purpose of this work was to compare the phenolic composition of three types of sherry wines differing only in their aging process, selecting the compounds and fractions most closely related to color development, with a view to expanding available knowledge about the resistance to browning of pale sherry wines during their biological aging.

MATERIALS AND METHODS

Wines. Nine samples of sherry wines obtained under biological and oxidative aging were selected as representative by expert tasters from the Montilla-Moriles region (southern Spain). Triplicates of three samples (F1, F2, and F3) were collected from wines that were biologically aged for 5 years under *Saccharomyces cerevisiae* race *capensis* yeasts (fino wines). Three other samples (O1, O2, and O3) were also obtained in triplicate from wines exclusively subjected to oxidative aging for 7 years (oloroso wines). Finally, three amontillado wine samples (A1, A2, and A3) were obtained by initial biological aging, similarly to the fino wines, followed by a second oxidative aging step such as that used with the oloroso wines. The ethanol contents for the fino, oloroso, and amontillado wines were 15.0 ± 0.174, 19.0 ± 0.175, and 19.0 ± 0.804% (v/v), respectively.

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Model Solution. (+)-Catechin, supplied by Sigma-Aldrich Chemical, SA (Madrid, Spain), was added at a 600 mg/L concentration to a solution of yeast nitrogen base (Difco, Detroit, MI) containing ethanol (15% v/v) and adjusted to pH 3.5 with tartaric acid. Following the procedure of Oszmianski et al. (1996), the solution was supplied with ferrous sulfate, at a 20 mg of Fe^{2+}/L concentration, as an oxidative catalyst. This solution was inoculated with 1×10^6 viable cells/mL of *S. cerevisiae* race *capensis* flor yeasts.

Analytical Procedures. Ethanol was quantified according to the Crowell and Ough (1979) method. Spectrophotometric measurements at 420 and 520 nm were made in a Beckman spectrophotometer, DU 600 model, on a 10 mm path length.

Extraction of Phenolic Compounds. A volume of 100 mL of wine was concentrated in a vacuum at 40 °C to 20 mL, which was adjusted to pH 7 with 0.1 N NaOH. The concentrate was passed through a Sep-Pak C18 cartridge, with 900 mg of filling (Long Body Sep-Pak Plus, Waters Associates), that was previously activated with 8 mL of methanol and washed with distilled water, which was adjusted to pH 7 with NaOH according to the method of Jaworski and Lee (1987). The cartridge was eluted with 8 mL of water at pH 7. This volume, in addition to the volume obtained as a result of the sample run-through prior to the elution, was used for the determination of the phenolic acid fraction. After preconditioning of the cartridge with 2 mL of water at pH 2, the flavan-3-ol fraction was eluted with 8 mL of 16% acetonitrile in water at pH 2 (Oszmianski et al., 1988). The two collected fractions were concentrated and passed through a filter of 0.45 μm pore size for injection into a Spectra-Physics SP880 HPLC instrument.

Identification and HPLC Analysis. The identification of the phenolic compounds was achieved by comparison of the retention times of the standards, UV spectra obtained by HPLC rapid scanning detector (Spectra-Physics model Focus), and calculation of UV absorbance ratios after co-injection of samples and standards (Mayen et al., 1995). Commercial standards were purchased from Sigma-Aldrich Chemical Co. (Madrid, Spain) and Sarsynthese Co. (Genay, France). Caftaric and coutaric acids were isolated according to the method described by Singleton et al. (1978). Procyanidins were obtained from a grape seed extract according to the procedure of Bourzeix et al. (1986). The standard's purity was 95−99%. Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard except the procyanidins, which were quantified as catechin.

Analyses were carried out on a C18 column (250 mm × 4.6 mm, 5 μm particle size) by using 2% aqueous acetic acid and acetonitrile as mobile phases at a flow rate of 2 mL/min and detection at 280 nm.

Phenolic Acids Fraction. The elution phases for this fraction were as follows: gradient elution from 0.1 to 5% CH_3CN in 5 min, isocratic elution for 10 min, gradient elution up to 15% CH_3CN in 5 min, and isocratic elution for 10 min. In this fraction were quantified the following compounds: hydroxybenzoic acids (gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, vanillic, and syringic acids); hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acids); esters of hydroxycinnamic acids (*trans*-caftaric, *cis*-coutaric, and *trans*-coutaric acids); and tyrosol and syringaldehyde.

Flavan-3-ol Fraction. The elution phases for this fraction were as follows: gradient elution from 0.1 to 15% CH_3CN in 15 min, isocratic elution for 5 min, gradient elution up to 20% CH_3CN in 5 min, and gradient elution up to 30% CH_3CN in 5 min. In this fraction were quantified the following compounds: catechins (catechin and epicatechin); and procyanidins (B1, B2, B3, and B4).

HPLC Direct Injection. To avoid possible retentions by the Sep-Pak cartridge affecting the condensed products, all of the wine samples and model solution were subjected to a direct injection. The elution conditions were the same as those used for the phenolic acids fraction, and detection was carried out at 280 and 420 nm. The unknown peaks were quantified as gallic acid.

Statistical Procedures. Variance analyses were performed on the replicated samples by using the Statgraphics Statistical Computer Package (Statistical Graphics Corp.).

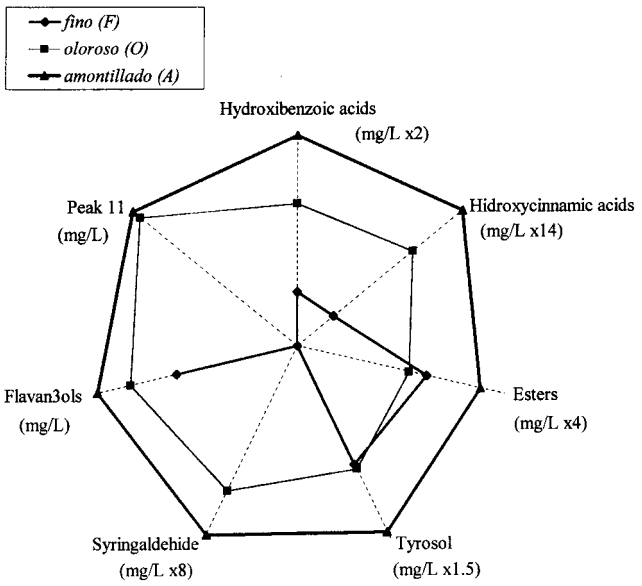

Figure 1. Average contents for the different phenolic fractions and compounds in the wines studied.

RESULTS AND DISCUSSION

Table 1 lists the absorbances at 420 and 520 nm and the contents of the different phenolic compounds in the wines, as well as the homogeneous groups resulting from the variance analysis carried out for each variable studied ($p < 0.01$). A_{420} exhibited significant differences among the three types of wine, particularly those for the fino wines (F) in relation to the oloroso (O) and amontillado (A) wines. This last type of wine showed an absorbance around 1 au that was even higher for the oloroso type. However, homogeneous results cannot reasonably be expected, even with wines of the same aging periods, due to natural variations. This explains, for example, the fact that F1 and F3 had A_{420} values markedly differing from those of F2, although with a substantially different order of magnitude than that of the O and A wines. Similar comments can be made on the absorbance in the red region of the spectrum (A_{520}) for the three types of wine.

Figure 1 shows the average contents in the different compounds studied, grouped in the following fractions: hydroxybenzoic acids, hydroxycinnamic acids, esters of hydroxycinnamic acids, flavan-3-ol derivatives, tyrosol and syringaldehyde. It is important to point out that in the chromatographic zone located at retention times between 33 and 36 min were observed several overlapping peaks, which reasonably can be attributed to slightly polar compounds, in a great part resulting from the browning. Therefore, the named peak 11 was calculated by addition of the areas corresponding to these compounds and quantified as gallic acid. As can be seen, the shapes of the phenol profiles for the O and A wines were similar, except for the vertex occupied by peak 11. On the other hand, the shape for the wine obtained under biological aging (F) was markedly different.

In general, the compounds of the hydroxybenzoic and hydroxycinnamic acid fractions increased in the wines subjected to prolonged aging due to the extraction from wood components (Miller et al., 1992; Fernandez de

Table 1. Absorbances (ua), Concentrations of Phenolic Compounds (mg/L), and Their Homogeneous Groups Resulting from Variance Analysis in the Wines Studied

	F1	F2	F3	O1	O2	O3	A1	A2	A3	F	O	A
gallic acid	7.60 ± 0.158	8.46 ± 0.256	8.48 ± 1.09	6.04 ± 0.890	7.22 ± 0.663	5.75 ± 0.479	8.44 ± 1.41	13.3 ± 0.264	8.47 ± 0.502	a	b	c
protocatechuic acid	3.40 ± 0.210	5.59 ± 0.415	5.08 ± 0.560	9.58 ± 1.18	7.86 ± 0.075	10.2 ± 0.100	8.73 ± 1.56	14.9 ± 1.70	8.67 ± 0.656	a	b	b
p-hydroxybenzoic acid	<0.001	<0.001	<0.001	1.33 ± 0.072	0.858 ± 0.036	1.87 ± 0.330	1.84 ± 0.041	5.03 ± 0.215	4.89 ± 0.217	a	b	c
m-hydroxybenzoic acid	<0.001	<0.001	<0.001	16.3 ± 1.46	15.4 ± 0.173	22.8 ± 2.27	29.9 ± 0.802	26.6 ± 2.79	20.7 ± 1.94	a	b	c
vanillic acid	0.891 ± 0.002	1.08 ± 0.036	0.613 ± 0.070	1.97 ± 0.165	1.49 ± 0.063	2.58 ± 0.215	3.93 ± 0.505	6.44 ± 0.106	2.58 ± 0.394	a	a	b
syringic acid	0.900 ± 0.078	1.85 ± 0.133	1.62 ± 0.100	3.24 ± 0.251	2.41 ± 0.034	2.70 ± 0.222	3.65 ± 0.140	5.50 ± 0.319	3.47 ± 0.042	a	b	c
caffeic acid	0.767 ± 0.038	1.20 ± 0.052	0.832 ± 0.025	3.45 ± 0.313	1.78 ± 0.080	2.68 ± 0.462	3.49 ± 0.137	5.58 ± 0.321	2.70 ± 0.178	a	b	c
p-coumaric acid	0.503 ± 0.047	0.304 ± 0.044	<0.001	1.90 ± 0.110	2.29 ± 0.340	2.74 ± 0.148	3.13 ± 0.445	5.28 ± 0.382	2.96 ± 0.168	a	b	c
ferulic acid	0.397 ± 0.065	0.777 ± 0.117	0.887 ± 0.063	1.41 ± 0.205	1.07 ± 0.126	0.669 ± 0.010	2.52 ± 0.234	<0.001	<0.001	a	a	a
trans-caftaric acid	8.56 ± 0.404	6.12 ± 0.145	7.60 ± 0.187	5.05 ± 0.825	4.46 ± 0.051	5.14 ± 0.687	7.77 ± 1.17	8.88 ± 0.823	9.03 ± 0.635	a	b	a
cis-coutaric acid	5.91 ± 0.105	4.12 ± 0.236	7.61 ± 0.736	4.51 ± 0.070	5.65 ± 0.542	6.35 ± 0.767	6.43 ± 0.386	8.11 ± 0.863	8.68 ± 0.461	a	a	b
trans-coutaric acid	4.56 ± 0.500	5.87 ± 0.507	5.80 ± 1.12	5.78 ± 0.954	5.20 ± 0.407	6.64 ± 0.066	7.23 ± 0.999	12.9 ± 1.11	10.7 ± 0.472	a	a	b
tyrosol	50.2 ± 4.42	55.4 ± 3.25	41.5 ± 3.93	48.2 ± 4.55	52.4 ± 6.02	52.3 ± 1.80	73.3 ± 0.607	75.6 ± 6.09	82.6 ± 3.80	a	a	b
syringaldehyde	<0.001	<0.001	<0.001	11.6 ± 1.25	7.73 ± 0.197	14.3 ± 2.37	11.5 ± 1.30	13.5 ± 0.222	18.9 ± 1.24	a	b	b
catechin	19.9 ± 0.655	16.7 ± 0.404	19.5 ± 0.230	27.7 ± 1.30	29.5 ± 4.32	27.8 ± 0.754	38.8 ± 2.74	35.3 ± 3.39	30.8 ± 1.59	a	b	c
epicatechin	10.7 ± 0.199	12.4 ± 1.10	8.99 ± 0.406	19.5 ± 1.91	15.7 ± 0.152	21.5 ± 2.20	18.8 ± 1.18	27.2 ± 2.86	30.0 ± 0.576	a	b	c
procyanidin B1	11.6 ± 0.230	21.5 ± 0.800	15.8 ± 1.00	16.1 ± 1.17	19.0 ± 0.953	26.7 ± 3.12	24.7 ± 0.818	31.3 ± 1.95	31.0 ± 0.208	a	a	b
procyanidin B2	5.92 ± 0.686	8.33 ± 0.321	7.54 ± 1.00	12.4 ± 1.84	12.0 ± 1.51	13.2 ± 2.52	13.3 ± 0.456	18.6 ± 0.632	14.4 ± 0.309	a	b	c
procyanidin B3	12.0 ± 1.76	15.8 ± 0.378	14.1 ± 0.100	12.8 ± 1.42	9.25 ± 0.161	12.5 ± 1.25	4.26 ± 0.270	4.92 ± 0.290	4.73 ± 0.321	a	b	c
procyanidin B4	3.12 ± 0.516	3.77 ± 0.455	2.38 ± 0.240	3.35 ± 0.319	4.29 ± 0.617	6.17 ± 0.880	4.46 ± 0.090	8.17 ± 1.11	6.14 ± 0.524	a	b	c
peak 11	<0.001	<0.001	<0.001	118 ± 4.62	108 ± 6.43	133 ± 6.11	103 ± 0.577	114 ± 3.76	126 ± 1.60	a	b	b
A_{420}	0.114 ± 0.002	0.141 ± 0.003	0.115 ± 0.002	1.53 ± 0.020	1.27 ± 0.020	1.57 ± 0.030	0.991 ± 0.023	1.11 ± 0.064	0.971 ± 0.037	a	b	c
A_{520}	0.017 ± 0.001	0.021 ± 0.002	0.020 ± 0.001	0.367 ± 0.010	0.326 ± 0.011	0.438 ± 0.008	0.231 ± 0.007	0.248 ± 0.025	0.260 ± 0.022	a	b	c

Figure 2. HPLC chromatograms registered at 420 nm for each type of wine studied.

Simon et al., 1996; Monedero et al., 1998) and the slow evaporation of water and/or ethanol. Therefore, their contents were significantly higher in the A and O wines than in the F wines. A similar behavior was observed in syringaldehyde, although no significant differences between the O and A wines were detected.

Regarding flavan-3-ol monomers and dimers, catechin showed the highest contents, followed by epicatechin and procyanidin B1. Except for procyanidin B3, the content in this fraction increased in the sequence F < O < A, consistent with the above-mentioned effect of evaporation of water and/or ethanol over the lengthy aging. Besides, aging favors the potential hydrolysis of flavan oligomers, as reported by some authors (Timberlake and Bridle, 1976; Dallas et al., 1995). On the other hand, peak 11 registered at 280 nm was especially high for the O and A wines, but it was absent in the F wines. The different behavior of the compounds included in this peak in relation to the visible spectral region demands some comments.

Figure 2 shows the chromatograms obtained at 420 nm for each type of wine studied. As can be seen, peak 11 exhibited a high absorbance for the O and A wines, which suggests that their colors comes largely from the compounds included in this peak. Taking into account that the F wines, without oxidative aging, exhibited a low absorbance at 420 nm in the region around 35 min, a direct or indirect effect of flor yeasts on the formation of some colored compounds during the aging of these wines is suggested.

Flavan-3-ol derivatives are known to play an important role in wine browning (Simpson, 1982; Oszmianski

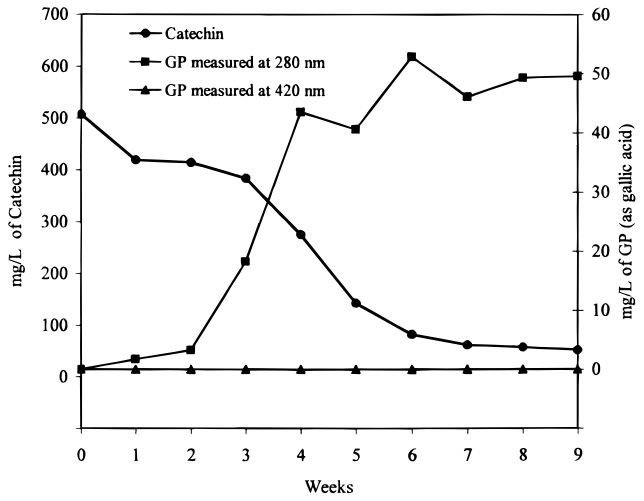

Figure 3. Changes in the contents of catechin and formed products in a model solution of catechin−Fe^{2+} inoculated with flor yeasts.

et al., 1985; Cheynier et al., 1989; Dallas et al., 1995; Fernandez-Zurbano et al., 1995). Of particular interest for the purpose of this work are the findings of Oszmianski et al. (1996). These authors, working on the oxidation of catechin−Fe^{2+} model solutions, found colored oxidation products eluted at retention times similar to those obtained in this work for the oxidatively aged wines (O and A). Because these compounds were not present in the biologically aged wines (F), this catechin degradation pathway must to some extent be altered by the presence of flor yeasts. To confirm this hypothesis, an experiment similar to that of the abovementioned authors was carried out but by inoculating the catechin model solution with *S. cerevisiae* race *capensis* flor yeasts.

Figure 3 shows the changes in catechin content versus time, as well as the concentration corresponding to the addition of the areas of the compounds eluted at retention times around 35 min (named GP), measured at 280 nm and expressed as gallic acid. As can be seen, the catechin content decreased with time, increasing that corresponding to GP. However, this increase resulted in no simultaneous increase of the area corresponding to GP peak measured at 420 nm, which suggests the formation of products absorbing in the UV region but not darkening the solution. It is premature to ascribe the increased contents in the GP absorbing at 280 nm only to catechin degradation products, so further research in this direction is needed for a safe assertion. On the one hand, flor yeasts have a complex metabolism that involves the excretion of non-phenol compounds absorbing in the UV region. On the other hand, the gradual disappearance of catechin may be an indirect result of the presence of flor yeasts, which typically produce acetaldehyde during their aerobic growth (Cortes et al., 1998), thereby favoring the formation of oligomers (Fulcrand et al., 1996). Besides, as suggested by Baron et al. (1997), the possibility of formation of colored oxidation compounds but subsequently retained by the yeasts, and thereby removed from the wine, should not be discarded. Obviously, although catechin is not the only compound responsible for color darkening in white wines, its behavior may partly account for the increased resistance to browning of pale sherry wine during its biological aging, even for >5 years. In our opinion, one of the key questions in this respect is whether the flor yeast promoted protection against browning is a direct result of the inhibition of the oxidation pathway or an indirect result through the adsorption of colored compounds already formed. The answer could be the basis to use yeasts as fining agents to decrease browning in white wines.

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