# Changes in Phenolic Compounds and Browning during Biological Aging of Sherry-Type Wine

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Contents of hydroxybenzoic and hydroxycinnamic acids, hydroxycinnamic esters, and monomeric and dimeric derivatives of flavan-3-ol during biological aging of dry pale sherry white wine were measured and compared between wine without aging and wines in five different stages of aging. The results show a significant decrease in the levels of vanillic and ferulic acids, catechin, epicatechin and procyanidins B2 and B4, as well as an increase in those of syringic acid and procyanidin B1. According to the changes observed in polyphenol contents and the absorbance data obtained at 420 nm, sherry-type wines seem to be protected from excessive browning during biological aging mainly as a result of the partial removal of flavan-3-ol derivatives, besides the effect of protection from air contact carried out by the flor yeasts growing on the wine surface.

**Keywords:** *Phenolic compounds; aging; browning; sherry wine*

## INTRODUCTION

As is well known, phenolic compounds are involved in white wine browning. Good reviews about this topic are made by Singleton (1987) and Macheix et al. (1991). In dry pale white sherry wines, browning results in progressive color darkening that is acceptable up to 9−12 months after bottling. Beyond that point, the brown color and the altered flavor of the wine make it advisable to withdraw it from market. Therefore, browning in this wine is a major source of economic losses and a serious trouble to exportation.

However, sherry wine does not brown as strongly as expected during its typical aging under flor yeasts (biological aging), even though the process takes at least 2 years in oak barrels. In this respect, aging of pale dry sherry wine is not static but dynamic, involving periodic transfers of a volume fraction of each barrel (usually one-fifth) from a younger wine to an older wine. Thus, from each barrel containing the wine in the oldest aging stage (barrel row named "solera"), a fraction is withdrawn and subjected to the fining and bottling operations. The solera is filled up with the same volume of wine from a barrel row at an earlier aging stage (the first "criadera"), which in turn receives wine from the previous aging stage (second criadera), and so on. The row at the lowest aging stage (the fourth criadera) is filled up with young wine without aging. These wine transfers are carried out every 5−6 months, so the minimum duration of the aging process in sherry wine is 2−2.5 years. Older wines can be produced by using wine from the solera to fill up a new fourth criadera, thereby starting a new aging process that duplicates the age of the wine. It should be noted that the wine is under flor yeasts, growing on its surface, at all aging levels (all criaderas and the solera). This and the contact with the barrel wood endow pale dry sherry wine with its characteristic sensory properties. Detailed descriptions about the solera system and the relevance of the veil yeasts for aging sherry wine can be found in papers by Casas (1985), García Maiquez (1988), and Domecq (1989).

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Oxygen is known to influence the browning progress in white wines (Singleton and Kramling, 1976; Singleton et al., 1979; Simpson, 1982; García-Barroso et al., 1989; Fernandez-Zurbano et al., 1995). Traditionally, the scarce browning observed during aging of pale dry sherry wine has been ascribed to the flor yeasts, growing on the wine surface and protecting it from atmospheric oxygen (Casas, 1985). However, this assumption is partially questionable in our opinion. On the one hand, wood is a porous material, so an aeration effect in the long run cannot be discarded. On the other, the protective effect of flor yeasts is not exerted throughout the year because of their stringent temperature demand (around 20 °C). Therefore, these yeasts grow strongly during spring and autumn, whereas the wine is partially exposed to the air during summer and winter. Finally, the above-mentioned periodic transfers of wine are performed in contact with the air; this causes the wine to be saturated with oxygen, which is consumed at the next aging level.

The purpose of this work was to study the browning and changes in nonflavonoid phenols and monomeric and dimeric derivatives of flavan-3-ol during biological aging of pale dry sherry wines under flor yeasts.

## MATERIALS AND METHODS

**Samples.** White wine samples from the Montilla-Moriles region (southern Spain), corresponding to young wine without aging, fourth, third, second, and first aging stages (fourth, third, second, and first criadera, respectively), and the oldest aging stage (solera), were prepared in triplicate by mixing wine from all the barrels (one-fifth from each) of a row at the same aging stage and before it was transferred to the next aging stage. All wines were found to have an ethanol content of 15 ± 0.1% (v/v).

**Analytical Procedures.** Ethanol was quantified by the Crowell and Ough (1979) method. Spectrophotometric measures were made at 420 and 280 nm in a Perkin-Elmer spectrophotometer, Lambda 3 model, on a 10 mm path length. The 280 nm data were corrected taking into account the dilutions performed.

**Extraction of Phenolic Compounds.** A volume of 100 mL of wine was concentrated in vacuum at 40 °C up to 20 mL, which was adjusted to pH 7 with 0.1 N NaOH. The concentrate was passed through a Sep-Pak $C_{18}$ cartridge, with

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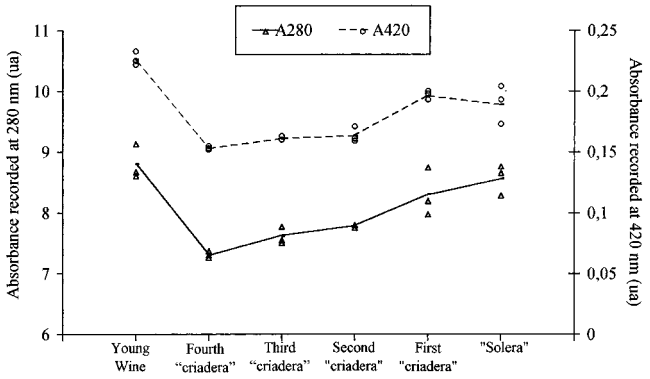


**Figure 1.** Absorbances recorded at 280 and 420 nm for the wines in the different aging stages.

900 mg of filling (Long Body Sep-Pak Plus, Water 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 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 $\mu$m pore size for injection into a Spectra-Physics SP880 HPLC instrument.

**Identification and HPLC Analysis.** The identification of the phenolic compounds was achieved by comparing with the retention times of the standards, UV spectra obtained by a HPLC rapid scanning detector (Spectra-Physics model Focus), and calculation of UV absorbance ratios after coinjection of samples and standards (Mayén 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 by the method described by Singleton et al. (1978). Procyanidins were obtained from a grape seed extract according to Bourzeix et al. (1986). The standard purity was 95−99%. Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard, except the procyanidins that were quantified as catechin.

Analyses were carried out on a $C_{18}$ column (250 mm × 4.6 mm, 5 $\mu$m 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, isocratic elution for 10 min, and gradient elution up to 100% $CH_3CN$ in 10 min. In this fraction were quantified the following compounds: hydroxybenzoic acids—gallic, protocatechuic, vanillic, and syringic acids; hydroxycinnamic acids—caffeic, *trans*-*p*-coumaric, and ferulic acids; esters of hydroxycinnamic acids—*trans*-caftaric, *cis*- and *trans*-coutaric, and feruloyl tartaric (feftaric acid) acids; tyrosol.

*Flavan-3-ol Fraction*. The elution phases for this fraction were as follows: gradient elution from 0.1% to 15% $CH_3CN$ in 5 min, isocratic elution for 5 min, gradient elution up to 20% $CH_3CN$ in 5 min, gradient elution up to 30% $CH_3CN$ in 5 min, and gradient elution up to 100% $CH_3CN$ in 10 min. In this fraction were quantified the following compounds: catechins—catechin and epicatechin; procyanidins—B1, B2, B3, and B4.

**Statistical Procedures.** Principal component analysis was performed on the replicated samples by using Statgraphics statistical computer package (Statistical Graphics Corp.).

## RESULTS AND DISCUSSION

Figure 1 shows the absorbances obtained at 280 and 420 nm for the wines at the different aging stages. As can be seen, both absorbances decreased markedly from the young wine to the fourth criadera. These decreases can be reasonably attributed to the precipitation of oxidized phenols present in the young wine, as well as of the phenol polymers. This polymerization is favored by the presence of acetaldehyde (Haslam and Lilley, 1988), produced during aging by flor yeasts growing on the wine surface. Likewise, both absorbances increased linearly ($p < 0.001$) from the fourth criadera to the solera, which suggests an increase in the amount of total polyphenols measured at 280 nm and in that of browning products at 420 nm. As regards the former, one can expect an extraction of phenolic compounds from wood barrels, basically lignin oligomers in old barrels, as shown for some liquors (Viriot et al., 1993). The increased absorbance at 420 nm is the result of the inevitable aeration involved in industrial transfers of wine during biological aging (sprinkling operations) and of wood porosity. This oxygenation leads to partial oxidation of the phenols that increase browning. The absorbance at 420 nm for the solera (0.196 absorbance unit) is considered somewhat high; however, it decreases markedly by the effect of the fining operations preceding bottling of sherry wine (Escolar et al., 1995).

Table 1 lists the contents in tyrosol, phenolic acids, hydroxycinnamic esters, and monomeric and dimeric derivatives of flavan-3-ol for the different aging stages studied. As can be seen, tyrosol was the major component in the young wine as a result of its production from tyrosine amino acid by the yeasts during alcoholic fermentation. This compound underwent no appreciable changes during aging. Of the other phenols coming from the grape, flavan-3-ol monomers and dimers were the most concentrated followed by hydroxycinnamic esters. By contrast, phenolic acids occurred at relatively low concentrations.

In order to study changes in the levels of the different phenolic compounds during aging, the previous results obtained in triplicate were subjected to principal component analysis. The first three components were selected, which accounted for 73% of the overall variance. Flavan-3-ol monomers and dimers (procyanidin B3 excepted) and some phenolic acids (ferulic, vanillic, and syringic) exhibited the largest statistical weights on component 1, which accounted for over 41% of the overall variance. Procyanidin B3 and *trans*-caftaric, *cis*-coutaric, and *trans*-coutaric acids had the largest weights on component 2, which accounted for 20% of the variance. Finally, protocatechuic and feftaric acids exhibited the highest contributions to component 3, which accounted only for a low fraction of the variance.

Figure 2 shows the scores for each of the samples in the plane defined by the first two components. Four different groups can be established from the sample distribution, namely: young wine, fourth and third criadera, second and first criadera, and solera. Based on component 1 only, the previous four groups can be reduced to two, including the young wine and the fourth and third criadera, on the one hand, and the second and first criadera besides the solera on the other. Taking into account the phenolic compounds that contribute most significantly to the first component, it would be interesting to analyze their individual changes during aging.

Figures 3 and 4 show the triplicate contents obtained for the flavan-3-ol derivatives at each aging stage, as well as their fitting to a straight line. The contents of procyanidins B1, B2, and B4, catechin, and epicatechin exhibited a significant correlation ($p < 0.001$). All except that of procyanidin B1, which increased gradually at least up to second criadera, decreased throughout

**Table 1. Content in Phenolic Compounds (mg/L) for Wine in Different Aging Stages**

| | | criadera | | | | |
|---|---|---|---|---|---|---|
| | young wine | fourth | third | second | first | solera |
| gallic | 6.01 ± 0.398 | 4.33 ± 0.368 | 5.23 ± 0.729 | 5.61 ± 0.098 | 6.23 ± 0.320 | 6.06 ± 0.810 |
| protocatechuic | 4.04 ± 0.456 | 3.94 ± 0.040 | 4.02 ± 0.740 | 3.25 ± 0.071 | 2.97 ± 0.232 | 5.65 ± 0.568 |
| vanillic | 1.78 ± 0.068 | 1.05 ± 0.231 | 1.03 ± 0.213 | 1.00 ± 0.041 | 0.928 ± 0.156 | 0.579 ± 0.034 |
| syringic | 0.936 ± 0.167 | 0.701 ± 0.098 | 0.869 ± 0.101 | 1.41 ± 0.172 | 1.06 ± 0.119 | 1.45 ± 0.310 |
| caffeic | 1.34 ± 0.285 | 1.43 ± 0.203 | 0.960 ± 0.263 | 0.352 ± 0.075 | 1.09 ± 0.129 | 1.08 ± 0.109 |
| *p*-coumaric | 0.647 ± 0.075 | 0.752 ± 0.019 | <0.001 | 0.481 ± 0.046 | <0.001 | 0.518 ± 0.021 |
| ferulic | 1.17 ± 0.222 | 0.729 ± 0.170 | 0.740 ± 0.092 | 0.430 ± 0.029 | 0.411 ± 0.123 | 0.294 ± 0.063 |
| tyrosol | 66.9 ± 8.09 | 65.6 ± 7.28 | 60.9 ± 0.321 | 65.0 ± 1.33 | 61.4 ± 4.76 | 57.3 ± 2.98 |
| *trans*-caftaric | 17.6 ± 1.92 | 20.3 ± 0.579 | 21.4 ± 2.61 | 18.5 ± 2.08 | 16.1 ± 0.816 | 17.5 ± 1.94 |
| *cis*-coutaric | 14.7 ± 1.33 | 16.2 ± 0.115 | 19.2 ± 2.52 | 13.3 ± 1.35 | 13.7 ± 0.149 | 14.7 ± 1.83 |
| *trans*-coutaric | 7.46 ± 0.316 | 9.18 ± 1.01 | 8.88 ± 1.57 | 5.64 ± 0.171 | 5.59 ± 0.286 | 9.53 ± 1.16 |
| feftaric | 5.98 ± 1.46 | 9.57 ± 1.18 | 7.65 ± 0.289 | 7.28 ± 0.302 | 6.83 ± 0.261 | 5.48 ± 1.27 |
| catechin | 36.9 ± 3.50 | 28.4 ± 2.29 | 27.5 ± 3.49 | 24.4 ± 0.456 | 22.4 ± 1.96 | 23.7 ± 3.17 |
| epicatechin | 18.8 ± 3.08 | 17.9 ± 1.45 | 20.8 ± 1.63 | 12.9 ± 1.44 | 12.3 ± 1.84 | 12.5 ± 0.404 |
| procyanidin B1 | 11.6 ± 2.25 | 16.7 ± 1.64 | 19.5 ± 1.95 | 19.7 ± 2.62 | 21.2 ± 0.712 | 20.0 ± 0.458 |
| procyanidin B2 | 13.3 ± 1.54 | 11.3 ± 0.306 | 12.6 ± 1.59 | 9.51 ± 1.85 | 7.79 ± 0.538 | 7.29 ± 1.44 |
| procyanidin B3 | 13.2 ± 2.75 | 13.9 ± 2.79 | 14.7 ± 0.735 | 11.9 ± 0.842 | 10.5 ± 0.360 | 14.7 ± 2.17 |
| procyanidin B4 | 9.25 ± 0.225 | 6.93 ± 1.52 | 7.56 ± 0.295 | 4.18 ± 0.872 | 3.18 ± 0.725 | 3.56 ± 0.419 |

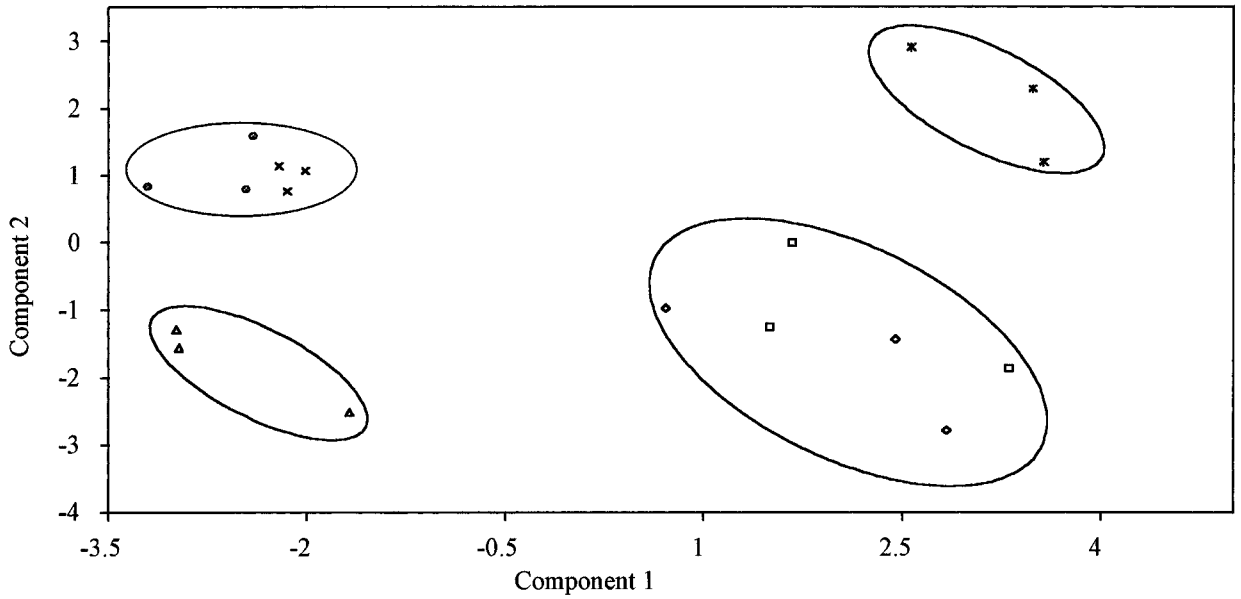


**Figure 2.** Principal component analysis. Score values of the wine samples and their grouping in the plane defined by the first two components: (*) young wine, (□) fourth criadera, (◇) third criadera, (×) second criadera, (○) first criadera, and (△) solera.

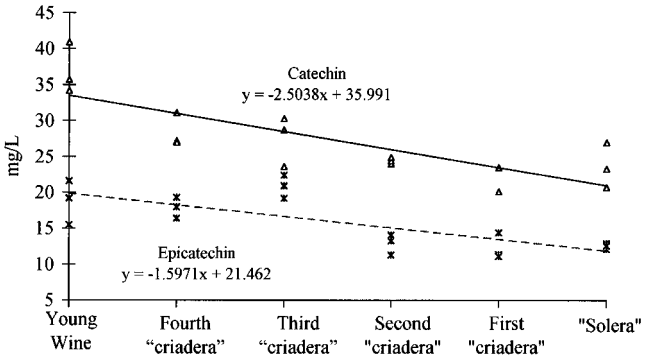


**Figure 3.** Catechin and epicatechin contents in the aging stages and their fitting to a straight line.

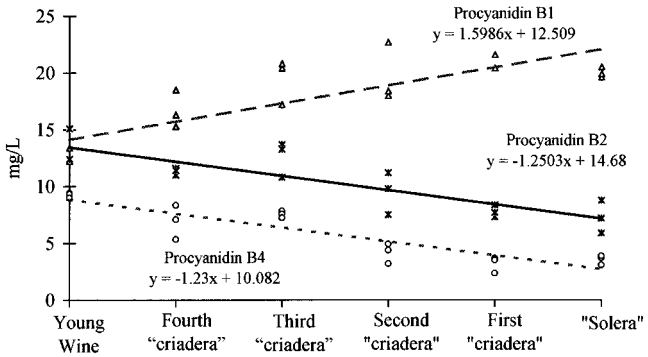


**Figure 4.** Procyanidin B1, B2, and B4 contents in the aging stages and their fitting to a straight line.

aging, although the catechin showed a trend to stabilize their contents after the first criadera. The contents of vanillic and ferulic acids decreased with a high linear correlation ($p < 0.001$), whereas that of syringic acid increased, also significantly ($p < 0.01$). However, the slopes of the straight lines corresponding to the last three compounds were very small, as well as their contents, relative to those obtained for the flavan-3-ol derivatives.

According to previous results, the decrease in the contents of flavan-3-ol monomers and dimers may be reasonably ascribed to oxidative processes, which were followed by condensation and subsequent precipitation in the oak barrels. This hypothesis is supported by several facts. Thus, these compounds are known to take an important role in wine oxidative browning (Simpson, 1982; Fernández-Zurbano et al., 1995) produced in sherry wine as a result of the aeration during biological

aging. This aeration is carried out through the wood and periodically by absence of the flor yeasts during some seasons of the year and as a result of the above-mentioned industrial transfers of the wine, typical of this aging. The aeration may lead to partial oxidation and polymerization of flavan-3-ol derivatives. Below a given molecular size, the polymers remain dissolved in the wine, thereby contributing to the darkening observed at 420 nm. As the polymers grow in size, they become insoluble and prevent an excessive increase of the absorbance at 420 nm. This may account for the typical presence of brown solids (in addition to dead flor yeasts) in the bottom of the oak barrels used to age sherry wines.

The differential behavior of procyanidin B1 suggests the possibility of its production from flavan-3-ol oligomers. In fact, the potential occurrence of this hydrolytic process has been discussed by several authors (Timberlake and Bridle, 1976; Dallas et al., 1995). This hypothesis does not exclude the involvement of this compound in the same above-described reactions as the other flavan-3-ol derivatives; however, the increase in the contents resulting from the hydrolysis of the oligomers should offset the losses.

The observed decreased in the flavan-3-ol derivatives could also be explained, in a complementary way, by adsorption of these compounds on the cellular walls of the flor yeasts. These retentions have been shown for some compounds, such as medium chain fatty acids in cellular walls of fermentative yeasts, named "yeast ghosts" (Larue et al., 1984; Lafon-Lafourcade et al., 1984; Ribereau-Gayon, 1985). In fact, the flor yeasts accumulating in the bottom of the oak barrels show a yellow-brown color that could be the result of the adsorption of these polyphenolic compounds. This hypothesis needs further researching in order to evaluate its importance, since the cellular walls of the flor yeasts could be applied in wine fining treatments, in order to decrease the levels of some phenolic compounds.

In conclusion, biological aging of sherry wines takes place with a general decrease in most of the monomeric and dimeric derivatives of flavan-3-ol, acting on the different aging stages as "cleaners" of these compounds. Taking into account their prominent role in the potential browning of white wines, besides the protection from air contact carried out by the flor yeasts, it allows for sherry wine to be aged under flor yeasts for many years without excessive browning.

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