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Influence of oxygen supply on the susceptibility of cv. Palomino fino must to browning

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Summary

Experiments have been conducted on the oxidation of must (cv. Palomino fino). At the initial decanting process prior to fermentation, wines produced from hyperoxidized must had a reduced content of oxidable polyphenolic compounds and a reduced tendency to browning; they maintained these characteristics after bottling. Intermediate doses and the combined use of oxygen and sulphur dioxide were not fully effective in resolving the problem of browning for must of this grape variety. The larger the dose of oxygen (30 mg·l^{-1}), the less the content of hydroxycinnamic esters and the lower the oxidizability. This dose was appropriate for producing a "fino" sherry wine with a low tendency to browning, retaining the sensory characteristics of this particular type of wine.

K e y w o r d s : must oxidation, Palomino fino, Sherry wine, browning, polyphenols.

Introduction

The typical "fino" Sherry wines of the Jerez region in Spain are submitted to a system of dynamic biological ageing (Casas 1985) under a surface layer of yeasts ("veil of flor"). This protects them from environmental oxygen and significantly influences their organoleptic properties.

After approximately two years of biological ageing, the wine is prepared for bottling and is no longer protected from oxygen; hence, browning problems occur, as with other white wines. Singleton (1987) and Macheix *et al.* (1991) have shown that the deterioration of the organoleptic and sensory properties caused by the phenomenon of browning is due to the oxidation of polyphenolic compounds. As for other white wines, a variety of techniques has been developed to stop this evolution, *e.g.* bottling under an inert gas atmosphere (Prass and Virgo 1976; Gai 1989), and the use of particular fining agents (Baron *et al.* 1998).

For some years, a technique known as "must hyperoxidation" has been used (Schneider 1998): By providing oxygen, some of the polyphenolic compounds are oxidized into polymers of low solubility which can be easily eliminated. This leads to a wine with a lower content in oxidable polyphenolic compounds, assumed to have a lowered tendency to browning. This enotechnical procedure has previously been employed to produce young white wines with good organoleptic characteristics and a higher resistance to browning (Guerzoni *et al.* 1981; Cheynier *et al.* 1991; Nicolini 1992). The capacity for oxygen consumption by must is highly variable and depends on the initial content of hydroxycinnamic acids, a group of polyphenols which differs depending on the grape variety (Schneider 1998).

In this paper, we studied the effect of hyperoxidation of must (cv. Palomino fino) with the aim to produce a "fino" Sherry wine which is resistant to browning and retains its sensory characteristics.

Material and Methods

J u i c e s : In 1996 and 1997 clusters (Palomino fino) were pressed (<1.5 kg·cm^{-2} pneumatic press) and calcium carbonate (2 g·l^{-1}) and tartaric acid (pH 3.25) were added.

P r o c e s s o f h y p e r o x i d a t i o n : For each harvest two trials were conducted in containers (30 l). In each trial, 5 vessels were used. In the hyperoxidized musts, oxygen was supplied before decanting by means of a diffuser, without prior addition of sulphur dioxide. The doses are as follows: A = 10 mg O_2·l^{-1} must; B = 30 mg·l^{-1}; C = 30 mg·l^{-1} followed by 100 mg·l^{-1} SO_2 1 h after addition of oxygen; D = SO_2 after decanting; E = control, with sulphur dioxide at the onset of conventional vinification. Oxygen diffused into the must was measured by an oxymeter (Cark electrode, Oxi-92, Crison Instrument, Barcelona, Spain). One hour later liquid gelatine (0.05 ml·l^{-1}, Gelsol ™, AEB Ibérica, Barcelona, Spain) and silica sol (0.5 ml·l^{-1}, Baykisol ™, AEB Ibérica, Barcelona, Spain) were added to each vessel as fining agents. All musts were decanted after 12 h. Before fermentation SO_2 (100 mg·l^{-1}) was added to musts A, B and D. After fermentation (7 days at 25 °C) and the analysis of the polyphenols and enological parameters, wines that had received the same dose were combined and kept in a vessel until it was racked off the lees. Thereafter, the wine was fortified to 15 % v.v. and then underwent the biological ageing process under yeasts ("veil of flor").

P o l y p h e n o l i c p r o f i l e : 80 µl of filtered wine (0.45 µm) was analysed, in triplicate, by HPLC. Mobile phases employed were: solvent A (95 % water, 5 % methanol) and B (95 % methanol, 5 % water) at pH 2.5 (super-pure sulphuric acid). Elution phases: gradient elution from 100 to 85 % solvent A (5 min), gradient elution from 85 to 50 % solvent A

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(40 min) and isocratic elution (35 min). The analyses were carried out using a C_{18} column (LiChrospher 100 RP-18, 250 x 3 mm, 5 µm particle size) at a flow rate of 0.5 ml·min^{-1}; and detection at 280 and 320 nm.

The polyphenolic compounds were identified by comparison with a library of DAD spectra and retention times of standards. Commercial standards were purchased from Fluka (Buchs, Switzerland) and East Kodak (Rochester, NY). Caftaric and coutaric acids were isolated as described by SINGLETON *et al.* (1978). Each compound was quantified using a calibration curve obtained with standards, except for GRP (2-S glutathionyl caftaric acid), which was quantified as caftaric acid.

Statistical treatment: Variance and principal component analyses were performed on data from the triplicated samples using the Statgraphics Statistical Computer Package (Statgraphics plus 3.1 for Windows 95).

Determination of susceptibility to browning: 100 ml of must were ultracentrifuged (30 min. at 12,000 *g* in a Beckman model J2-21 centrifuge with JA-14 rotor) and subjected to a process of electrochemical oxidation developed by our research group. The susceptibility to browning was quantified as follows: increase in absorbance recorded at 420 nm related to the amount of electricity supplied (PALMA *et al.* 2000). Curves were obtained which fitted to equations of the following type:

$$y = -b + \frac{1}{2 + a^{-CX}},$$

where y equals the increase in absorbance (420 nm), x the electricity supplied (coulombs), a the slope of the curve, representing the rate of browning; b the horizontal asymptote to which the curve tends. For high values of electricity this parameter represents the maximum degree of predicted browning (the higher b, the lower browning); c is a parameter with no chemical meaning varying between 0.3 and 0.4.

Results and Discussion

Tab. 1 presents average values of the main polyphenols after fermentation of must of the 1997 harvest. To identify significant differences between the control and the hyperoxidized wines, the results were subjected to an analysis of variance for each phenolic compound of the two tests of the 1997 harvest. The main polyphenolic compounds which differed significantly between control and hyperoxidized wines are gallic acid, caftaric acid, *cis-p*-coutaric acid, *trans-p*-coutaric acid, feftaric acid, 2S-glutathionyl caftaric acid (GRP), chlorogenic acid, caffeic acid, catechin and epicatechin.

A general decrease of the polyphenolic content can be observed for wines produced from hyperoxidized musts, with the exception of tyrosol; this compound is not modified by the oxygen supplied since it is present only in wine as a result of fermentation of tyrosine by yeasts (SINGLETON and TROUSDALE 1983). GUNATA *et al.* (1987), CHEYNIER and OSSE (1988) and CHEYNIER *et al.* (1989) have demonstrated that

Table 1

Polyphenols (mg·l^{-1}) after fermentation (mean values) and analysis of variance applied to two trials (harvest 1997). GRP = 2S-glutathionyl caftaric acid. For details see Fig. 1

Compound	Concentration (mg·l^{-1}, first test)					Analysis of Variance			
						First test		Second test	
	A	B	C	D	E	*F*	*p*	*F*	*p*
gallic acid	4.81	4.40	4.56	6.01	6.11	11.91	0.009^{A}	25.55	0.002^{A}
tyrosol	19.65	22.50	20.95	16.15	18.82	0.77	0.588	10.53	0.060
catechin	5.14	4.20	5.08	6.13	7.28	10.10	0.013^{a}	0.70	0.058
p-hydroxybenzoic acid	1.31	1.03	1.43	0.97	2.09	7.13	0.027^{a}	0.60	0.678
p-hydroxybenzaldehyde	0.57	0.57	0.59	0.32	0.68	2.35	0.187	1.85	0.257
syringic acid	2.16	1.52	2.37	0.57	2.10	0.99	0.488	19.18	0.003^{A}
epicatechin	3.20	3.80	5.10	4.73	5.91	32.37	0.000^{A}	28.52	0.001^{A}
caftaric acid	7.43	1.97	3.06	12.72	21.72	86.9	0.000^{A}	18.53	0.003^{A}
cis p-coutaric acid	7.74	3.47	5.53	8.84	11.86	68.16	0.000^{A}	25.52	0.002^{A}
trans p-coutaric acid	7.08	1.76	2.46	10.40	17.31	32.6	0.000^{A}	34.38	0.000^{A}
feftaric acid	0.34	0.14	0.22	0.39	0.49	2.65	0.007^{A}	11.74	0.009^{A}
GRP	7.12	5.05	7.10	9.13	13.06	26.76	0.000^{A}	81.14	0.000^{A}
chlorogenic acid	1.43	0.49	1.14	1.84	2.83	0.99	0.000^{A}	6.23	0.035^{a}
caffeic acid	1.23	1.22	1.71	2.08	2.36	8.61	0.018^{a}	2.47	0.008^{A}
cis p-coumaric acid	1.12	1.12	1.13	1.06	1.22	0.98	0.493	1.21	0.411
trans p-coumaric acid	0.96	1.00	1.05	1.05	1.33	1.10	0.447	2.06	0.224
i-ferulic acid	1.06	0.99	1.84	1.12	1.21	11.12	0.051	2.90	0.136
ferulic acid	2.76	2.30	2.63	2.14	2.24	4.26	0.072	9.74	0.014^{a}

^{a} Values are significantly different at $p < 0.05$
^{A} Values are significantly different at $p < 0.01$

oxygen provided early in the process causes oxidation of the hydroxycinnamic esters into quinones, which in turn facilitate oxidation of other polyphenolic compounds present in the must, such as catechin and epicatechin.

In order to examine the overall effect of each dose of oxygen on the phenolic compounds studied, a multivariate principal component analysis was carried out for those compounds that were significantly affected by hyperoxidation ($p < 0.01$) in comparison with control wine (E). For the first test (1997 harvest), Fig. 1 shows the plane defined by the first two components, which represents the statistical weights of the samples corresponding to the control wine and those treated with different doses of oxygen as well as the vectors which reflect the contribution of each phenolic compound.

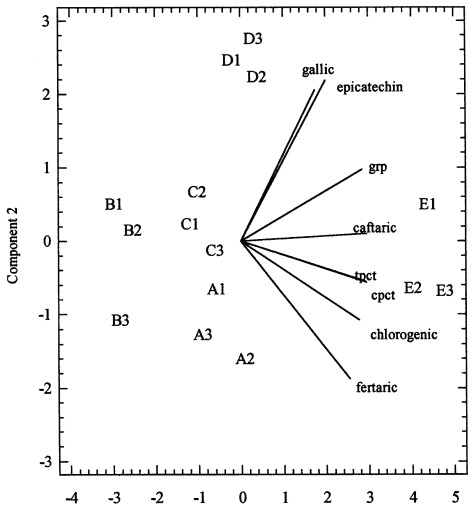

Fig. 1: Principal component analysis. Biplot representation of wine samples and statistical variables (phenolic compounds). The plot contains a scatterplot of principal components and a line for each variable that reflects how each of them contributes to the components. A = 10 mg·l^{-1}, B = 30 mg·l^{-1}, C = 30 mg·l^{-1} followed by 100 mg·l^{-1} SO_2 1 h after addition of oxygen, D = SO_2 after decanting, E = Control. GRP = 2S-glutathionyl caftaric acid, tpct = *trans p*-coutaric acid, cpct = *cis p*-coutaric acid.

Component 1, which accounted for 76.0 % of the overall variance, divided the wines in three groups: control wine (E); wines treated with oxygen (A, B and C); and wine without protection against browning (D). Taking into account that all phenolic compounds considered contributed with a positive sign to component 1, wines from must treated with the largest dose of oxygen (B) were those which led to the largest overall decrease in phenolic compounds. Component 2, accounting for a much lower proportion of the overall variance (13.1 %), provided no clear results because some phenols contributed to it with a positive sign and others with a negative sign.

Within the group of treated wines, no significant differences were found between wines A (10 mg·l^{-1}) and C (30 mg·l^{-1} oxygen + sulphur dioxide added 1 h later). In view of these results, it would appear that, after the rapid initial action of the supplied oxygen, the addition of sulphur dioxide diminished the efficiency of the hyperoxidation process; it possibly reduced some of the oxidized compounds (CHEYNIER *et al.* 1991).

D wines, subjected to environmental oxygen, demonstrated an intermediate position between untreated and treated wines. We therefore suggest that, due to their lower phenolic content wines produced from hyperoxidized musts, will show a lower susceptibility to browning. To determine the susceptibility of wines from the two harvests, samples taken during the process of vinification and during a 12 months period of ageing under the "flor" yeasts, were subjected, in duplicate, to the electrochemical test of accelerated browning. No significant differences were observed between musts of different harvests subjected to the same dose of oxygen and sulphur dioxide.

Fig. 2 presents the results obtained from samples taken after decanting and fermentation (first test, harvest 1997).

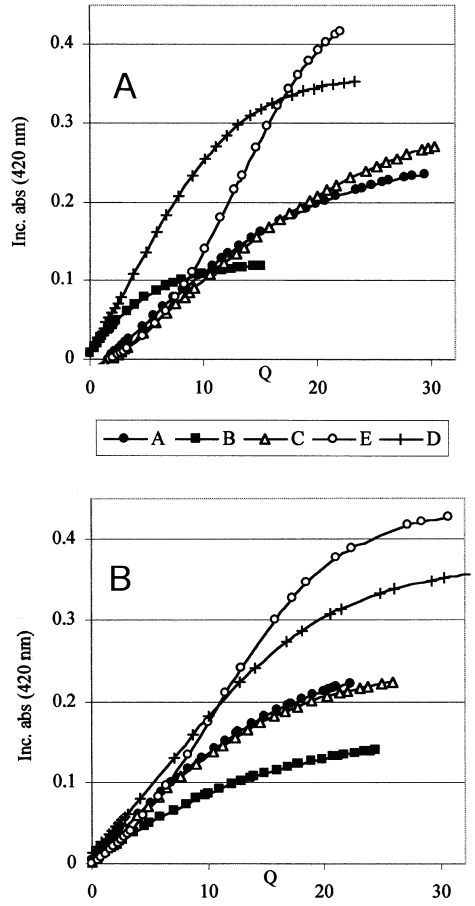

Fig. 2: Electrochemical test for accelerated browning of samples taken after decanting (**A**) and after fermentation (**B**). Harvest 1997. For details see Fig. 1. Q: Electric charge (C).

After decanting the sample subjected to conventional vinification (E) showed the highest tendency to browning. After pressing SO_2 had been added to this sample by which the start of oxidation was delayed. This must showed the maximum degree of oxidizability. Musts A and C were quite similar reaching comparable levels of oxidizability; this was expected given the similarity with respect to their phenolic content. The must without sulphur dioxide, D, in which oxidation was enhanced by providing environmental oxygen, presented levels of oxidizability which were intermediate between the control must and the samples A, B and C subjected to hyperoxidation. The reduced tendency to browning observed for musts subjected to hyperoxidation, after

Table 2

Values of the parameters "a" and "b" found for the experimental wines from the 1996 and 1997 harvests, after 6 and 12 months of biological ageing under the "flor". Colour (absorbance at 420 nm) for bottled wines after 6 months under biological ageing and then kept another 6 months in bottle

Wine	Harvest 1996					Harvest 1997				
	6th month			12th month		6th month			12th month	
	abs (420 nm)	a	b	a	b	abs (420 nm)	a	b	a	b
A	0.182	1.76	0.27	0.99	0.33	0.180	1.28	0.31	1.34	0.30
B	0.150	0.80	0.36	0.77	0.36	0.152	0.67	0.38	0.93	0.35
C	0.179	2.45	0.24	3.77	0.18	0.180	1.83	0.26	1.50	0.30
D	0.198	2.42	0.23	2.41	0.24	0.195	4.58	0.15	4.43	0.16
E	0.214	4.55	0.15	5.06	0.15	0.220	5.37	0.14	7.98	0.10

they have been decanted, implies that oxygen affects the oxidizable phenolic compounds rapidly (CAPRISI *et al.* 1995), *e.g.* hyperoxidation very rapidly led to a darkening of the must. As expected the must subjected to the largest dose of oxygen demonstrated the least tendency to browning.

After fermentation experimental wines showed a similar behaviour; during their ageing under the "flor" yeasts, samples were taken every two months throughout the first year, for the trials on wine produced from the 1996 and 1997 harvests.

Tab. 2 presents values obtained for the parameter "a" (rate of oxidation) and "b" (tendency to browning). The data correspond to the samples taken after 6 and 12 months of biological ageing under the "flor", for the two harvests. Similar to the results obtained after decanting and after fermentation, the not hyperoxidized samples of both harvests showed higher oxidation rates and a higher tendency to browning after 6 and 12 months under the "flor".

These results obtained by the electrochemical test of accelerated browning agree with colour evolution in samples taken after 6 months of biological ageing and another 6 months in bottle (Tab. 2): 6 months after bottling, the wines produced from hyperoxidized musts had a less intense colour, thus demonstrating a reduced tendency to browning.

With regard to the organoleptic characteristics, at the time of bottling a group of experts found no alteration of the sensory properties of the experimental wines, even not if the largest dose of oxygen (30 $mg \cdot l^{-1}$) had been used.

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