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Influence of the addition of sulphur dioxide and must hyperoxidation on the phenolic fractions during vinification of Sherry wines

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Changes in some polyphenol fractions after prefermentative must treatments, during alcoholic fermentation and postfermentation standing of Sherry wines were studied. The prefermentative treatments were hyperoxidation, sulphur dioxide addition or both simultaneously. In general, all the fractions increased in concentration in every type of must, possibly as a result of their extraction from the residual sludges. These residual sludges are characteristics of Sherry wine industrial vinification. Wines from non-hyperoxidized and sulphited musts had the lowest contents of all phenolic fractions. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Must and wine browning is one of the greatest problems encountered during the vinification of white wines as it alters not only their colour, but also their sensory properties during the wine making process and/or after a short period following bottling. The origin of this alteration is the oxidation and subsequent condensation of polyphenol fractions in musts and wines (Singleton, 1982, 1985, 1987; Singleton *et al.*, 1984, 1985; Cheynier *et al.*, 1989*a,b,c*, 1990*a,b*, 1991; Cheynier & Ricardo Da Silva, 1991). As well as by the addition of an antioxidant such as sulphur dioxide, browning is typically avoided by the following: soft pressed systems of grapes, use of nitrogen to avoid contact of the must and wine with atmospheric oxygen, more effective systems for the prefermentation sludge removal and hyperoxidation of musts with gaseous oxygen. This latter procedure involves the forced oxidation of polyphenols and their removal prior to fermentation in order to decrease the concentration of oxidizable substrates that give rise to browning, which is thus delayed or reduced. However, the addition of oxygen to must initially proved to be detrimental to the aroma fraction of the resulting wine (Singleton *et al.*, 1980). More recent hyperoxidation procedures using variable amounts of oxygen frequently avoid this problem, even though their effectiveness depends on the particular grape variety (Nagel & Graber, 1988; Cheynier *et al.*, 1989*c*, 1991). Likewise, the use of sulphur dioxide in conjunction with an oxidation procedure can decrease the hyperoxidation efficiency (Cheynier *et al.*, 1991; Sims *et al.*, 1991), on account of the reducing character of sulphur dioxide.

On the other hand, the musts from which Sherry wines are produced are usually subjected only to a simple decanting process prior to fermentation. So, the industrial fermentation of Sherry wines is carried out in the presence of residual sludges. In fact, it is well known that more thorough sludge removal increases the fraction of fruity aroma compounds in the wine, which is not a desirable organoleptic characteristic in Sherry wines. However, must sludges are a source of polyphenols, so their partial presence during alcoholic fermentation can increase the contents of these compounds in the resulting wine.

The aim of this work was to study comparative changes in some polyphenol fractions after treatment of musts with oxygen, sulphur dioxide or both, during alcoholic fermentation and postfermentation standing of Sherry wines, in order to identify variations of these compounds at the different vinification stages. The purpose is to determine the prefermentative treatments (or combination of them) for decreasing the amount of polyphenols in the wines, making them more resistant to browning under the typical sludge removal conditions used by the winemaking industry of Sherry wines.

MATERIALS AND METHODS

Samples and fermentations

Grapes of *Vitis vinifera* c.v. Pedro Ximénez grown in the Montilla–Moriles (Southern Spain) were used. After

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pressing, the must thus obtained was split into four batches, namely:

- batch 1, was subjected to a decanting process for 24 h, after which sludges were eliminated. The final must had a suspended solids concentration (residual sludges) of 1.7% ±0.35 (v/v) (treatment without O_2, without SO_2);
- batch 2, was subjected to a decanting process similar to the previous one and supplied with 75 mg SO_2/litre as potassium metabisulphite (treatment without O_2, with SO_2);
- batch 3, must without sludge removal was subjected to hyperoxidation by circulation of gaseous oxygen (purity higher than 99.9%) up to saturation measured by stabilization of an oximeter provided with Clark electrode (Oxi-92, Crison Instrument, Barcelona, Spain). After 24 h, the hyperoxidated must was separated from the sludge. The final must had a suspended solids concentration of 2.0% ±0.3 (v/v) (treatment with O_2, without SO_2);
- batch 4, was hyperoxidized and subsequently subjected to sludge removal, like the previous one, and supplied with 75 mg SO_2/litre (treatment with O_2, with SO_2).

The four batches of musts were adjusted to pH 3.2 by adding tartaric acid, and inoculated with *Saccharomyces cerevisiae* race *cerevisiae* yeast strains. Each must batch was subjected to alcoholic fermentation in 10-litre thermostatted (25°C) tanks. All experiments were carried out in triplicate. After the fermentations were finished (14 days), the resulting wines were stored at room temperature (20°C) until 30 days after the experiments were started.

Samples were collected from each of the three tanks of each batch at the start (initial), after hyperoxidation (wherever applicable), after SO_2 addition (in the applicable musts), at the end of alcoholic fermentation (end fermentation) and at 30 days.

Analytical procedures

Ethanol was quantified by the Crowell & Ough (1979) method. The levels obtained (% v/v) for the four wines at the end of the study were: 15.2±1.03 (without O_2, without SO_2), 15.3±0.763 (without O_2, with SO_2), 16.1±0.212 (with O_2, without SO_2) and 16.0±0.251 (with O_2, with SO_2).

Extraction and identification

Samples of 100 ml were adjusted to pH 7 with NaOH, prior to extraction with ethyl acetate, in order to separate neutral from acidic polyphenols (Ramey *et al.*, 1986). After that, Sep-Pak C18 cartridges (Water Associates), previously activated with methanol and adjusted to pH 7 (by passing water adjusted to pH 7 with NaOH) according to Jaworski & Lee (1987), were used to retain neutral polyphenols contained in the ethyl acetate. The retained polyphenols are subsequently eluted by using solvents of variable polarity, 16% acetonitrile at pH 2 to collect flavan-3-ols fraction (catechins and procyanidins), and ethyl acetate to collect the flavonols fraction (Oszmianski *et al.*, 1988).

After the extraction with ethyl acetate, the pH of the aqueous phase was adjusted to 2 with HCl and then extracted again with ethyl acetate to obtain the polyphenols acidic fractions (hydroxybenzoic and hydroxycinnamic acids and esters of hydroxycinnamic acids). Finally, all the fractions were evaporated to dryness and then dissolved in 2 ml of methanol.

The identification of the phenolic compounds was achieved by comparing with the retention times of the standards, UV spectra obtained by HPLC Diode Array detector (Spectra-Physics mod. Focus) and calculation of UV absorbance ratios after coinjection of samples and standards (Mayén *et al.*, 1995). Commercial standards were purchased from Sigma-Aldrich Chem. Co. (Madrid, Spain) and Sarsynthese Co. (Genay, France). Caftaric and Coutaric acids were isolated of the method described by Singleton *et al.* (1978). Procyanidins were obtained from a grape seed extract according to Bourzeix *et al.* (1986). The standard's purity was 95–99%. The quantified compounds in each fraction were as follows.

Phenolic acids fraction:

- hydroxybenzoic acids: gallic, protocatechuic, *p*-hydroxybenzoic, *m*-hydroxybenzoic, vanillic and siringic;
- hydroxycinnamic acids: caffeic, *p*-coumaric and ferulic;
- esters of hydroxycinnamic acids: caftaric and coutaric.

Flavan-3-ols fraction:

- catechins: catechin and epicatechin;
- procyanidins: B1, B2, B3 and B4.

Flavonols fraction:

- myr-3-rhamnoside, que-3-galactoside, que-3-glucoside, que-3-rhamnoside, myricetin (myr), quercetin (que) and kampherol (kam).

Each compound was quantified by comparison with a calibration curve obtained with the corresponding standard, except the procyanidins which were quantified as catechin.

HPLC analyses

A 25 μl aliquot of each fraction was injected into an HPLC (Spectra-Physics, mod. SP880). The chromatographic conditions employed were as follows.

C18 column (220×4.6 mm, 5 μm size particle). Mobile phase: 2% acetic acid and acetonitrile

	Phenolic acids fraction	Flavan-3-ols fraction	Flavonols fraction
Flow-rate	2 ml/min	2 ml/min	1.5 ml/min
Wavelength	280 nm.	280 nm.	365 nm.

Elution	%CH_3CN	%CH_3CN	%CH_3CN
0 min	0.1	0.1	0.1
5 min	5	—	—
15 min	5	15	15
20 min	15	15	15
25 min	—	20	20
30 min	15	30	30
40 min	100	100	40
50 min	—	—	100

Statistical procedures

Student *t*-test and principal component analyses were performed on the replicated samples by using a Statgraphics Statistical Computer Package.

RESULTS AND DISCUSSION

In order to observe changes in the different polyphenol fractions after each prefermentative treatment, during alcoholic fermentation and during postfermentation standing, the per cent change for each such fraction in each period, *viz.* [(final content−initial content)/initial content]×100 was calculated. Previously the significance of the differences between the samples used for calculation (final and initial value for each period) using the Student's *t*-test (Tables 1 and 2) was determined. Only the per cent changes corresponding to significantly different samples ($P<0.01$) were used for a graphical plot. The plus and minus signs in the tabulated values denote an increase or decrease, respectively, in the fraction concerned during the stated period.

Figure 1 shows the per cent change of the contents in the different polyphenol fractions resulting from hyperoxidation of the applicable must (initial—after hyperoxidation). The content of hydroxybenzoic and hydroxycinnamic acids and procyanidins increased after oxidation, probably as a result of the partial extraction of the must on the sludge pressing (most sludges were removed 24 h after oxygen was supplied). However, the ester of hydroxycinnamic acids fraction decreased by *ca* 50% relative to that in the initial must, which can be ascribed to its higher sensitivity to oxygen as a result of its greater liability to oxidation reaction (Romeyer *et al.*, 1985; Gunata *et al.*, 1987; Cheynier & Van Hulst, 1988; Cheynier *et al.*, 1989*a*). The addition of oxygen to the must caused no significant changes in the catechin and flavonol fractions, which does not necessarily imply that they were not oxidized—particularly the former—since their possible oxidation may be overlapped with the extraction effect from sludge.

Both hyperoxidized and non-hyperoxidized musts (wherever applicable), were supplied with SO_2 after sludge removal. Figure 2 shows the per cent change of the different fractions studied as a result of this treatment. No significant changes in the catechin, procyanidin or flavonol contents, on addition of the sulphur dioxide, were observed. Non-oxidized musts markedly increased the concentrations of hydroxycinnamic esters and (more moderately) the content of hydroxybenzoic and hydroxycinnamic acids. Oxidized musts only featured moderately increased levels of hydroxybenzoic acids. Based on previous interpretations (Cheynier *et al.*, 1991), the increased contents of these fractions as a result of the addition of SO_2 can be ascribed to the reversible character of the oxidation process in the presence of a reductant such as SO_2. On this assumption, the large difference in the ester fraction between the two types of must suggests that, in those subjected to accelerated oxidation, oxygen gave rise to oxidized forms that could not be reduced by SO_2 and/or incorporated into the sludge and subsequently eliminated with sludge removal, so only a portion (significant at $P<0.05$, however) was re-incorporated into the must in its reduced forms. Reduction of oxidized forms in musts that were not subjected to accelerated oxidation, but

Table 1. Student's *t* values and probability for the initial and final samples in each period

Period	Treatments	Benzoics T(*P*)	Cinnamics T(*P*)	Esters T(*P*)	Catechins T(*P*)	Procyanidins T(*P*)	Flavonols T(*P*)
Initial—after O_2	with O_2–without SO_2	+7.31***	+6.66**	−8.57***	−0.20	+5.90**	+1.33
Initial—after SO_2	without O_2–with SO_2	+5.74**	+3.82**	+11.4***	+1.41	−0.747	+1.79
	with O_2–with SO_2	+4.24**	+0.019	+2.80*	+1.45	−3.10*	+0.445
Initial—end fermentation	without O_2–without SO_2	+5.08**	+5.71**	+21.1***	+6.42**	+22.1***	+1.43
	without O_2–with SO_2	−1.27	+2.24*	−3.67*	+3.70*	+22.7***	+3.27*
	with O_2–without SO_2	+4.67**	+7.61***	+11.5***	+10.2***	+7.08**	−0.744
	with O_2–with SO_2	+9.36***	+6.16***	+9.04***	+12.8***	+7.87***	+0.940
End fermentation—30 days	without O_2–without SO_2	+1.96	+2.05	+0.289	−2.80*	−2.48*	−1.82
	without O_2–with SO_2	−0.306	−3.01*	+2.19*	−6.25**	−5.17**	−6.63**
	with O_2–without SO_2	+0.258	−2.46*	+0.328	−6.27**	−1.49	−1.45
	with O_2–with SO_2	−1.73	−1.16	+0.010	−3.14*	+0.278	−1.34
Initial—30 days	without O_2–without SO_2	+6.10**	+9.89***	+10.9***	+4.63**	+95.8***	−1.41
	without O_2–with SO_2	+3.79**	+8.65***	+26.7***	−2.48*	+150***	−1.84
	with O_2–without SO_2	+5.49**	+3.99**	+7.07**	+2.95*	+658***	−0.636
	with O_2–withSO_2	+15.8***	+9.17***	+20.5***	+2.80*	+41.9**	+1.43

***$P<0.001$, **$P<0.01$, *$P<0.05$.

only to that resulting from contact with atmospheric oxygen during mechanical pressing of the grapes, was much more marked.

Figure 3 shows the per cent change in the contents of the different polyphenol fractions during alcoholic fermentation. This period was considered from the end of each treatment (oxidation or SO_2 addition) to the end of alcoholic fermentation (14 days). As can be seen, all the fractions studied increased in concentration in all types of must, with the exception of the flavonols fraction in all musts, and those of hydroxybenzoic and hydroxycinnamic acids, hydroxycinnamic esters and catechins in the must without oxidation and SO_2 treated (without O_2, with SO_2). We should emphasize the large increase in the procyanidin fraction content in all types of must, followed by those of hydroxycinnamic acids and catechins.

Table 2. Absolute values of phenolics (mg/100 ml)

	Benzoics	Cinnamics	Esters	Catechins	Procyanidins	Flavonols
Treatments: without O_2–without SO_2						
Initial	3.69	0.327	1.06	0.882	2.31	6.99
	2.69	0.267	0.744	0.711	2.71	7.24
	3.02	0.288	0.924	0.747	2.28	7.69
End fermentation	4.52	0.627	3.45	1.96	33.0	12.5
	4.94	0.744	3.21	1.80	37.8	9.22
	5.04	0.900	3.46	1.48	34.0	7.07
30 Days	5.30	0.975	3.50	1.09	31.5	6.65
	5.02	0.852	3.04	1.39	31.5	7.36
	5.76	1.08	3.78	1.34	30.7	5.79
Treatments without O_2–with SO_2						
Initial	3.69	0.327	1.06	0.882	2.31	6.99
	2.69	0.267	0.744	0.711	2.71	7.24
	3.02	0.288	0.924	0.747	2.28	7.69
After SO_2	6.94	0.534	5.63	1.01	2.35	8.28
	6.73	0.744	4.78	0.855	2.37	7.42
	5.41	0.501	4.48	0.792	2.26	9.20
End fermentation	6.00	0.855	3.61	1.59	34.4	10.3
	4.90	0.714	2.93	1.41	30.2	9.82
	5.91	0.792	3.70	1.18	30.9	11.2
30 Days	6.41	0.558	4.08	0.639	25.0	6.58
	4.65	0.672	4.02	0.651	25.2	5.51
	5.17	0.624	3.82	0.660	25.1	7.06
Treatments: with O_2–without SO_2						
Initial	1.95	0.294	1.48	0.645	1.12	6.94
	2.49	0.228	1.46	0.205	1.27	7.95
	1.86	0.306	1.45	0.205	1.26	8.46
After O_2	3.71	0.450	0.762	0.306	1.66	8.77
	3.92	0.471	0.642	0.312	1.54	8.19
	3.56	0.435	0.918	0.348	1.72	8.31
End fermentation	7.00	1.08	6.01	2.10	37.2	7.87
	5.24	0.888	5.15	1.79	52.7	8.10
	6.69	1.14	6.67	2.36	34.7	8.65
30 Days	7.80	0.651	5.08	0.678	33.1	6.22
	5.10	0.567	6.14	0.948	39.7	7.90
	6.69	1.14	6.67	2.36	34.7	8.65
Treatments: with O_2–with SO_2						
Initial	1.95	0.294	1.48	0.645	1.12	6.94
	2.49	0.228	1.46	0.205	1.27	7.95
	1.86	0.306	1.45	0.205	1.26	8.46
After O_2	3.71	0.450	0.762	0.306	1.66	8.77
	3.92	0.471	0.642	0.312	1.54	8.19
	3.56	0.435	0.918	0.348	1.72	8.31
After SO_2	5.12	0.381	1.68	0.348	1.41	9.67
	4.76	0.552	1.26	0.390	1.51	7.81
	4.37	0.426	1.06	0.327	1.43	8.55
End fermentation	6.76	0.834	7.72	1.46	46.4	10.1
	6.84	0.774	6.97	1.44	31.1	9.68
	6.97	0.894	5.74	1.42	35.1	8.37
30 Days	6.66	0.827	7.27	1.26	40.4	8.30
	6.73	0.798	6.81	0.777	37.3	9.19
	6.11	0.672	6.37	0.816	38.8	8.21

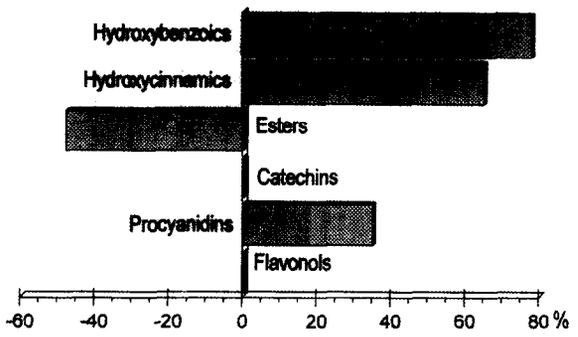

Fig. 1. Changes (%) in the polyphenol fractions in the period initial—after hyperoxidation (wherever applicable).

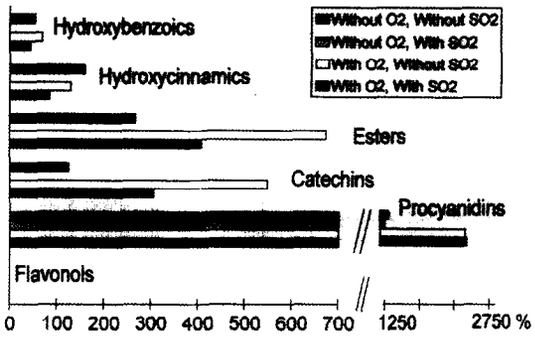

Fig. 3. Changes (%) in the polyphenol fractions during fermentation.

Before the fermentative process, in the musts to which no SO_2 was added, greater amounts of oxidized forms can be expected than those supplied with this antioxidant, particularly in the must with oxidation treatment. Consequently, a higher content of these forms could be potentially reduced during fermentation (reducing medium) in the musts without SO_2 treatment. However, in the must that was both oxidized and supplied with antioxidant (with O_2, with SO_2), part of the oxidized forms were immediately reduced on addition of SO_2, so this must contained smaller amounts of such forms than did the oxidized must containing no SO_2 (with O_2, without SO_2), but greater than the must without oxidation and without SO_2 treatments. Greater or smaller amounts of oxidized forms existing in the musts before fermentation can explain greater or lesser increases in the phenol contents during fermentation, owing to the existing reducing medium. In any case, these redox equilibria were overlapped with the potential extraction of musts on the solids remaining after sludge removal, residual sludges (*ca* 2% *v/v*), so the catechins and procyanidins increases—particularly the latter—may be more closely related to this effect than to the previous one.

Figure 4 shows the per cent change of the different phenolic fractions during postfermentation standing of the wines (end fermentation—30 days). As can be seen, the contents of hydroxybenzoic and hydroxycinnamic acids, and hydroxycinnamic esters, remained virtually unchanged, with no significant decreases or increases. However, the content of catechins, procyanidins and flavonols fractions tended to decrease in all the wines, though only significantly in the wine coming from must supplied with SO_2 but not oxidized (without O_2, with SO_2). Of special note is also the significant decrease in the catechins content of wine coming from must oxidized but with no SO_2 added (with O_2, without SO_2).

Figure 5 shows the per cent change of the polyphenol fractions during the overall study (from the initial must to 30 days). As can be seen, the content of all the fractions studied (except for catechins in wines coming from treated musts and flavonols in all wines) increased significantly in the four types of wine, as a result of the increasing trend observed during fermentation (Fig. 3). We should emphasize the significant but relatively low variation of the catechin fraction as compared to other fractions during the overall study, notwithstanding its rise during fermentation, as a result of its marked decrease during postfermentation standing (from the end of fermentation to 30 days). Several apparent contradictions also warrant some comment. Thus, the flavonols fraction did not change significantly between the beginning and end of the study (30 days), even though it decreased appreciably during postfermentation standing (between the end of fermentation and the 30th day) in the non-oxidized, SO_2-supplied must (without O_2, with SO_2). In order to interpret these observations, one should bear in mind that only those percentages calculated from significantly different values were plotted, so other increases and/or decreases, however statistically insignificant, are real (Tables 1 and 2), but have not been plotted.

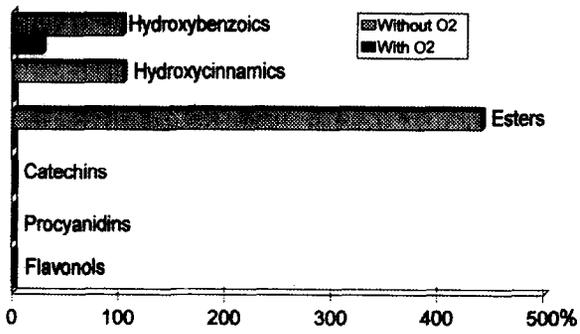

Fig. 2. Changes (%) in the polyphenol fractions in the period initial—after sulphitation (wherever applicable).

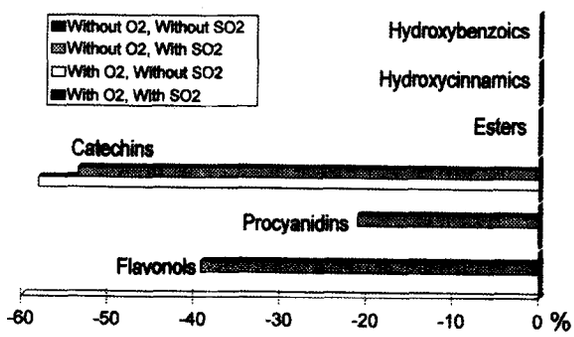

Fig. 4. Changes (%) in the polyphenol fractions during postfermentation standing.

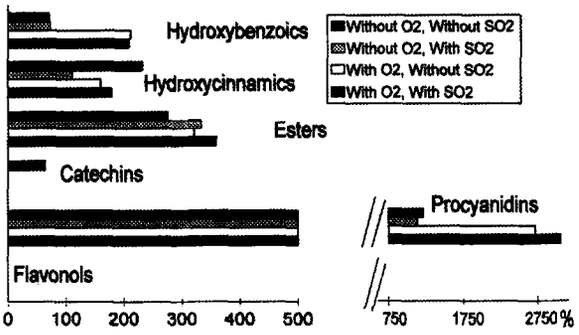

Fig. 5. Changes (%) in the polyphenol fractions during the overall study.

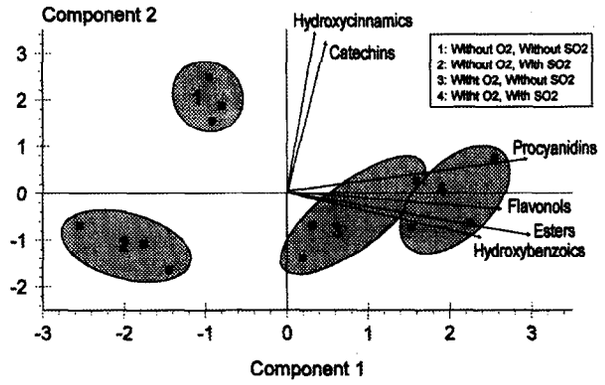

Fig. 6. Principal component analysis. Biplot representation of final samples and variables.

In order to compare the content of the different polyphenol fractions in the final wines coming from various prefermentative treatments, the results were subjected to principal component analysis (PCA). Figure 6 shows the representation of the results obtained for each triplicate sample on the plane defined by the first two components, in addition to the vectors reflecting the contributions of the different polyphenol fractions of each component. The greatest relative weight on component 1 corresponded to the hydroxybenzoic acids, hydroxycinnamic esters, procyanidins and flavonols fractions. This component accounted for 46.76% of the variance. Component 2 was mainly determined by the hydroxycinnamic acids and catechins fractions and accounted for 31.02% of the variance.

As can be seen, the wines coming from different prefermentative treatments can be grouped in four clusters. Group 2, corresponding to the wine samples from unoxidized musts and SO_2 supplied (without O_2, with SO_2), featured the lowest content in all the polyphenol fractions studied. Group 1, which included the wines from musts subjected to no treatment (without O_2, without SO_2), exhibited the highest content of catechin and hydroxycinnamic acid fractions. Finally, groups 3 and 4, partially overlapped in relation to components 1 or 2, included the wines from oxidized musts (with O_2), whether or not they were supplied with SO_2 (with SO_2 or without SO_2), and featured the highest content of hydroxybenzoic acid, hydroxycinnamic esters, flavonols and procyanidins.

In conclusion, hyperoxidation of musts, with a partial sludge removal, with a view to producing Sherry wines is seemingly less effective than is reported for the production of table wines, relative to musts from which sludge is thoroughly removed. In fact, the content of most polyphenol fractions increased during the study in the four must batches. In addition, the hyperoxidation treatment provided worse results than did the traditional treatment (without O_2, with SO_2) and even no treatment (without O_2, without SO_2). As noted earlier, the worse results provided by the oxidative treatments might arise from reduction of the oxidized forms of polyphenols, the concentration of which may have been increased by the oxygen treatment. In our opinion, the must oxygenation for production of Sherry wines could be useful if sludges have been thoroughly removed, even though in this case the wines are known to inherit some uncharacteristic fruity aroma compounds from the musts. However, such fruity aromas may be lost during biological aging of the wines under "flor" film yeasts. If such was the case, hyperoxidation treatments might help prevent browning, with no adverse effect to the characteristic aroma of Sherry wines. Further research is required, however, in order to check this hypothesis in wines aged over a period of at least 2 years, the minimum time for biological aging in this type of wine.

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