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# Hyperoxidation: influence of various oxygen supply levels on oxidation kinetics of phenolic compounds and wine quality

by

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## Hyperoxygénation: influence de différents apports d'oxygène sur la cinétique d'oxydation des composés phénoliques et la qualité des vins

R é s u m é : Des moûts de Chenin blanc, de Mauzac et de Chardonnay ont été hyperoxygénés chacun avec trois doses différentes d'oxygène, déterminées d'après leur capacité maximale de consommation d'oxygène. La composition phénolique des moûts témoins et traités a été analysée par HPLC ainsi que celle des vins correspondants. Ces derniers ont également été soumis à une analyse sensorielle. L'hyperoxygénation n'a pratiquement pas modifié la composition phénolique des moûts de Chenin et de Mauzac qui se sont révélés en grande partie oxydés au terme du pressurage. De même, l'analyse sensorielle n'a mis en évidence aucun effet du traitement sur la qualité des vins de ces deux cépages. Dans les moûts de Chardonnay, la concentration en acides caféoyl et p-coumaroyl tartrique, acide S-glutathionyl,2,caféoyl tartrique et catéchine diminue avec les doses croissantes d'oxygène. Les vins contiennent de plus grandes quantités de composés phénoliques à cause de la réduction d'une partie des quinones présentes sous forme libre lors du sulfitage. Ce dernier devrait donc être supprimé ou du moins différé pour favoriser la transformation des quinones en produits condensés et améliorer l'efficacité de la technique d'hyperoxygénation. Les vins de Chardonnay issus de moûts hyperoxygénés ont été jugés moins colorés et de meilleure qualité que le témoin.

K e y w o r d s : must, wine, phenol, oxygen, sulphur, hyperoxidation, polyphenol, wine quality, sensory rating, variety of vine.

## Introduction

Enologists traditionally recommended careful protection of white musts against oxidation, in particular by early sulfuring, to avoid undesirable browning during storage. However, as the regulation concerning sulfur dioxide addition in the food industry become stricter, the need for alternative technology increases.

The technique referred to as 'hyperoxidation' has been proposed recently by Italian researchers (GUERZONI *et al.* 1981) and successfully applied to produce stable white wines with respect to color from mechanically harvested grapes. Must conditioning with oxygen fastens the transformation of phenolic precursors into brown polymers which are then easily eliminated during the normal clarification and fermentation processes. Thus, and although thoroughly oxidized musts are often very dark, the color of the resulting wines is usually lighter and much more stable than that of those produced by conventional technology (MÜLLER-SPÄTH 1977; SINGLETON *et al.* 1980; NAGEL and GRABER 1988; CHEYNIER *et al.* 1989 b; DUBOURDIEU and LAVIGNE 1990). However, some of the wines produced using this technique lacked varietal aroma and were considered low in quality (SINGLETON *et al.* 1980; DUBOURDIEU and LAVIGNE 1990), whereas, according to other authors, oxidation exerted no advserse effect on wine flavor (MÜLLER-SPÄTH 1977; NAGEL and GRABER 1988; CHEYNIER *et al.* 1989 b). These conflicting results

are not surprising given the large variability in must composition and amounts of oxygen supplied by the various experimentators. However, they suggest that the optimal hyperoxidation conditions should be determined for each particular must.

The mechanisms responsible for must oxidative browning are better understood since the discovery, by SINGLETON and coworkers, of 2-S-glutathionyl caffeoyl tartaric acid, also called 'Grape Reaction Product' (GRP), in oxidizing musts (SINGLETON *et al.* 1984, 1985; CHEYNIER *et al.* 1986). This compound results from the reaction of glutathione with caffeoyl tartaric acid o-quinone generated by enzymatic oxidation of caffeoyl- and p-coumaroyl-tartaric acids, the major phenols of white musts. After glutathione depletion, the excess caffeoyl tartaric acid o-quinones can oxidize other must constituents, including GRP (CHEYNIER and VAN HULST 1988) and flavanols (catechins and procyanidins) (CHEYNIER *et al.* 1988), and be simultaneously reduced back to caffeoyl tartaric acid. The regeneration of caffeoyl tartaric acid in this reaction enables its reoxidation by polyphenoloxidase with further oxygen consumption.

An experimental device allowing the determination of the amount of oxygen which can be absorbed by a given must has been developed by RIGAUD *et al.* (1988). Fast (i.e. due essentially to polyphenoloxidase activity) oxygen consumption capacity of musts has been shown to be highly variable and correlated with the initial hydroxycinnamic acid content, whereas the oxidation kinetics appeared more related to the hydroxycinnamic acids to glutathione molar ratio (CHEYNIER *et al.* 1990 a). Besides, increased flavanol extraction due to skin contact or drastic pressing induced larger oxygen consumption (MOUTOUNET *et al.* 1990).

The purpose of our work was to compare the effect of three different oxygen levels, adapted for each variety tested according to its particular oxygen consumption capacity, on must phenolic composition and wine quality.

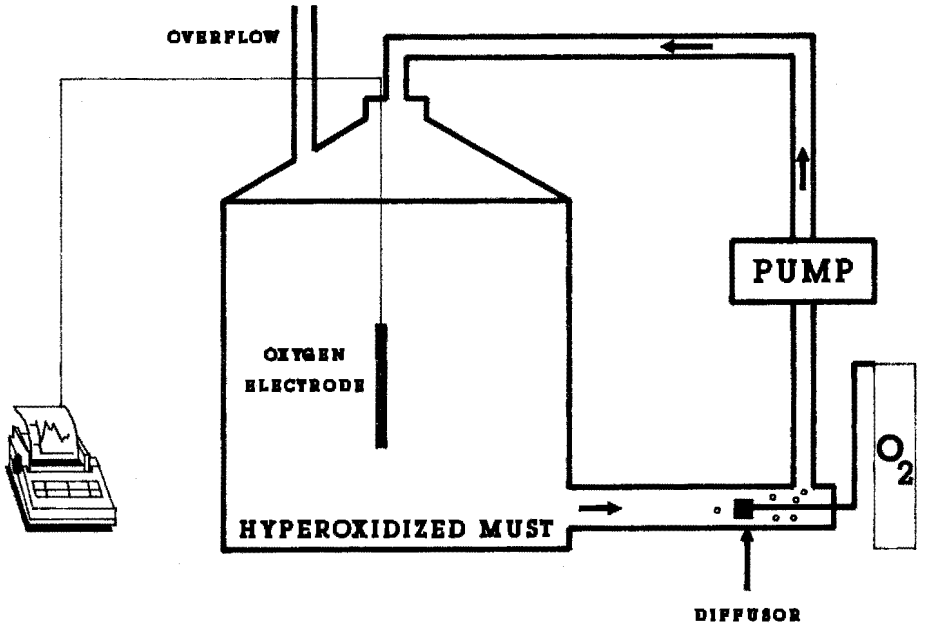



Fig. 1: Experimental device used for must hyperoxidation.

Dispositif expérimental utilisé pour l'hyperoxygénation des moûts.

## Materials and methods

### Grapes and wines

The wines were made in 1988 at the INRA experimental winery in Pech Rouge (Gruissan, France) from Chardonnay, Chenin blanc and Mauzac grapes, harvested at commercial maturity in Limoux. Grenache blanc grapes were harvested during the 1989 season from the INRA vineyard at Pech Rouge.

For each variety, 1500 l must were obtained from 2500 kg grapes and divided into four lots. The first three were supplied with three oxygen quantities corresponding, respectively, to the total fast oxygen absorption capacitiy determined as described by RIGAUD *et al.* (1988) on musts prepared from the same grapes, half this value, and a quarter of it. The fourth lot, supplied immediately with 50 mg/l sulfur dioxide, served as control. Hyperoxidation was performed using the experimental device shown in Fig. 1, by circulating must through the full tank and pipes while diffusing oxygen and by varying the experiment duration so as to obtain the required oxygen supply with constant must and gas flow rates. After completion of the oxygen consumption, controlled by means of a WTW Ox 91 oxymeter equipped with a Clark electrode, each tank received 50 mg/l sulfur dioxide. All four musts were then allowed to settle overnight at 15 °C, centrifuged, inoculated with 200 mg/l dried Fermivin yeast (*Saccharomyces cerevisiae*), and fermented to dryness (less than 2 g/l residual sugar). Sulfur dioxide (40 mg/l) was systematically added at the end of the alcoholic fermentation to prevent malolactic fermentation. The wines were clarified and bottled 4 months later.

### Chemical analysis

Must samples were taken from each lot before and after hyperoxidation, sodium benzene sulfinate added (approximately 2.5 g/l) to trap the free o-quinones eventually present as the corresponding benzene sulfones (CHEYNIER *et al.* 1989 a), stabilized by addition of sodium metabisulfite and stored in the dark at 4 °C.

HPLC assays of caffeoyl tartaric acid, p-coumaroyl tartaric acid, 2-S-glutathionyl caffeoyl tartaric acid (GRP), catechin, and of the corresponding o-quinones were performed as described previously (CHEYNIER *et al.* 1989 a) by direct injections of the must samples, after filtration through 0.45 µm membrane filters. Wines were analyzed in the same way, shortly after bottling.

### Sensory analysis

All wines were evaluated after 1 year storage by a panel of 16 judges. Each variety was presented in a different session. The wines were served cool (8—10 °C) in clear wine glasses. The tasters were required to rate the color (COL) on a 1 (green) to 5 (gold) arbitrary scale and the olfactory frankness (OFR) (no off-odor), odor intensity (INT), aroma quality (ARQ), frankness by mouth (FRM) (no off-taste), acidity (ACI), mellowness (MEL), equilibrium (EQU), persistance (PER) and overall quality (QUA), each on a 1 (low) to 5 (high) arbitrary scale for the four wines plus one replicate of two randomly selected wines.

## Results and discussion

Oxygen absorption capacity was approximately 15 mg/l for each of the three varieties studied. Therefore, the amounts of oxygen added were 3.75 mg/l, 7.5 mg/l and 15 mg/l in all three cases.

1. Influence of hyperoxidation on must phenolic composition

Comparison of the caffeoyl (plus p-coumaroyl) tartaric acid concentration in control and hyperoxidized musts with that of the non-oxidized musts obtained in the laboratory from the same grapes (Fig. 2) indicates that most of the oxygen consumable by the polyphenoloxidase system (about 50 % in the case of Chardonnay, over 90 % in Chenin blanc and Mauzac) was consumed during crushing and pressing. Thus, hyperoxidation hardly modified phenolic composition of the last two musts, whereas caffeoyl tartaric acid losses reached 60 %, 75 % and 90 %, respectively, in the Chardonnay musts supplied with 3.75 mg/l, 7.5 mg/l and 15 mg/l oxygen. As well, the exhaustion of phenolic substrates after pressing explains the very slow decrease of oxygen concentration observed in hyperoxidized Chenin blanc and Mauzac musts.

Initial concentrations of caftaric acid and glutathione were approximately the same (215 µM and 119 µM, 218 µM and 112 µM, respectively) in Chenin blanc and Chardonnay. However, the oxidation kinetics of hydroxycinnamic derivatives in Chenin blanc resembled that of model solutions containing enough glutathione to trap all the caffeoyl tartaric acid o-quinones formed as GRP (CHEYNIER *et al.* 1990 a, b). On the opposite, Chardonnay behaved like model solutions containing two- to threefold excess of caffeoyl tartaric acid, as expected from its composition. Therefore, the differences observed among the two varieties, although they may indicate higher polyphenoloxidase activity in Chenin blanc, are more likely to be due to its larger content of quinone-trapping compounds other than glutathione. Another possibility is that the presence of rather large amounts of reductants such as ascorbic acid delays phenolic compounds oxidation in Chardonnay (CHEYNIER *et al.* 1990 a).

It should be noted that Chardonnay musts oxidized with 3.75 and 7.5 mg/l oxygen still contained large amounts of free caffeoyl tartaric acid o-quinones after the available oxygen was consumed. Besides, caffeoyl tartaric acid concentration in the corresponding wines (Table) exceeded that measured in musts, meaning that sulfiting at this stage reduced part of the quinones present, thus preventing them from proceeding to polymers. This was confirmed in 1989 by oxidizing Grenache blanc must with

Phenolic composition of control and hyperoxidized Chardonnay wines

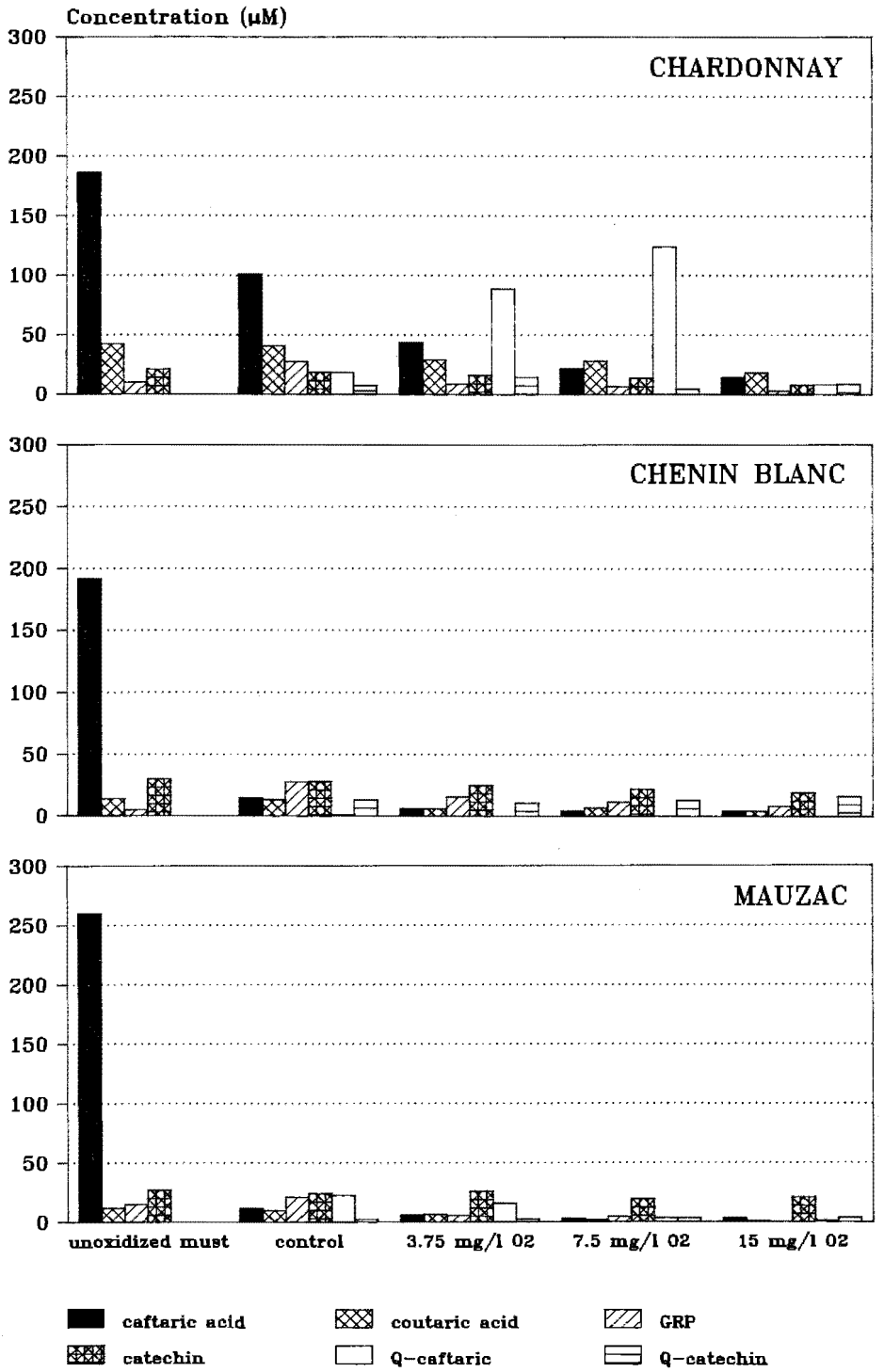
Composition phénolique des vins de Chardonnay témoin et hyperoxygénés

| Prefermentation treatment | Concentration of phenolic compounds (µM) | | |
|---|---|---|---|
| | Caffeoyl tartaric acid | GRP[1]) | p-Coumaroyl tartaric acid |
| Control | 96.5 | 21.5 | 24.5 |
| 3.75 mg/l $O_2$ | 91.5 | 20.4 | 19.2 |
| 7.5 mg/l $O_2$ | 67.2 | 14.1 | 10.4 |
| 15 mg/l $O_2$ | 19.1 | 4.0 | 7.2 |

[1]) GRP: 2-S-glutathionyl caffeoyl tartaric acid.

Fig. 2: Phenolic composition of unoxidized, control and hyperoxidized musts. — Abbreviations: caftaric acid: caffeoyl tartaric acid; coutaric acid: p-coumaroyl tartaric acid; GRP: 2-S-glutathionyl caffeoyl tartaric acid; Q-caftaric: caffeoyl tartaric acid o-quinone; Q-catechin: catechin o-quinone.

Composition phénolique des moûts non oxydés, témoins et hyperoxygénés.


Concentration (µM)
CHARDONNAY
CHENIN BLANC
MAUZAC
300
250
200
150
100
50
0
unoxidized must
control
3.75 mg/l 02
7.5 mg/l 02
15 mg/l 02
caftaric acid
coutaric acid
GRP
catechin
Q-caftaric
Q-catechin

50 mg/l oxygen and taking samples after each step of the winemaking process. Under these conditions, the oxygen added was consumed in a few minutes, the amount of caffeoyl tartaric acid free o-quinones was very high and half the initial caffeoyl tartaric acid concentration was recovered after sulfiting (Fig. 3). Catechin and GRP were also partially regenerated by sulfur dioxide.

On the other hand, hyperoxidized Chenin blanc and Mauzac musts as well as the Chardonnay must supplied with 15 mg/l oxygen exhibited very low free quinone levels, probably because the much slower decrease of oxygen concentration down to zero allowed enough time for reactions of quinones to take place. This leads to the conclusion that sulfur dioxide addition should be avoided or at least delayed to achieve maximum efficacy of the hyperoxidation technique.

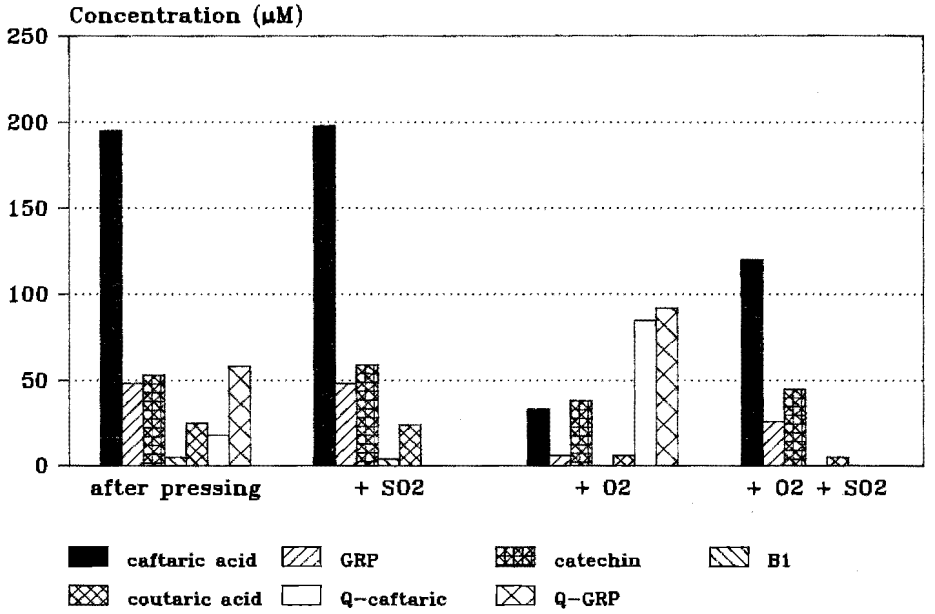



Fig. 3: Influence of hyperoxidation (50 mg/l $O_2$) and sulfiting on the phenolic composition of Grenache blanc must. — Abbreviations: caftaric acid: caffeoyl tartaric acid; coutaric acid: p-coumaroyl tartaric acid; GRP: 2-S-glutathionyl caffeoyl tartaric acid; Q-caftaric: caffeoyl tartaric acid o-quinone; Q-GRP: GRP o-quinone; B1: procyanidin B1.

Influence de l'hyperoxygénation (50 mg/l $O_2$) et du sulfitage sur la composition phénolique d'un moût de Grenache blanc.

## 2. Results of sensory analysis

Tests of similarity probabilities performed for each variety showed no difference among the four Chenin blanc wines. This indicates that the treatment had no effect on this particular must, presumably because it was almost completely oxidized after pressing, as shown by the compositional data. On the contrary, at least one sample differed from others for olfactory frankness and frankness by mouth in the case of Mauzac wines, and for olfactory frankness, aroma intensity, aroma quality, frankness by mouth and overall quality in the case of Chardonnay wines.

Prinicipal component analysis (PCA) was performed on the correlation matrix generated using the mean scores obtained for the sensory attributes by each Chardonnay and Mauzac wine.

The first three principal components (PCs), which, according to the Kaiser criterion were the only significant ones, accounted together for 79,6 % of the total variance. The best separation of the wines was achieved along the first two PCs. The factor loadings for the sensory parameters (shown as vectors) and the wine mean scores are presented in Fig. 4.

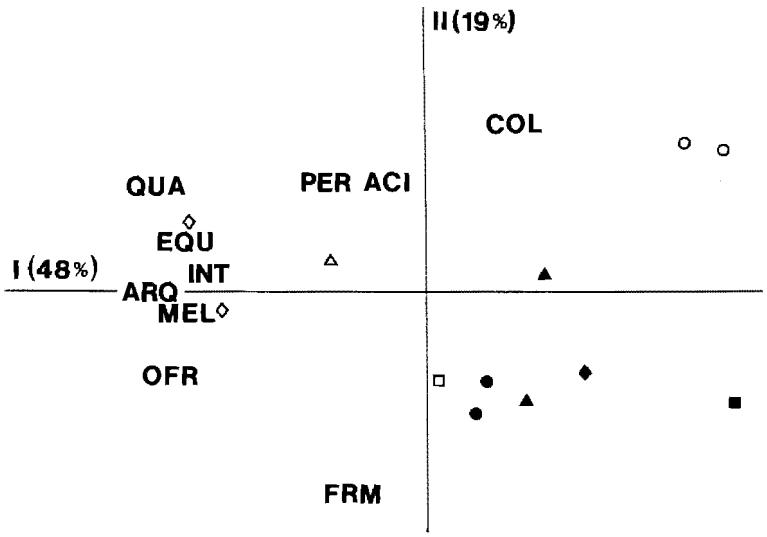



Fig. 4: Projection of wine sensory data on principal components I and II. Sensory attributes loadings and factor scores for control wines (○), wines obtained from musts treated with 3.75 (△), 7.5 (□), and 15 (◇) mg/l oxygen, where Chardonnay wines are denoted by open symbols and Mauzac wines by solid symbols. — Abbreviations: ACI: acidity; ARQ: aroma quality: COL: color; EQU: equilibrium; FRM: frankness by mouth (no off-taste); INT: odor intensity; OFR: olfactory frankness (no off-odor); MEL: mellowness; PER: persistence; QUA: overall quality.

Projection des variables sensorielles et des vins de Chardonnay (symboles évidés) et de Mauzac (symboles pleins) témoins (○), et issus de moûts ayant reçu 3,75 (△), 7,5 (□) et 15 (◇) mg/l d'oxygène sur les deux premiers axes principaux.

The first axis (48 % of the total variance) was a function of aroma quality, intensity and frankness, overall quality, mellowness and equilibrium which were all positively correlated. The second PC (19 % of the total variance) contrasted color and frankness by mouth. The Chardonnay wine treated with 15 mg/l oxygen and, to a lesser extent, those treated with 3.75 and 7.5 mg/l oxygen were rated higher in frankness and quality and well separated from control Chardonnay which had higher color scores. Mauzac wines occupied an intermediate position and were very close together except for that oxidized with 7.5 mg/l oxygen, meaning, since the latter was noticeable by an off-flavor presumably not related to hyperoxidation, that the treatment was ineffective on Mauzac musts, as expected from the compositional data.

## Conclusions

Analysis of must phenolic composition showed that Chenin blanc and Mauzac musts were almost totally oxidized after pressing so that hyperoxidation was efficient on Chardonnay must only. As well, sensory analysis established no treatment related difference among the Mauzac and Chenin blanc musts.

Although systematic recourse to hyperoxidation cannot be recommended, this technique induced no aroma or quality degradation in the three varieties tested, under the experimental conditions described herein. It may therefore be of use to avoid defects resulting from excessive phenolic extraction.

However, from our observations it appears essential to evaluate the residual oxygen absorption capacity of each must after pressing in order to determine its optimal hyperoxidation conditions. Besides, it was demonstrated that sulfiting, which reduced part of the quinones formed, partially cancelled the effect of hyperoxidation and should therefore be delayed to favor condensation reactions, elimination of browning precursors and precipitation of the undesirable polymers.

## Summary

Chenin blanc, Mauzac and Chardonnay musts were hyperoxidized using three different oxygen levels fixed according to the maximum oxygen consumption capacity determined for each must. Phenolic composition of the control and oxidized musts and that of the corresponding wines were analyzed by HPLC. The wines were also submitted to sensory evaluation. Hyperoxidation hardly modified phenolic composition of Chenin blanc and Mauzac musts which were in fact almost totally oxidized after pressing. As well, it induced no change in wine quality in these two varieties. In Chardonnay musts, concentrations of caffeoyl and p-coumaroyl tartaric acid, 2-S-glutathionyl caffeoyl tartaric acid and catechin decreased with increasing oxygen supplies. Wines contained larger amounts of phenolic compounds than musts because part of the quinones formed during oxidation were reduced when sulfiting, especially for intermediate oxygen levels. Thus, sulfiting should be omitted or delayed to allow quinone condensation and maximum efficiency of the hyperoxidation technique. Hyperoxidized Chardonnay wines were rated higher in quality and lower in color than the control.

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