Must Hyperoxidation: A Review

V. SCHNEIDER*

When white grape juice is processed without sulfur dioxide, enzymatically induced oxidation occurs and leads to a precipitation of phenolic compounds as insoluble brown pigments. Wines obtained from oxidized must are reported to display more resistance against oxidative quality degradation during aging. Hyperoxidation makes use of deliberate oxidation prior to fermentation in order to improve wines' shelf-life. Sensory results are derived basically from the flavonoid removal involved. This review deals with the reactions involved, technical applications, analytical control, chemical and sensory consequences, and the reasons for conflicting results of hyperoxidation.

KEY WORDS: hyperoxidation, oxygen consumption, flavonoid phenols, oxidative aging, browning, flavor stability

The very first experiments on must hyperoxidation were reported in the 1970s [37,38,39]. Traditionally, careful protection of must against oxidation has been recommended to avoid browning. Thus, the purpose of these experiments was to demonstrate that oxidation of must prior to fermentation was not so detrimental to white wine quality as expected. In many instances, however, wines made from oxidized musts were rated even better than those obtained from non-oxidized or sulfited musts. Consequently, there was a tendency in several European countries to reduce or omit the use of sulfur dioxide during pre-fermentation operations.

The development of mechanical harvesting, associated with early crushing and longer pomace contact time, often led to an increase of phenol content and resulted in bitter, coarse wines. This was when systematic studies on hyperoxidation were instigated in order to precipitate phenols responsible for bitterness, astringency, and browning during wine aging [22]. The technique was based on the assumption that oxidation of must is different from oxidation of wine. When phenols are eliminated from must by enzymatic oxidation, they do not affect wine quality when undergoing chemical oxidation. Must processing with oxygen fastens the transformation of phenolic precursors to brown, insoluble polymers which are easily removed during the normal clarification process. Thus, although oxidized musts are very dark, the resulting wines are lighter and more stable in sensory parameters than those produced by conventional technology.

Enological interest of flavonoid removal: The two major classifications of phenolic compounds in grapes are non-flavonoids and flavonoids. Non-flavonoids are concentrated in the pulp cell vacuoles and are present in all juices. The flavonoid phenols reside in the firmer tissues of the skin, seeds, and stems. White wines are usually made from juices pressed from the skins quickly and, therefore, contain relatively low levels of total phenols, almost all of which are non-flavonoids in nature. The extent to which the total phenols are increased is largely related to the additional extraction of flavonoids from the firmer tissues. Grinding and shredding of these tissues by severe mechanical treatments and pomace contact time make flavonoids much more available to the must. Flavonoid extraction is further enhanced by temperature and sulfur dioxide [7,11,59,73,74,75].

The phenolic regime of juices is related to sulfur dioxide management. When enzymatic activity is inhibited by SO_2, phenols are protected against oxidation and stabilized in solution. When white grape musts are left on their own, phenols undergo oxidative browning and precipitate. Winemaking practices without SO_2 are already fairly aerative. Passive oxidation is only limited by natural oxygen uptake. It makes the transition from SO_2-protected juice processing to deliberate oxidation.

Hyperoxidation reduces the amounts of all phenolic compounds investigated [7,8,9,10,11,51], but sensory results are derived mainly from the removal of flavonoid phenols. Flavonoids have been proven to be primarily responsible for the development of bitterness, astringency, browning, and aroma alterations during oxidative aging of white table wines [25,46, 54,59,60,63,70,71]. They reduce shelf-life and typical cultivar aroma. The non-flavonoid phenol fraction is unable to generate this kind of deterioration. It does not contribute to bitterness at concentrations found in wine [79]. White wines produced in such a way as to contain no significant amounts of flavonoids resist browning in absence of SO_2 when exposed to air. By introducing flavonoid phenols in such wines, oxidative aging is induced.

When high amounts of suspended yeast maintain reducing conditions in young white wines after fermentation, flavonoids are hardly perceptible by mouth. Oxidative alterations occur markedly after bottling when oxygen diffuses through the cork into the bottle. Under these conditions, the larger part of absorbed oxygen reacts with phenols and a minor part with SO_2 [19]. Increasing free SO_2 from 30 to 60 mg/L has no significant influence on the percentage of oxygen consumed by phenols [59,60]. From the absorption curves in the UV- and visible range it could be deduced that only free

*Müller & Schneider Corp., Am Holderbusch, 55411 Bingen, Germany.

Manuscript submitted for publication 2 May 1997.

SO_2 concentrations of more than approximately 500 mg/L were able to completely inhibit phenol oxidation when oxygen was absorbed [58]. In bottles closed with screw-caps, white wines containing even high amounts of flavonoids withstand development of bitterness and astringency.

Flavonoids undergo regenerative polymerization when oxygen is consumed [72], and their flavor thresholds are lowered as polymerization progresses [1,14, 26,29,30,31,55]. That is why flavonoids can hardly be detected by sensorial means in young white wine, but turn out to develop flavor after a certain aging period. Thus, hyperoxidation must be seen as a technique to remove flavonoid phenols using the natural enzymatic equipment of grape and to contribute to sensory stability of white wine. It acts contrarily to seed enhancement techniques [81].

Reactions involved: The oxidation of must is an enzymically induced reaction. The enzymes involved are the natural tyrosinase of grapes and, for grapes infected with molds, laccase. Both kinds of polyphenoloxidase (PPO) catalyze the oxidation of phenols to the quinones. Laccase has a broader spectrum of possible substrates than tyrosinase [15,16]. Further reactions leading to brown pigments are nonenzymic.

The most abundant phenolic compounds in white grapes are hydroxycinnamic acids; their major derivate is caftaric acid. Furthermore, must contains variable amounts of the tripeptide glutathione which is involved in the post-enzymic reactions.

Tyrosinase has a high activity with caftaric acid. In the first stage of oxidation, it is converted to caftaric acid quinone. This primary oxidation product is, owing to its high concentration and reactivity, at the origin of three further nonenzymic reactions [8,9,11,32,52, 53,76].

(a) It combines with glutathione to a colorless compound initially called Grape Reaction Product (GRP) and identified as 2-S-glutathionyl caftaric acid.

(b) After glutathione depletion, the excess caftaric acid quinone can oxidize other must constituents, including GRP and flavanols, and be simultaneously reduced back to caftaric acid. The partial regeneration of caftaric acid enables its re-oxidation by PPO and further oxygen consumption.

(c) It polymerizes with its own precursor, caftaric acid, regenerating the original phenol form able to be re-oxidized.

All three reactions are interdependent. Particularly, trapping of caftaric acid quinone by glutathione *(a)* or reduction with sulfite limits the other reactions. Oxygen consumption capacity of musts is highly variable and depends on the initial hydroxycinnamic acids content, whereas the oxidation kinetics are more correlated to the hydroxycinnamic acids to glutathione molar ratio. This ratio is typical for the grape variety. Three groups of varieties have been distinguished. Each group is characterized by the hydroxycinnamic acids to glutathione ratio, oxygen consumption capacity and browning capacity [52]. On the other hand, it was reported for 20 cultivars and two years that an average level of 50 mg/L of oxygen was absorbed under identical conditions; 95% of the values ranged from 30 to 65 mg/L oxygen [13].

When flavanols are oxidized by caftaric acid quinone *(b)*, the respective quinones polymerize rapidly and precipitate as brown pigments. The polymerization reaction is essentially the same as in wine. The main difference between enzymically induced polymerization in must and nonenzymic browning in wine lies in the faster rate of quinone production by the former. Furthermore, while the pigments are more or less soluble in an alcoholic medium, they are insoluble in must.

Oxygen supply required for flavonoid precipitation: Oxygen consumption by the PPO system in must is referred to be generally very fast in absence of sulfur dioxide, ranging from 30 to 200 mg/L/hr [4,15,16,27,42,47,48]. On the other hand, there are reports emphasizing that the uptake rate may be as low as 4 mg/L/hr at ambient temperature though oxygen supply was not limited [20,63]. Variable hydroxycinnamic acids contents may account for so different uptake kinetics, but also partial depletion of the phenolic substrates by accidental oxygen uptake before starting the measurements. Important oxidation occurs inside the presses, depending on the kind of press and pressing management. Oxygen uptake during pressing of whole clusters was estimated at 10 to 15 mg/L [10]. Further consumption by previous crushing is difficult to estimate. When laccase is present, the uptake rate does not change significantly, but total oxygen uptake increases. Adding SO_2 inhibits tyrosinase activity, and oxygen consumption is reduced drastically [15].

Uptake rate is higher at the beginning and decreases when the phenolic substrate depletes (Fig. 1). After rapid processing of must by moderate mechanical

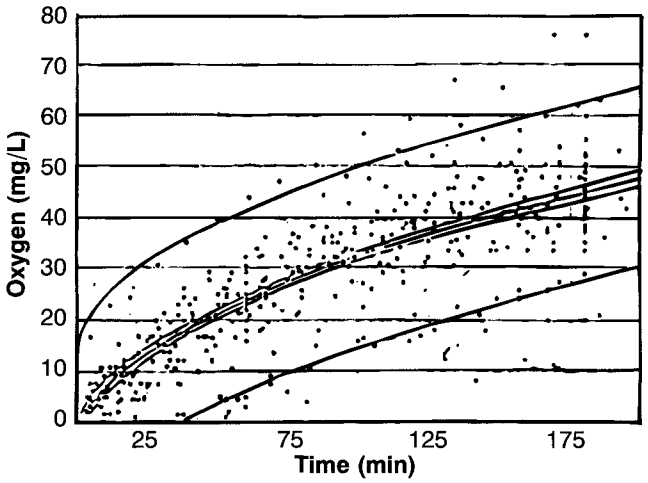

Fig. 1. Oxygen consumption rate of musts from 20 cultivars over two years [13].

treatment and soft pressing, the substrate may be exhausted as juice leaves the press. Since flavonoids are located in grape solids, pomace contact and mechanical treatments like pumping, stirring, and severe pressing [46,59] enhance their extraction, thus increasing the phenolic substrate and the oxygen consumption capacity of the juice. For practical reasons, it is important to know how much oxygen a juice needs to complete flavonoid precipitation after leaving the press and how much time it requires when oxygen is not limited.

Monitoring oxygen demand of musts from 20 grape varieties in a manometric device at atmospheric pressure for 180 minutes, showed that half of the oxygen is consumed during the first hour of contact. Catechin removal was 83% to 99% after two hours [13]. Correlating oxygen supply with the decrease of total phenolics measured by the Folin-Ciocalteu procedure, a saturation level where no further precipitation of phenols occurs was met after 15 minutes for most of the cultivars tested [27].

Based on flavonoid evaluations by the HCl-methanal/Folin-Ciocalteu method [28], it was shown that with a continuous oxygen supply for two hours under industrial conditions, flavonoids may be precipitated completely or partially. When the precipitation is incomplete, the residual flavonoid content is low enough to be absorbed by yeast during fermentation. Maximum flavonoid precipitation took 30 to 60 minutes. Reduction of total phenolics as assayed by the Folin-Ciocalteu procedure was consistent with flavonoid removal [63].

Monitoring oxygen consumption of industrially pressed musts in a manometric device for 2.25 to 13.5 hours, flavonoid precipitation appeared to depend more on other must specific kinetics than on the amount of oxygen consumed. Flavonoids precipitated by 1 mg/L oxygen consumed ranged from 0 to 8.6 mg/L (as catechin). Low initial flavonoid contents were not affected by precipitation. Incomplete flavonoid precipitation was not due to decreasing oxygen consumption rate. Under the same conditions, oxygen consumed initially produced the most drastic flavonoid precipitation (Fig. 2). As consumption continues, the precipitation rate, expressed as mg/L flavonoids to mg/L oxygen, decreases. In order to precipitate must flavonoid contents lower than 100 mg/L (as catechin), one saturation concentration (9 mg/L) of oxygen may be sufficient. Higher contents as they may be obtained by pomace contact require an oxy consumption of about 30 mg/L, corresponding to approximately three saturation concentrations [63]. There is some evidence that an excessive oxygen supply may be highly detrimental to wine quality [4].

The amounts referred to are within the range of natural oxygen uptake possible during processing of non-sulfited musts. Additional oxygen supply is a means to reestablish the flavonoid-oxygen balance when flavonoid content is too high to be removed by naturally available oxygen. This means the commonly used term "hyperoxidation" is not adequate to describe what really occurs. The oxygen amount that must

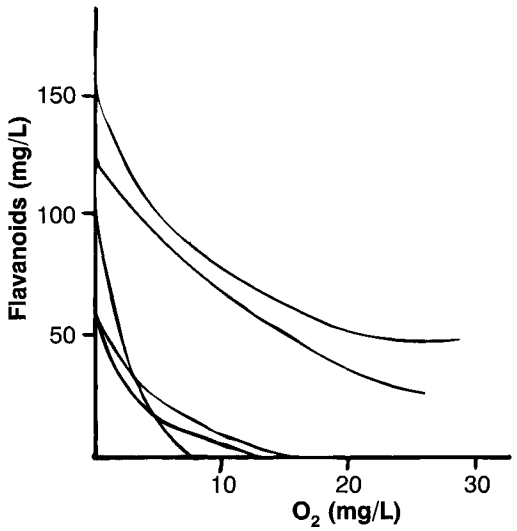

Fig. 2. Decrease in flavonoid phenols during oxygen consumption of five grape musts [63].

needs for phenol stabilization may be considered as enzymic oxygen demand and the technique to meet it as oxygenation.

Technical application: Hyperoxidation may be performed by various technical means:

(a) Must is pumped from one tank to another or from the press to a tank, and oxygen is added during the transfer by means of a diffuser introduced in the transfer pipe. The oxygen flow rate has to be adjusted to the must flow rate, for example, 100 g/hr oxygen have to be added at a flow rate of 10 000 L/hr in order to dissolve 10 mg/L oxygen in must. The diffuser can be designed as a carbonating apparatus.

(b) Must is circulated through the full tank, leaving at the bottom and entering at the top, while oxygen is added by a diffuser introduced in the circulation pipe. At constant flow rates for oxygen and must, oxygen supply is controlled by varying the circulation duration.

(c) A diffuser connected directly to the gas hose is submerged in a tank while stirring the must. Gas supply may be controlled and adapted to the batch volume. Working on small volumes, gas uptake is somewhat difficult to control. However, after charging the must with gas during some minutes in a way to produce a visible degassing at the surface, it is saturated with oxygen (9 mg/L O_2 at 15°C) or slightly supersaturated. Excessive oxygen is not dissolved and escapes without any further effects. If one oxygen saturation is considered not sufficient, the process can be repeated half an hour or so later when dissolved oxygen has been used up in the oxidation [62].

(d) Pumping into top-filled tanks is not effective to supply more than 3 to 4 mg/L oxygen to must.

(e) If flotation is used for must clarification, oxygen or air may be used instead of nitrogen. As the liquid is pressured by the gas, oxygen uptake is several times the saturation concentration at normal pressure. Maximal flavonoid precipitation is achieved during the time

must remains in the flotation unit. The precipitate is discarded with other must solids. Thus, must hyperoxidation and clarification are performed simultaneously [18,64,78].

Instead of pure oxygen stored in steel tanks, compressed air may be used when it is not contaminated by oil and volatile products obtained from lubricants used in some compressors. Since it contains only 21% oxygen, the gas flow rate has to be five-fold. Occasionally, foaming creates a problem owing to escaping of the nitrogen contained in the air.

The time injected oxygen needs to dissolve in the juice depends on the bubble size as determined by the porosity of the diffuser [49]. Pore size of the diffuser medium should not exceed 0.003 mm. Organic deposit from must on the diffuser surface has to be removed periodically since it increases bubble size. Sintered stainless steel diffusers are preferred because they withstand frequent mechanical cleaning [62].

Oxygen enzymatically combined in must under industrial conditions may be less than the quantity supplied when not all oxygen injected is dissolved. Furthermore, although it is possible by in-line injection to achieve a temporary supersaturation, oxygen dissolved in this way tends to decrease slowly by desorption through the liquid surface in tanks at atmospheric pressure. This is one of the causes of conflicting recommendations for oxygen need in hyperoxidation. Unlike oxygen consumption monitoring in hermetically closed experimental devices, there are no reliable means to predict how much of the supplied oxygen must really is combining on an industrial scale. It is known empirically that in-line injection of about 20 to 30 mg/L oxygen, or charging the must twice with oxygen using the batch procedure *(c)*, is sufficient to make sure maximal flavonoid precipitation [63].

Further technical parameters: As must oxidation kinetics require the presence of PPO, all technical means of lowering enzymatic activity have to be avoided before the hyperoxidation procedure is accomplished. Natural tyrosinase activity found in grape must is not limiting flavonoid removal when technical parameters are adequate. Press juice has more tyrosinase activity than free-run juice. A major part of the enzyme is associated with the particulate matter of grape must, another part is present in soluble form. Activity of particulate bound tyrosinase is 8 to 50% of total activity. When juice is racked or centrifuged, bound activity is largely removed. Oxidation products include polymer flavonoids that inactivate and precipitate PPO and other proteins. Tyrosinase activity loss is about 20% per saturation concentration of oxygen consumed. Bentonite-fining by 100 g/hL leads to a loss of 30% of the initial activity, eliminating soluble enzyme [15]. Flavonoid precipitation is not affected when PPO activity is reduced by bentonite because glutathione is removed simultaneously. Since excessive glutathione is competitive to flavonoid oxidation in must, its partial removal may compensate for loss of PPO activity [66].

Sulfur dioxide acts in three different ways to inactivate the mechanism of flavonoid precipitation in must. It inhibits and destroys tyrosinase; total activity decrease is 75% to 90 % when 50 mg/L SO_2 are added [15]. Caftaric acid quinone, primary oxidation product of PPO and directly responsible for flavonoid oxidation, is reduced back by SO_2 [36]. Solubility of all phenolic compounds is enhanced in sulfite containing medium [59]. It thus appears that even if not all oxidation is cancelled [57], the flavonoid precipitation is reduced to zero or a few percent when must is sulfited.

Temperature effects have been shown to have no significant effect on flavonoid precipitation, though PPO activity increases when temperature rises to 45°C. Enhancing PPO activity by laccase addition leads to lower total phenolics in the wine, but there seems to be no advantage for specific flavonoid precipitation [4,33]. Previous pasteurization excludes hyperoxidation since PPO destruction occurs at 65°C.

The combined effect of SO_2, bentonite, and clarification on PPO activity explains why must hyperoxidation has to be carried out directly after pressing and before any further juice processing. Spontaneous sedimentation of oxidized must is delayed if compared to the sulfited reference. When flavonoid precipitation is accomplished, or at least two hours after oxygen supply, juice treatment may be performed following standard methods. Clarification by racking or centrifugation is an important step and has to reduce suspended solids to less than 1% by weight in order to eliminate the major part of the phenolic precipitate. The precipitate is subject to dissolution when the medium contains alcohol and/or SO_2 [66]. Thus, the effect of hyperoxidation is cancelled when unclarified or poorly clarified juice is fermented. Sulfur dioxide may be added to juice after clarification and is sometimes applied for microbiological reasons, though fermentation without previous sulfiting is current practice in many wineries using hyperoxidation. Poor juice clarification techniques account for many contradictory results obtained by hyperoxidation. Residual flavonoid content, analytical and sensory properties of wine depend largely on clarification quality and residual turbidity [3,66].

Clarified juice continues to display a brown color. The reducing conditions during fermentation and absorption by yeast neutralize the oxidation effects. Wines obtained from oxidized musts show the normal bright color after fermentation. Furthermore, if an oxidized must is clarified by filtration, the filtrate is bright. This observation demonstrates that the compounds responsible for dark color are precipitated solids since they can be removed mechanically.

Analytical control: As outlined above, sensory benefits of hyperoxidation result from the removal of flavonoid phenols. Therefore, analytical techniques to control flavonoid content are of interest in order to judge effectiveness of the hyperoxidation procedure applied, to predict flavor stability of the resulting wine, and to decide if hyperoxidation may improve wine quality lowering flavonoid levels considered too high.

HPLC equipment is not common and usually too time consuming for routine quality control. Ultraviolet absorption measurements [77] are difficult to correlate with concentration units. Total phenol content as determined by the Folin-Ciocalteu procedure shows no close correlation to flavonoid content [59,60]. The flavonoid assay as carried out by the HCl-methanal/ Folin-Ciocalteu method described by Kramling and Singleton [28] is useful when color development is performed under carefully controlled pH conditions [65]. However, reproducibility and sensitivity are poor for the low concentration range [68]. Accelerated browning tests are run under conditions not comparable to real aging conditions, although they allow for a rough estimation of relative flavonoid content [65]. The vanillin assay for flavanols leads to a high overestimation [61]. The 4-(dimethylamino)-cinnamaldehyde assay outlined by Zironi, Buiatti, and Celotti [82] displays high photometric revelation, good reproducibility, and simple execution in routine control of the low concentration range [68].

Hyperoxidation is considered giving satisfactory results when residual flavonoids in the wine are approximately 0 mg/L by the HCl-methanal/Folin-Ciocalteu method, less than 20 mg/L by the vanillin assay, and less than 5 mg/L by the 4-(dimethylamino)-cinnamaldehyde assay (values as catechin or gallic acid) [65,68]. Under these conditions, sterile-filtered white wines without SO_2 do not display color increase at 420 nm when exposed to air over a 15-hour period (Fig. 3) [63]. When screening flavonoid content of musts, samples have to be stabilized with SO_2 to prevent flavonoid precipitation before the assay is run.

For monitoring oxygen consumption kinetics in must, the classical oxygen measurement with the Clarke membrane electrode is not the best way. It only allows the monitoring of the amount of consumption of oxygen previously dissolved in the sample. Oxygen absorption can be measured continuously with a manometric method, using glass flasks containing defined volumes of must and air. A well, filled with NaOH, is inserted in order to absorb CO_2 released from grape tissue respiration. During constant stirring, oxygen is absorbed from the headspace. The pressure decrease produced is converted in mg/L O_2 consumed [20].

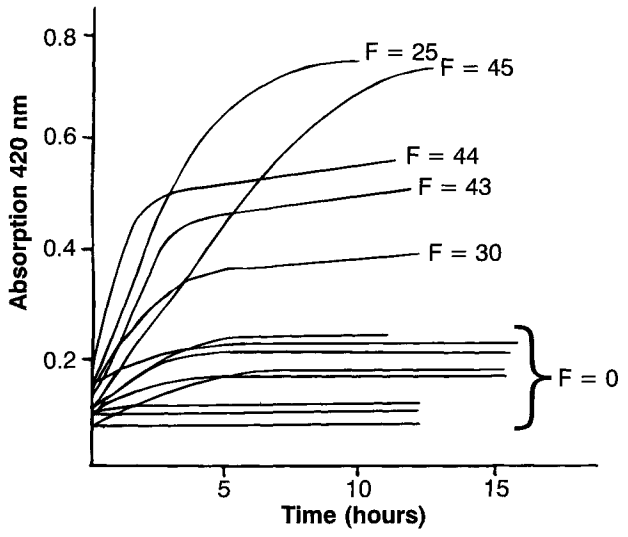

Fig. 3. Browning behavior of non-sulfited young white wines exposed to air [63]. F = flavonoids as mg/L catechin.

Microbiological consequences: Molecular oxygen has been proven to enhance yeast activity and fermentation rate. Under identical fermentation conditions, oxygen supply to fermenting must improves vitality and fermentation rate per yeast cell all over the fermentation. Of particular technical interest, however, is its action as a survival factor for yeast during the declining phase of fermentation, leading to less residual sugar. These phenomena are explained by partial removal of toxic medium length (C8-C12) fatty acid chains and accelerated synthesis of long chain (C16-C18) fatty acids and sterols, both factors contributing to a better sugar uptake through the cell membrane. Furthermore, proline, not assimilable under anaerobic conditions, can be used as a supplementary nitrogen source in presence of molecular oxygen. These effects seem to be more pronounced upon indigenous yeast than on active dry yeast multiplied under aerobic conditions [24,50].

There seems to be general agreement that oxygen supply to must has the most influence on fermentation kinetics when carried out during exponential growth or stationary phase of yeast. Under absolutely anaerobic conditions, yeast activity breaks down soon after starting, explaining thus that alcoholic fermentation actually does need some oxygen. Yeast's oxygen consumption was roughly estimated at 10 to 20 mg/L under optimal conditions [56].

It is quite evident that oxygen can only act as a survival factor on yeast cells which already exist in the medium. On the other hand, oxygen supplied to must by means of hyperoxidation is consumed enzymatically within a short period of time or, at least, before the start of yeast multiplication. Thus, hyperoxidation has no influence upon fermentation kinetics. The formation of acetaldehyde, a main secondary product of yeast metabolism and which is responsible for SO_2 combination, is not subject to any alteration by must hyperoxidation when compared to traditional vinification without the addition of SO_2 to must. To take advantage of oxygen for fermentation purposes, the oxygen supply has to be renewed after the beginning of yeast activity.

Bacterial activity like that of acetobacter has never been observed to be enhanced by must hyperoxidation [4]. Apparently, there is enough oxygen dissolved in juice to allow bacterial growth when all other environmental factors are favorable to its growth. Additional oxygen quantities cannot be used by bacteria. Formation of volatile acidity and ethylacetate prior to fermentation seems to be controlled mainly by the time between crushing and beginning of CO_2 production regardless of any supplementary oxygen uptake. Thus, when vinification is carried out using hyperoxidation,

bacterial activity is usually repressed by inducing a rapid start of alcoholic fermentation, or by sulfiting after removal of the phenolic precipitate by juice clarification.

Sensory results: There is general agreement upon improved color stability of wines produced by hyperoxidation [2,4,5,7,11,37,38,39,40,43,45,60,63]. Although initial color intensity determined as A_{420} is often slightly higher than in the reference obtained by conventional winemaking practices, browning capacity during oxidative aging is significantly reduced or eliminated. Browning is highly correlated to flavanols. Based on enzymic oxidation of individual phenolic compounds at equal molar concentrations, catechin, epicatechin, procyanidin B2, and procyanidin B3 have a browning potential about 10-fold higher than hydroxycinnamic acid derivates [32]. When browning capacity was correlated to flavonoid content, filtered young wines exposed to air without SO_2 resisted browning when flavonoids had been reduced to zero as assayed by the HCl-methanal/Folin-Ciocalteu method (Fig. 3). Under the same conditions, the non-hyperoxidized wines containing flavonoids browned. Laccase activity carried over into the wine did not induce browning when flavonoids were absent [63].

Browning under accelerating conditions is only the visual indication of further profound flavor and odor alterations to expect during oxidative aging. Development of bitterness and astringency can be avoided or at least drastically reduced by must hyperoxidation. They were rated significantly lower in hyperoxidized lots when compared to a flavonoid-containing reference, and the differences increased with the chemical age of the reference [60,63]. This is logical since flavonoid precursors of polymeric tannic compounds are eliminated, but there are other reports [7,9,21,41] stressing no difference in sensory preference.

The oxidation of juice is much more complex than the simple elimination of readily oxidizable phenols. Oxidation can affect other wine constituents. However, reports on olfactory changes generated by hyperoxidation are conflicting.

For Chardonnay, Mauzac, and Chenin blanc from France, hyperoxidation induced no aroma degradation, being Chardonnay treated with oxygen even rated higher in aroma quality and aroma frankness than the sulfited control [9]. For Chardonnay, Parellada, and Muscat from Spain, higher ratings of aroma intensity and frankness were reported for several of the hyperoxidized lots and explained by an increase of acetates of higher alcohols, fatty acids, and their esters, and free volatile terpenes as measured by GC [2]. For Faberrebe from Germany made by skin contact, hyperoxidation increased aroma intensity, but did not affect aroma in the lots made without skin contact [67].

Evaluated by Quantitative Descriptive Analysis (QDA), Riesling from Germany [69] displayed an increase in lemon, a decrease in peach, and similar ratings for apple and pear attributes when juice without pomace contact time was hyperoxidized. Aroma was slightly modified, but not less fruity. An increase of aroma intensity obtained by pomace contact was cancelled by hyperoxidation. After further aging of one year, the lots made without skin contact showed no differences as induced by hyperoxidation, whereas the skin contact lots had higher ratings for all fruity attributes when made by hyperoxidation. No lot displayed oxidative off-flavor since initial flavonoid content was very low.

Some reports [6,17,21,44,75] refer to less aroma intensity and less varietal aroma in the hyperoxidized lots, depending on cultivar. Several European grape varieties showed an increase of acetates and higher aldehydes (C3-C10), and a decrease in higher alcohol after hyperoxidation. Increase of higher aldehydes accounted for slightly higher vegetative flavor ratings [21]. Formation of C6-aldehydes and alcohols has been shown to increase under oxidative pre-fermentation conditions [12,23].

There seems to be a tendency towards lower contents of volatile phenols [17,43] and sulfur compounds [21,37,38,39] in wines made with hyperoxidation.

Other experiments [7,9,34,41,43], however, did not show significant preference differences of hyperoxidized lots when compared to sulfited or passively oxidized references. These conflicting results may be explained by several hypothesis:

(a) The natural oxygen uptake by must may be sufficient to exhaust flavonoid content when phenol extraction is reduced by absence of pomace contact time, gentle must processing and soft pressing [9,62]. Consequently, further oxygen supply cannot improve wine's resistance to develop bitterness and astringency.

(b) Bitterness and astringency do not exist *a priori* in white wine containing flavonoids, but turn out to be perceptible as flavonoid polymerization [72] occurs during oxidative aging. Perceived intensity is not correlated to concentration; it depends on the chemical age and the timing of sensory evaluation [60].

(c) Flavonoid removal by hyperoxidation can be cancelled by dissolution of the precipitate due to poor clarification prior to fermentation [66].

(d) When quality evaluation is run as a preference test, results are hedonic. Therefore, sensory perception of flavonoids can be judged according to the type of wine one is accustomed to. Tasting panels are split in preference tests. Compared to a flavonoid-containing control, wine made by hyperoxidation displays less body and mouth-feel. That may make a wine delicate, in one case, or thin in another. A high flavonoid content wine may be a rich, luscious one to one person while to another, it is harsh, heavy, and unsuitable for aging. Quantitative descriptive analysis is a useful tool to specify sensory data when unequivocally defined terms are used.

Flavonoids have been proven to induce odor degradation, although they are not volatile themselves.

When a low flavonoid wine was enriched with catechin or flavonoids extracted from grape seeds, negative aroma attributes like mushroom, earth, straw, and black tea as rated by QDA increased strongly within five months under normal bottle-storage conditions, whereas the reference had higher ratings for fruity attributes. Free acetaldehyde was not involved. During accelerated oxygen consumption in a manometric device, the flavonoid enhanced lot consumed more oxygen, lost more sulfur dioxide, and produced more acetaldehyde than the reference [70]. The more oxygen must absorbs, the less oxygen is consumed by wine, and a lower percentage of oxygen consumed by wine reacts with phenols [4]. Since oxidation of flavonoids in wine yields peroxide oxidizing ethanol to acetaldehyde [80], it is assumed that this mechanism of coupled oxidation also produces off-flavor by oxidation of unknown precursors. This reaction may explain how nonvolatile flavonoid compounds participate in olfactory alterations during aging [70]. The behavior of flavonoids as a catalyst for odor degradation accounts for the remarkable flavor stability of hyperoxidized wines reported in the literature [25,59,60,63]. It also explains why aroma intensity enhanced by pomace contact may be very short-lived if simultaneously extracted flavonoids are not removed.

Conclusions

Hyperoxidation is a more recent technique in prefermentation processing of white grape musts. Many psychological factors have been involved in discussion of its benefits or disadvantages, since traditional recommendations have advocated careful protection of must against oxidation. In fact, it is hard to believe that wines made from strongly browned juices are usually of the same and more stable color than their counterparts treated with SO_2 prior to fermentation. Yet, oxidation of must reduces oxidation capacity of wine. Phenols are the major oxygen-consuming substrate. When they are allowed to oxidize in must, they precipitate. When they oxidize in wine, they may induce drastic flavor alterations during aging.

There is no doubt about elimination of bitterness and astringency producing capacity since monomeric precursors of tannin are largely removed. This original goal of hyperoxidation is unequivocally met. Sensory data correlate well with flavonoid phenols content, but not with total phenolics. More systematic and specific flavonoid assaying would explain why some wines do not respond in this way. In fact, if flavonoid release is minimal due to soft must processing, there is no sensorially relevant substrate to eliminate by hyperoxidation. There is need of more sensory research upon long-time behavior of hyperoxidized wines. Most experimentations were carried out over a reduced period of time, but sensory differences are insignificant or neither observed in young wines. Comparing a hyperoxidized lot with a flavonoid-containing reference, sensory differences increase during oxidative aging. The flavonoid-containing reference undergoes a more dynamic evolution which the hyperoxidized lot does not display.

Oxidation of must involves more constituents than phenols. Olfactory changes may be induced. Aroma intensity and quality were observed to improve or to decrease depending on grape variety. More information is needed about the specific olfactory response of cultivars. Oxygen supply must be restricted to the amount required for flavonoid removal. Data are given. Excessive oxygen consumption has been shown to generate aroma losses.

Flavonoids catalyze the production of unidentified volatile compounds responsible for the occurrence of oxidative off-flavor in white wines in which free acetaldehyde is not involved. This phenomena accounts for improved flavor stability of hyperoxidized wines. When aroma intensity is observed to be reduced in a hyperoxidized lot at an early stage of aging, this assessment may not be true after some aging due to aroma degradation of the flavonoid containing reference.

One feature which has been stressed is the difficulty in defining quality. Preference tests are based on hedonics and not able to identify unequivocally sensory differences generated by hyperoxidation. An additional problem is that the sense one tasting panel may have of wine quality does not transfer to another panel. Quantitative descriptive analysis is useful to describe how hyperoxidation really changes the wine.

Current winemaking practices like malolactic fermentation, sur lie, aging in oak, *etc.* are used deliberately to modify wine style. From a more practical point of view, hyperoxidation is an additional mean to differentiate wines. We trust that this paper will stimulate winemakers and scientists to devote more attention to the management of the phenolic regime by must oxidation.

Literature Cited

1. Arnold, R. A., A. C. Noble, and V. L. Singleton. Bitterness and astringency of phenolic fractions in wine. J. Agric. Food Chem. 28:675-678 (1980).

2. Artajona, J., R. Bobet, J. Marco, and F. Sabat. Expériences d'hyperoxygénation au Penedés: Rev. Fr. Oenol. 124:65-67 (1990).

3. Bach, H. P., and P. Nobis. Einfluß der Mostoxidation auf den Wein. Weinwirtschaft-Technik, No. 9:294-311 (1985).

4. Bagnoud, X. Etude de la réactivité, des moûts et des vins blancs vis-à-vis de l'oxygène: Méthodes d'analyse et applications pratiques. Thesis, Ecole d'Ingénieurs ETS, Changins (Switzerland) (1993).

5. Bailly, B. Essai d'hyperoxygénation des moûts sur cépages locaux en Alsace. Rev. Fr. Oenol. 127:7-14 (1990).

6. Blanck, G. Utilisation de l'hyperoxidation pour la valorisation des moûts de tailles en Champagne. Rev. Fr. Oenol. 124:50-57 (1990).

7. Cheynier, V., J. Rigaud, J. M. Souquet, J. M. Barillère, and M. Moutounet. Effect of pomace contact and hyperoxidation on the phenolic composition and quality of Grenache and Chardonnay wines. Am. J. Enol. Vitic. 40:36-42 (1989).

8. Cheynier, V., J. Rigaud, J. M. Souquet, F. Duprat, and M. Moutounet. Must browning in relation to the behavior of phenolic compounds during oxidation. Am. J. Enol. Vitic. 41:346-349 (1990).

9. Cheynier, V., J. M. Souquet, A. Samson, and M. Moutounet. Hyperoxidation: influence of various oxygen supply levels on oxidation kinetics of phenolic compounds and wine quality. Vitis 30:107-115 (1991).

10. Cheynier, V., G. Masson, J. Rigaud, and M. Moutounet. Estimation of must oxidation during pressing in Champagne. Am. J. Enol. Vitic. 44:393-399 (1993).

11. Cheynier, V., H. Fulcrand, S. Guyot, J. M. Souquet, and M. Moutounet. Oxidation enzymatique et chimique des polyphénols au cours de la vinification. *In:* Proceedings 4th Intnl. Symposium on Innovations in Wine Technology, Stuttgart, Germany. pp 50-59 (1995).

12. Cordonnier, R. E., and C. L. Bayonove. Etude de la phase préfermentaire de la vinification: extraction et formation de certains composés de l'arôme, cas des terpénols, des aldéhydes et des alcools en C6. Connaiss. Vigne Vin 15:269-286 (1982).

13. Crapisi, A., R. Ferrarini, R. Zironi, A. Lante, G. Pasini, and P. Spettoli. Prefermentative treatments interaction on the white must polyphenolic composition. *In:* Proceedings 4th Intnl. Symposium on Innovations in Wine Technology, Stuttgart, Germany. pp 79-84 (1995).

14. Delcour, J. A., M. M. Vanderberghe, P. F. Corten, and P. Daudeyne. Flavor thresholds of polyphenolics in water. Am. J. Enol. Vitic. 35:134-136 (1984).

15. Dubernet, M. Recherches sur la tyrosinase de *Vitis vinifera* et la laccase de *Botrytis cinerea*. Thesis, Université Bordeaux (1974).

16. Dubernet, M., and P. Ribéreau-Gayon. Causes et conséquences de la consommation de l'oxygène par les moûts de raisin. Vitis 13:233-244 (1974).

17. Dubourdieu, D., and V. Lavigne. Incidence de l'hyperoxygénation sur la composition chimique et les qualités organoleptiques des vins blancs secs du Bordelais. Rev. Fr. Oenol. 124:58-61 (1990).

18. Establet, G., and W. Mulinazzi: Innovations dans les procédés de clarification des moûts. Rev. Oenol. No. 77:35-40 (1994).

19. Fabre, S. Destination de l'oxygène consommé par le vin. Rev. Fr. Oenol. 71:23-26 (1993).

20. Gétaz, J., and S. Fabre. Mesure de l'absorption d'oxygène dans les moûts par la méthode D.B.O. Rev. Fr. Oenol. 124:21-26 (1990).

21. Guedes de Pinho, P., A. Bertrand, and I. Guillou. Influence de l'hyperoxygénation des moûts sur la composition chimique et sensorielle des vins blancs. Rev. Fr. Oenol. 145:9-17 (1994).

22. Guerzoni, M. E., R. Zironi, C. Intrieri, and E. Magnanini: Stabilisation of white wine by early hyperoxydation of must. Food Technol. Austral. 33:442-446 (1981).

23. Guillot, I., and A. Bertrand. Dosage des aldéhydes des vins - influence de l'hyperoxygénation des moûts sur leurs teneurs dans les vins. Rapport des activités de recherches 1990-92, Institut d'Oenologie, Université de Bordeaux II, pp 96-98.

24. Ingledew, W. M., and R. E. Kunkee. Factors influencing sluggish fermentation of grape juice. Am. J. Enol Vitic. 36:65-76 (1985).

25. Johner, H., and V. Schneider: Mostoxidation-Erfahrungen aus Baden. Der Badische Winzer 10:359-361 (1991).

26. Joslyn, M. A., and J. L. Goldstein. Astringency of fruits and fruit products in relation to phenolic content. Wall. Lab. Comm. 28:143-159 (1965).

27. Koch, T., and G. F. Baumgarten: Research report on the influence of grape varietals and the oxidation thereof on polyphenols of South African grape juice. *In:* Proceedings 4th Intnl. Symposium on Innovations in Wine Technology, Stuttgart, Germany, pp 60-66 (1995).

28. Kramling, T. E., and V. L. Singleton. An estimate of the nonflavonoid phenols in wine. Am. J. Enol. Vitic. 20:86-92 (1969).

29. Lea, A. G. H. The analysis of cider phenolics. Ann. Nutr. Alim. 32:1051-1061 (1978).

30. Lea, A. G. H., and G. M. Arnold. The phenolics of ciders: bitterness and astringency. J. Sci. Food Agric. 29:478-83 (1978).

31. Lea, A. G. H., P. Bridle, C. F. Timberlake, and V. L. Singleton. The procyanidins of white grapes and wines. Am. J. Enol. Vitic. 30:289-300 (1979).

32. Lee, C. Y., and A. W. Jaworski. Phenols and browning potential of white grapes grown in New York. Am. J. Enol. Vitic. 39:337-340 (1988).

33. Mayer, G., H. Dietrich, and K. Wucherpfennig. Weinausbau ohne SO_2 mit Enzymen möglich? Weinwirtschaft-Technik 7:18-22 (1990).

34. Meistermann, E. Hyperoxygénation des moûts; essais réalisés en Alsace. Rev. Fr. Oenol. 124:62-64 (1990).

35. Moutounet, M., V. Cheynier, J. Rigaud, and J. M. Souquet. Les mécanismes d'oxydation mis en jeu lors de la préparation des moûts destinés à l'élaboration de vins blancs. Rev. Fr. Oenol. 117:23-29 (1989).

36. Moutounet, M., J. Rigaud, J. M. Souquet, and V. Cheynier. Influence de quelques paramètres sur l'oxydation des moûts de raisin. Interprétations technologiques. Rev. Fr. Oenol. 124:32-38 (1990).

37. Müller-Späth, H. Neueste Erkenntnisse über den Sauerstoffeinfluß bei der Weinbereitung aus der Sicht der Praxis. Weinwirtschaft 113:144-157 (1977).

38. Müller-Späth, H., T. Löscher, and G. Schöfer. Einfluß des Sauerstoffs bei der Weinbereitung von der Traube bis zur Flaschenfüllung. Deut. Weinbau 32:384-392 (1977).

39. Müller-Späth, H., N. Moschtert, and G. Schäfer. Beobachtungen bei der Weinbereitung. Eine Bestandsaufnahme. Weinwirtschaft 114:1084-1089 (1978).

40. Müller-Späth, H. Der POM-Test. Deut. Weinbau, No. 23: 1099-1100 (1992).

41. Nagel, C. W., and W. R. Graber. Effect of must oxidation on quality of white wines. Am. J. Enol. Vitic. 39:1-4 (1988).

42. Neradt, F. Sauerstoffbindungsgeschwindigkeit bei Traubenmost. Weinberg u. Keller 17:519-526 (1970).

43. Nicolini, G., F. Mattivi, and A. Dalla Serra. Iperossigenazione dei mosti: conseguenze analitiche e sensoriali su vini della vendemmia 1989. Riv. Vitic. Enol. 3:45-56 (1991).

44. Ough, C. S., and E. A. Crowell: Use of sulfur dioxide in winemaking. J. Food Sci. 52:386-388 (1987).

45. Perez-Bustamante, J. A., C. G. Barroso, R. Castro, and J. Gomez. Influence of controlled oxydation on the polyphenolic contents of sherry must. *In:* Proceedings 4th Intnl. Symposium on Innovations in Wine Technology, Stuttgart, Germany. pp 152-160 (1995).

46. Peri, C., C. Pompei, G. Montedoro, and C. Cantarelli. Madeirization of white wines. Influence of pressing on the susceptibility of grapes to oxidative browning. J. Sci. Food Agric. 22:24-28 (1971).

47. Perscheid, M., and F. Zürn: Über die Bedeutung des Sauerstoffs bei der Weinbereitung. Weinwissenschaft 31(4):287-300 (1976).

48. Perscheid, M., and F. Zürn: Der Einfluß von Oxidationsvorgängen auf die Weinqualität. Weinwirtschaft 113:10-12 (1977).

49. Riba, J. P. L'oxygène en solution: Solubilité et lois de transfert. Rev. Fr. Oenol. 124:14-19 (1990).

50. Ribéreau-Gayon, P. New developments in wine microbiology. Am. J. Enol. Vitic. 36:1-10 (1985).

51. Ricardo da Silva, J. M., V. Cheynier, A. Samson, and M. Bourzeix. Effect of pomace contact, carbonic maceration and hyperoxidation on the procyanidin composition of Grenache blanc wines. Am. J. Enol. Vitic. 44:168-172 (1993).

52. Rigaud, J., V. Cheynier, J. M Souquet, and M. Moutounet. Mécanismes d'oxydation des polyphénols dans les moûts blancs. Rev. Fr. Oenol. 124:27-31 (1990).

53. Rigaud, J., V. Cheynier, J. M. Souquet, and M. Moutounet. Influence of must composition on phenolic oxidation kinetics. J. Sci. Food Agric. 57:55-63 (1991).

54. Rossi, J. A., and V. L. Singleton. Contribution of grape phenols to oxygen absorption and browning of wines. Am. J. Enol. Vitic. 17:231-239 (1966).

55. Rossi, J. A., and V. L. Singleton. Flavor effects and adsorptive properties of purified fractions of grape seed phenols. Am. J. Enol. Vitic. 17:240-246 (1966).

56. Sablayrolles, J. M. Besoins en oxygène lors des fermentations oenologiques. Rev. Fr. Oenol. 124:77-79 (1990).

57. Schmitt, A., H. Köhler, R. Miltenberger, and K. Curschmann: Über

den Einfluß des Sauerstoffs auf Maische und Most unter Bercksichtigung der Reduktionsmittel SO_2 und Ascorbinsäure. Deut. Weinbau 23:856-859, 24:890-897 (1980).

58. Schneider, V. Die Entwicklung der Farbe in Rotwein, Farbsüßreserve und rotem Traubensaft. Weinwirtschaft-Technik 121(7):208-212 (1985).

59. Schneider, V. Verhalten phenolischer Substanzen. Weinwirtschaft-Technik 124(2):12-16; (3):16-20 (1988).

60. Schneider, V. Weinalterung, Part III. Weinwirtschaft-Technik 125(10):23-27 (1989).

61. Schneider, V. Aussagekraft des Catechinwertes. Weinwirtschaft-Technik 125(2):17-19 (1989).

62. Schneider, V. Forcierte Mostoxidation - technische Anwendung. Weinwirtschaft-Technik 126 (8):35-36 (1990).

63. Schneider, V. Comportement des vins obtenus par oxygénation des moüts blancs. Rev. Fr. Oenol. 130:33-42 (1991).

64. Schneider, V., and P. Chapron. Mostvorklärung durch Flotation. Weinwirtschaft-Technik 128(2):17-19 (1992).

65. Schneider, V. Oxidative Weinalterung. Analytische Ansätze. Das Deutsche Weinmagazin 17:18-26 (1993).

66. Schneider, V. Neuere Erkenntnisse zur Mostoxidation. Die Winzer-Zeitung 10:20-23 (1994).

67. Schneider, V. Primäraroma. Die Winzer-Zeitung 10:24-25 (1994).

68. Schneider, V. Evaluation of small amounts of flavonoid phenols in white wines by colorimetric assays. Am. J. Enol. Vitic. 46:274-7 (1995).

69. Schneider, V. Einfluß von Maischestandzeit und Mostoxidation auf die Sensorik von Riesling. Die Winzer-Zeitung 7:22-25 (1996).

70. Schneider, V. Entstehung und Charakterisierung von Altersfirne. Das Deutsche Weinmagazin 14:19-21 (1996).

71. Simpson, R. F. Factors affecting oxidative browning in white wine. Vitis 21:233-9 (1982).

72. Singleton, V. L. Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical implications. Am. J. Enol. Vitic. 38:69-77 (1987).

73. Singleton, V. L, and P. Esau. Phenolic substances in grapes and wine and their significance. *In:* Advances in Food Research. Academic Press, London (1969).

74. Singleton, V. L., and A. C. Noble: Wine flavor and phenolic substances. Am. Chem. Soc. Symposium Series 26:47-70 (1976).

75. Singleton, V. L., J. Zaya, and E. Trousdale. White table wine quality and polyphenol composition as affected by must SO_2 content and pomace contact time. Am. J. Enol. Vitic. 31:14-20 (1980).

76. Singleton, V. L., M. Salgues, J. Zaya, and E. Trousdale. Caftaric acid disappearance and conversion to products of enzymic oxidation in grape must and wine. Am. J. Enol. Vitic. 36:50-56 (1985).

77. Somers, T. C., and G. Ziemelis. Interpretation of ultraviolet absorption in white wines. J. Sci. Food Agric. 23:441-453 (1972).

78. Tournier, R. Conditions de mise en oeuvre de l'hyperoxygénation. Rev. Fr. Oenol. 124:39-41 (1990).

79. Verette, E., A. C. Noble, and T. C. Somers. Hydroxycinnamates of *Vitis vinifera:* Sensory assessment in relation to bitterness in white wine. J. Sci. Food Agric. 45:267-272 (1988).

80. Wildenradt, H. L., and V. L. Singleton: The production of aldehydes as a result of oxidation of phenolic compounds and its relation to wine aging. Am. J. Enol. Vitic. 25:119-126 (1974).

81. Williams, R. L., J. M. Duvernay, and J. Recht. Influencing polyphenols in white wine. Seed enhancement studies. *In:* Proceedings 4th Intnl. Symposium on Innovations in Wine Technology, Stuttgart, Germany. pp 233-239 (1995).

82. Zironi, R., S. Buiatti, and E. Zelotti. Evaluation of a new colourimetric method for the determination of catechins in musts and wines. Vitic. Enol. Sci. 47:1-7 (1992).