



## Assessing the Oxidative Sensitivity of Wines Using an Air Resistance Test Followed by Voltammetry: The Capacity of Phenolic Compounds to Form Complexes with Fe(II) and Fe(III) Ions Could Explain the Longevity of Wines

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One of the winemaker's major challenges is to assess a wine's capacity to resist the appearance of signs of oxidation, in order to adapt the technical itinerary to its profile and shelf-life objectives.

Oxidative signs in wines are typically described as a color evolution, with the appearance of orange hues, an aromatic evolution marked by the loss of varietal notes (boxwood, passionfruit), and/or the emergence of oxidative notes (honey, wax, chocolate, dried fruit, dry herbs, nuts...). Sometimes, there is an evolution in the taste balance with an increase in astringency and/or loss of roundness. These various oxidative signs can appear successively or simultaneously, with one sign not necessarily indicating the onset of another.

The point at which a wine is considered oxidized depends on the acceptability of these characteristics relative to the type of product and the winemaker's intention. For example, it is acceptable, or even desirable, to have dried fruit notes in sweet wines, whereas it is undesirable in dry Sauvignon Blanc wines from certain appellations that must retain citrus or exotic fruit notes.

Evaluating a wine's ability to resist oxidation means using a predictive test to determine its capacity to age, its longevity, provided it is stored in the right conditions.

In wine, a number of molecules are known for their antioxidant capacities, for example phenolic compounds, or antioxidants added during the process, such as sulfites.

Consequently, the evaluation of antioxidant concentration has been considered as a means of predicting the longevity of a wine. This can be assessed by a number of different methods, generally consisting of evaluating a wine's capacity to trap radicals, to react with different types of oxidants (DPPH test, ABTS, FRAP, Folin Ciocalteu analysis), or to oxidize on electrodes of a given nature (voltametry). It has finally been shown that the antioxidant content of a wine is strongly correlated with the concentration of phenolic compounds (Büyüktuncel *et al.* 2014; Romanet *et al.* 2019; de Beer *et al.* 2004, 2006; Kilmartin 2001).



Several studies have shown that antioxidant concentration decreases during wine oxidation (Rodrigues *et al.* 2007, Ugliano 2013 and 2019). Overall, older wines contain fewer antioxidants than younger wines (Rodrigues *et al.* 2007), but the variability of vintages and winemaking processes (particularly phenolic compound extraction stages) make it impossible to determine the age of a wine from its antioxidant concentration (Romanet *et al.* 2019). Finally, a wine's low antioxidant content has been correlated with the presence in sensory profiles of notes associated with oxidative evolution of wines (Romanet *et al.* 2019). However, these studies do not show that antioxidant content is predictive of an ability to resist oxidation.

To make a link with winemaking practices, if the intrinsic content of phenolic compounds (major contributors to antioxidant content) were an image of a wine's ability to resist the onset of oxidative notes, winemakers would use the Folin index or even the Total Polyphenol Index for this purpose. To date, however, winemakers have assessed this capacity empirically by tasting before and after contact with air (air resistance test). The evolution of the wine's sensory profile is evaluated, in particular the appearance of notes considered oxidative.

This article presents the development of a method for determining the sensitivity of wines to oxidation. To this end, wine samples were empirically selected by winemakers and subjected to an air resistance test monitored by linear sweep voltametry. A different voltammetric signal evolution was observed for wines empirically categorized as oxidation-resistant and oxidation-sensitive. Finally, the voltammetric signal of catechin in hydroalcoholic solution in the presence and absence of Fe(II) and Fe(III) ions under inert atmosphere was recorded, enabling us to hypothesize on the involvement of phenolic compounds - Fe(II) or Fe(III) ion complexes in the recordings obtained and the mechanisms underlying this test.

## Material and method

134 wine samples (87 reds, 32 whites and 15 rosés from various French and Spanish regions) were selected at the end of alcoholic or malolactic fermentation by experienced winemakers (cellar masters or consultant oenologists with several years' experience in the production area in question, and who generally incorporate micro-oxygenation into some of their technical itineraries). For each sample, the winemaker empirically evaluated the risk of rapid oxidation by tasting and categorizing the wine as either resistant or sensitive to oxidation. Wine samples were taken from inerted 750 mL bottles, filled to overflow and capped with a screw cap. These wines were tested for air resistance by taking 50 mL of sample with a volumetric pipette and placing it in a 125 mL bottle. Linear sweep voltammetry measurements (0-600 mV, 100 mV/s, 10 mV steps) were taken when the sample bottle was opened, then after 2 hours, using a potentiostat (WQS Polyscan, Vinventions), on printed electrodes (carbon working electrode, Vinventions). Potentials are expressed relative to an Ag/AgCl reference electrode.

Solutions of catechin (1150.0 mg/L and 1165.0 mg/L), iron sulfate (10.3 mg/L) and iron chloride (10.7 mg/L) were prepared in 13% ethanol, containing 4.0 g/L tartaric acid and adjusted to pH 3.3 with 1N and 0.1N HCl. These stock solutions were deaerated by nitrogen bubbling and placed in a fume cupboard inerted by nitrogen flushing. The % O<sub>2</sub> in the fume cupboard was monitored by a Nomasense O<sub>2</sub> P300 (Vinventions) and maintained at less than 2% throughout the test. Intensity-potential curves for freshly prepared stock solutions and 50/50 v/v catechin/Fe ion mixtures were recorded in triplicate as previously described by linear sweep voltametry.



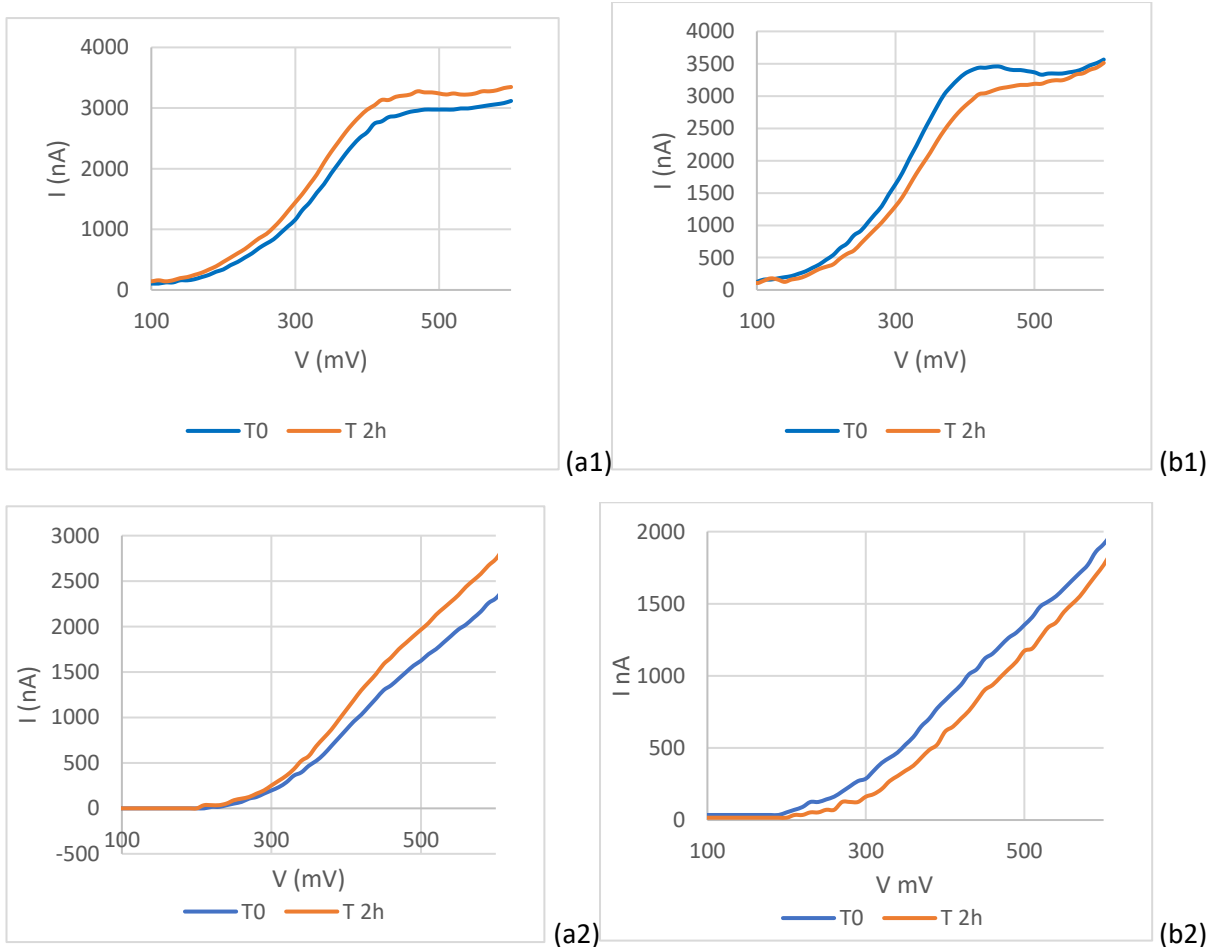
## Results

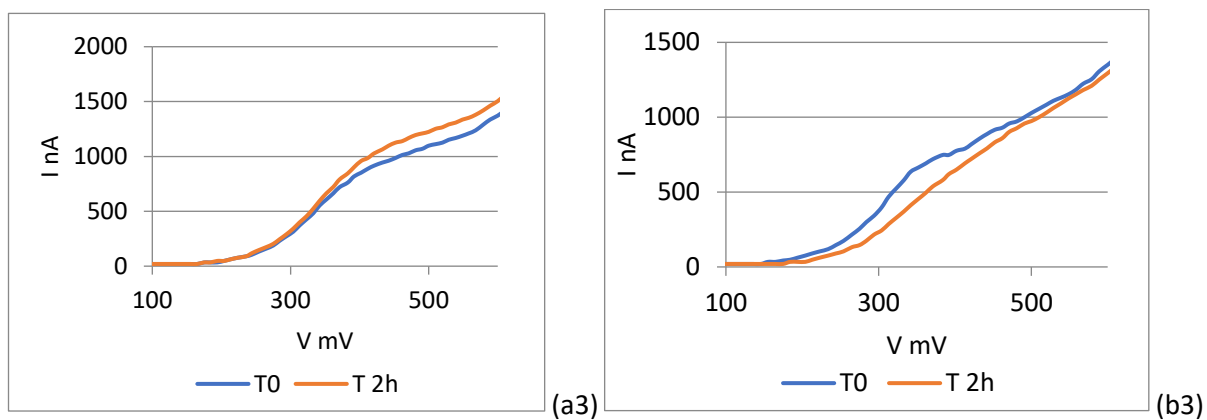
### Observation of voltammetric signal changes during air resistance tests:

Empirically, the air resistance test is only carried out by winemakers on samples that have not been subjected to contact with oxygen in the preceding days. To replicate to these conditions, air resistance tests were carried out here only on samples stored for 3 weeks prior to measurement in the absence of O<sub>2</sub>. 58% of the samples selected by the winemakers were empirically categorized as resistant to oxidation.

Comparison of the intensity/potential curves obtained on a wine immediately after opening the sample bottle and those obtained after 2h of contact with air revealed several signal evolutions.

For 47 of the 134 wines, a significant increase in intensity (greater than 5%, the method's measurement uncertainty) in the 100-600 mV range was observed on the measurement taken after 2h of contact with air (figure 1 (a)). All 47 samples in this group had been characterized as resistant to oxidation by the winemakers. None of the wines categorized as "sensitive to oxidation" exhibited this behavior..





**Figure 1: Intensity/potential curves of a wine measured when the sample is placed in contact with air (T0) and then after 2h of contact. (a) samples described as resistant to oxidation by winemakers (b) samples described as sensitive to oxidation by winemakers. (a1) and (b1): red wines. (a2) and (b2): rosé wines. (a3) and (b3): white wines.**

For 38 of the 147 samples, on the other hand, a significant decrease in intensity (greater than 5%, the method's measurement uncertainty) in the 100-600 mV range was observed on the measurement taken after 2h of contact with air (Figure 1 (b)). These 38 wines had all been characterized as sensitive to oxidation by the winemakers. None of the wines categorized as "resistant to oxidation" exhibited this behavior.

For the remaining 49 wines, the variation in intensity between the initial measurement and that recorded after 2 hours of contact with air is not significant. These wines could potentially have less pronounced oxidation behaviors than the 2 categories described above, making the test insensitive to their discrimination. Furthermore, in the categorization of the winemakers, no scale of intensity of resistance or sensitivity to oxidation was requested, meaning that this category of wine subsequently described as "intermediate" could not be distinguished.

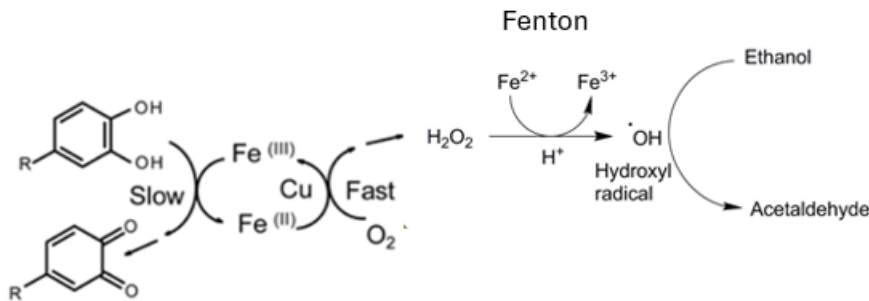
The test does seem to be able to categorize wines:

- those showing an increase in signal between the initial measurement and the one taken after 2h of contact with air appear to be systematically resistant to oxidation,
- those showing a drop in signal between these two measurements appear to be systematically sensitive to oxidation.

#### *Impact of Fe(II) and Fe(III) ion complexation by phenolic compounds on the voltammetric signal:*

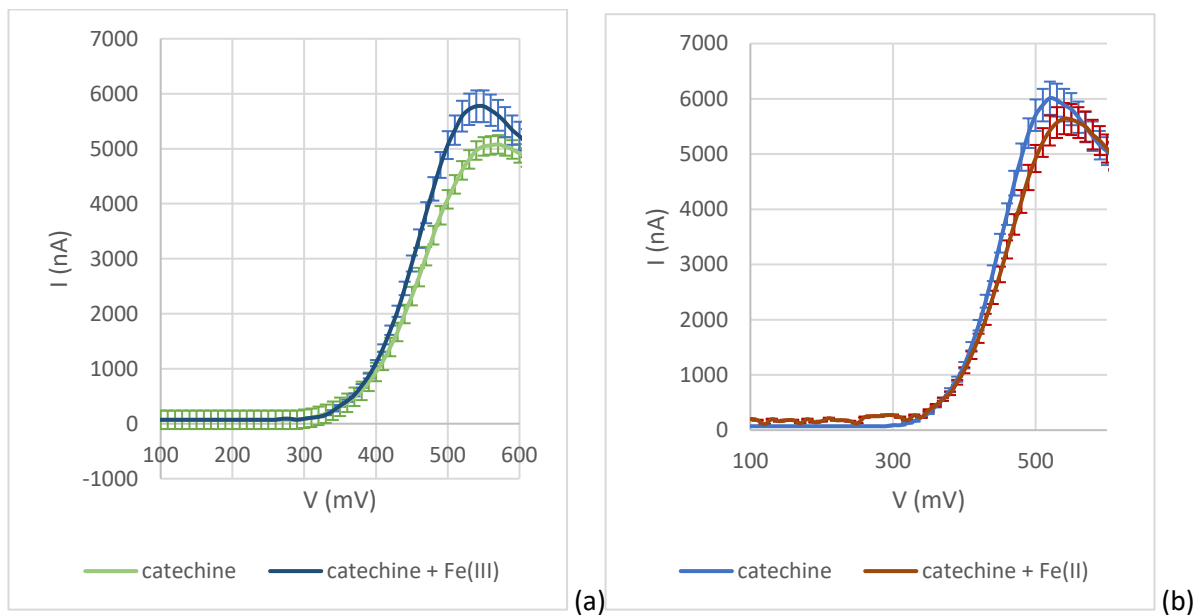
In an attempt to explain the chemical mechanism underlying the test, additional experiments were conducted in model solution under inert conditions. In literature, it has been shown (Kilmartin *et al.* 2002, Ugliano *et al.* 2019) that carbon electrode voltametry can be used to analyze phenolic compounds.

It has also been hypothesized that the oxidation mechanism in wines (Figure 2) is driven by the redox cycle of the Fe(II)/Fe(III) couple (Ribereau Gayon 1931, Danilewicz 2018, du Toit *et al.* 2006, Waterhouse and Laurie 2006).



**Figure 2: Wine oxidation mechanism, adapted from Danilewicz (2007)**

In addition, these same Fe(II) and Fe(III) ions are known to form complexes with phenolic compounds, particularly structures with a di- or tri-hydroxylated B ring (Amorim Porfírio *et al* 2014). Recently, several studies (Le Nest *et al.* 2004, Amorim Porfírio *et al* 2014) have highlighted that complexes between phenolic compounds and certain metal ions (Zn<sup>2+</sup>, Fe<sup>2+</sup>) can present different intensity/potential curves from the one of the phenolic compound in the absence of metal ions. The signal of the complex is quite frequently higher. Several model phenolic compounds have been investigated in these studies (morine, quercetin, fisetin, catechin, chrysin, taxifolin...) showing different impacts of complexation on their respective voltammetric signal. However, these studies were carried out at neutral pH to reproduce physiological conditions, and do not present the signal of the phenolic compound-Fe(III) complex. Since wine has an acidic pH and organic acids, notably tartaric acid, are also known to complex Fe(II) and Fe(III) ions, the voltammetric signal of catechin, chosen as the model phenolic compound for this study, was recorded in wine model solution (13% hydroalcoholic solution, 4 g/L tartaric acid, pH 3.5) before and after independent addition of Fe(II) and Fe(III) ions, in an inert atmosphere to prevent the oxidation mechanism from setting in (performed in a fume cupboard inerted by continuous nitrogen flushing, solutions deaerated beforehand). The independent addition of Fe(II) and Fe(III) ions to the catechin solution led to changes in the intensity-potential curve recorded for catechin alone (Fig. 3), suggesting that catechin - Fe(II) and Fe(III) ion complexation had indeed taken place under model wine conditions and had an impact on the voltammetric signal. It was observed that the voltammetric signal of the catechin - Fe(II) complex was close to that of catechin (Figure 3 (b)), as observed by Porfírio *et al* (2014), or even slightly lower between 450 and 500 mV. On the contrary, the signal of the catechin - Fe(III) complex was significantly higher than that of catechin alone between 450 and 600 mV (figure 3 (a)).



**Figure 3: Potential intensity curves for : (a) catechin (1150mg/L, 13% hydroalcoholic solution, pH 3.3, 4g/L tartaric acid) in the absence or presence of Fe(III) ions (5 mg/L) (b) catechin (1165mg/L, 13% hydroalcoholic solution, pH 3.3, 4g/L tartaric acid) in the absence or presence of Fe(II) ions (5 mg/L)**

*The complexation of Fe(II) ions may make them less available to take part in the Fenton reaction:*

Consequently, the following hypotheses have been put forward to explain the evolution of the intensity-potential curves of a given wine after 2h of contact with air.

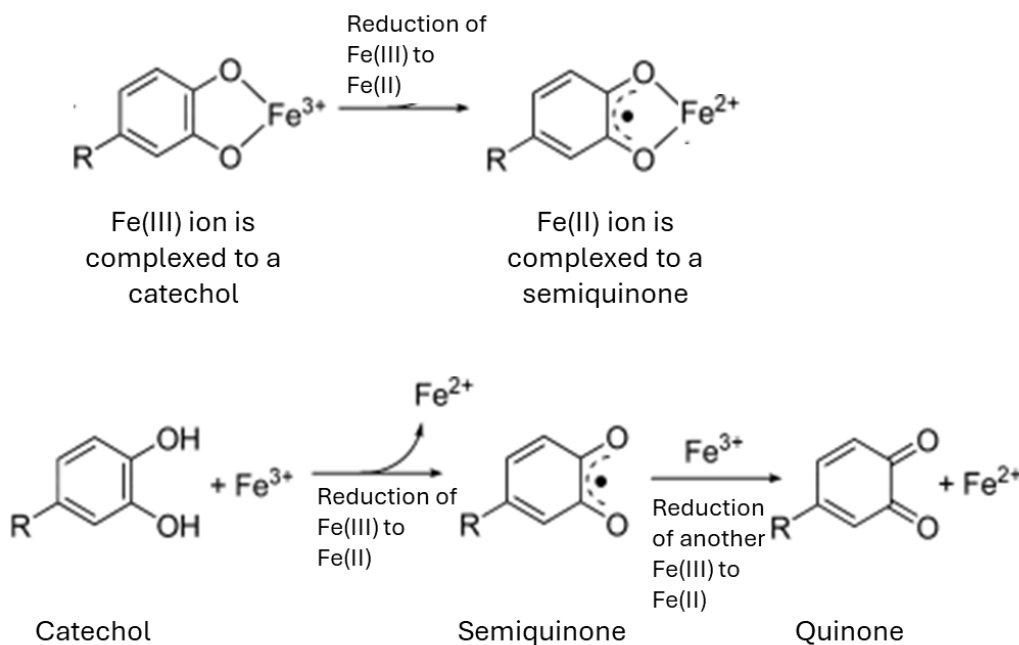
The first measurement is taken immediately after opening the sample, which has been stored for a minimum of 3 weeks in anoxic conditions following sampling under inert conditions. Under these conditions, iron ions are predominantly in the Fe(II) form (Danilewicz, 2016 and 2018, Nguyen & Waterhouse 2019). The voltammetric signal therefore corresponds to that of phenolic compounds or phenolic compound-Fe(II) complexes, depending on the ability of the phenolic compounds present to complex Fe ions.

After 2h of contact with air, the oxidation mechanism (Figure 3) began, materializing as an oxidation of Fe(II) to Fe(III) coupled with a reduction of oxygen to hydrogen peroxide (Ribereau Gayon 1931, Danilewicz 2018, du Toit *et al.* 2006, Waterhouse and Laurie 2006). When an increase in signal is observed, this could be attributed to the contribution of phenolic compound-Fe(III) complexes, whose signal appears higher than that of phenolic compound-Fe(II) complexes based on recordings made on catechin solutions. This would mean that wines classified as resistant to oxidation by this test have a pool of phenolic compounds capable of complexing Fe ions under the environmental conditions of these wines. The effect of exogenous metal chelators in stabilizing wines against oxidation was considered by Kreitman *et al.* (2013) following a model solution study. Furthermore, in a physiological context, Lopes *et al.* (1999) envisaged that tannic acid limits the oxidation of 2-desoxyribose, a component of DNA, through its ability to chelate Fe ions making Fe(II) ions unavailable to participate in the Fenton reaction and thus limiting the formation of  $\text{HO}^\bullet$  hydroxyl radicals. In the case of resistant wines, it could be that Fe(II) ions (figure 4)



remain complexed to semiquinone (Perron *et al.* 2009 and 2010), the oxidized form of the phenolic compound that has reduced an Fe(III) ion to Fe(II). There would be no continuation of the mechanism towards a quinone form having reduced 2 Fe(III) ions, and the Fe(II) ions would remain complexed to the phenolic compounds. Perron *et al.* (2010) also envisaged that the stability of the phenolic compound-Fe(III) complex would favor autoxidation of the complexed Fe(II). Fe(II) ions could thus be less available to react with hydrogen peroxide and give rise to the Fenton reaction, which would explain the lesser appearance of notes described as oxidative when these wines are in contact with oxygen.

On the contrary, when the wine signal decreases after 2h of contact with air, it is likely that the oxidation mechanism has resulted in the formation of quinones, oxidized forms of phenolic compounds that cannot contribute to the voltammetric signal in the potential zone under consideration. The pool of phenolic compounds under the environmental conditions of these wines would not be able to maintain complexed Fe ions (Figure 4). Fe(II) ions could therefore react with hydrogen peroxide to initiate the Fenton reaction (Singleton 1987) and give rise to very high-energy radicals, the HO° hydroxyl radicals that oxidize ethanol to ethanal, for example. Without being exhaustive, it has also been envisaged that hydroxyl radicals generate the appearance of brown/yellow/orange pigments linked to condensation reactions of flavan-3-ols (tannins) in the presence of ethanal or pyruvic acid (Oszmianski 1996, Fulcrand 1996, Guyot 1996) or attack compounds with alcohol functions, leading to the formation of so-called oxidation aldehydes (methional, phenylacetaldehyde) (Nikolantonaki and Waterhouse 2012).



**Figure 4: Mechanism of oxidation of a catechol on the B ring of a flavonoid and reduction of Fe(III) to Fe(II). Top: Fe ions remain complexed, with Fe(II) less available to generate the Fenton reaction. Proposed mechanism for oxidation-resistant wines. Bottom: Fe ions are not complexed and Fe(II) are more likely to generate the Fenton reaction. Proposed mechanism for oxidation-sensitive wines. R: A and C nuclei of a flavonoid.**



In conclusion, determining a wine's sensitivity to oxidation is of major interest to winemakers in order to best adapt the winemaking method and storage conditions to each product's desired profile. Air resistance testing is an empirical method for simply assessing this resistance. When this test is followed by linear sweep voltametry, the evolution of the signal between the moment of contact with air and 2 hours of contact enables us to identify oxidation-resistant wines, showing an increase in the voltammetric signal, and oxidation-sensitive wines, showing a decrease in the latter. These signal changes could be linked to the varying capacity of the wine's phenolic compounds to complex Fe ions, with complexation leading to a higher voltammetric signal for the phenolic compound -Fe(III) complex and making Fe(II) ions less available to generate the Fenton reaction. Beyond the link between voltammetric measurement results and empirical wine grading by winemakers, white and red wines were subjected to this test, then bottled in a controlled manner and preserved in order to test the predictive character of the test. These studies, confirming the possibility of assessing the oxidation sensitivity of wines, will be published in a forthcoming article.





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