RED WINE BARREL AGEING ON LEES. ADVANTAGES OF OXIDATIONS UNDER REDUCTIVE CONDITIONS.

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Introduction

During wine ageing, oxido-reductive reactions are mainly determined by the frequency of oxygen additions. Oxidations are caused either by the exposure to oxygen during racking, headspaces, or other operations, or during micro-oxygenations by diffusing pure oxygen slowly into the wine at pre-determined rates.

Today, the different effects of oxygen are better understood. For example, it is known that the resulting transformations are essential for colour stabilization and to smooth tannins in red wines. However, in spite of the enhancing effects of oxidations, some undesirable effects can not be neglected. At international level, markets are focussed on fruity wines with fresh, intense colours and round tannins, which allow these wines to be consumed early. Nevertheless, oxidations often cause decreases in fruity aromas. At the same time, depending on the frequency and intensity of oxygen additions, the phenolic structure and colour of the wine can evolve prematurely. This leads to thinner wines and dryness caused by the precipitation of the most polymerized tannins, and the colour evolves towards brick-red hues from colour degradation. In fact, the challenge of oxidative ageing lies in both the adjustment of oxygen additions to the wine phenol profile, as well as the utilization of reducing substances allowing to limit the unwanted effects of oxygen, such as colour destruction and loss of fruity aromas. If modern wine ageing had to be summarized in few words, we would choose "*Oxidations under Reductive Conditions*". However, this concept is not innovative but rather the return to ancient practices, which recommended early ageing combined with extended yeast-lees contact. This procedure is still in use in Burgundy.

Yeast lees, also called fine lees, thus seem to play a central role in red wine ageing. In previous studies, we underlined the advantages of this procedure, mainly because of the supply of polysaccharides, which contribute to the round and full characters of wines (Vivas *et al.,* 2001a and b). In line with early assumptions, we suggested the preeminent importance of nitrogenous compounds in the control of the oxidative phenomena (Vivas *et al.,* 2003 ; Vivas *et al.,* 2004). Our results have been confirmed by studies from Lavigne-Cruège *et al.* (2003), which demonstrated the role of glutathione in the stabilization of the molecules with fruity aromas in white wines. The reducing role of sulphur amino acids and peptides, as well as their use to control oxidations is well known in the agrifood industry. Thus, instead of using traditional reducing agents (SO_2, ascorbic acid), it seemed of interest to study the possibilities of controlling oxidations with the nitrogenous compounds, which can originate from yeast autolysis in the fresh lees.

1.- Definition of controlled oxidation, coupled oxidation, and oxidations under reductive conditions

1.1.- Controlled oxidation

Controlled oxidations are a distinctive feature of barrel ageing. Simultaneously, oxygen diffuses slowly and constantly because of the wood porosity, and through the extraction of ellagitannins, very active molecules, which contribute to increased oxido-reduction potentials in total absence of oxygen.

In the past, the porosity of barrels has been much debated. However, today, this question has been definitively resolved thanks to the development of a specific device. This device consists of a double-chamber: The first chamber is in contact with the environment, the second is attached to a closed circuit containing pure nitrogen. Between the two chambers, a piece of wood (attached to an object holder with a non-porous glue) is positioned in the same orientation as staves in a barrel. Air-tightness is ensured by an O-ring, and is controlled by the installation of a piece of steel instead of the wooden one. Thus, the oxygen (measured with a specific probe) appearing in the nitrogen filled chamber over time comes from the direct transfer of air in the first chamber through the wood fibres. The entire structure works under atmospheric pressure. In fact, Figure 1 shows that oxygen is accumulated over time thus demonstrating the porosity of the wood and also, that coarser grains are less porous than tight grains. Finally, wood thickness influences the transfer rate.

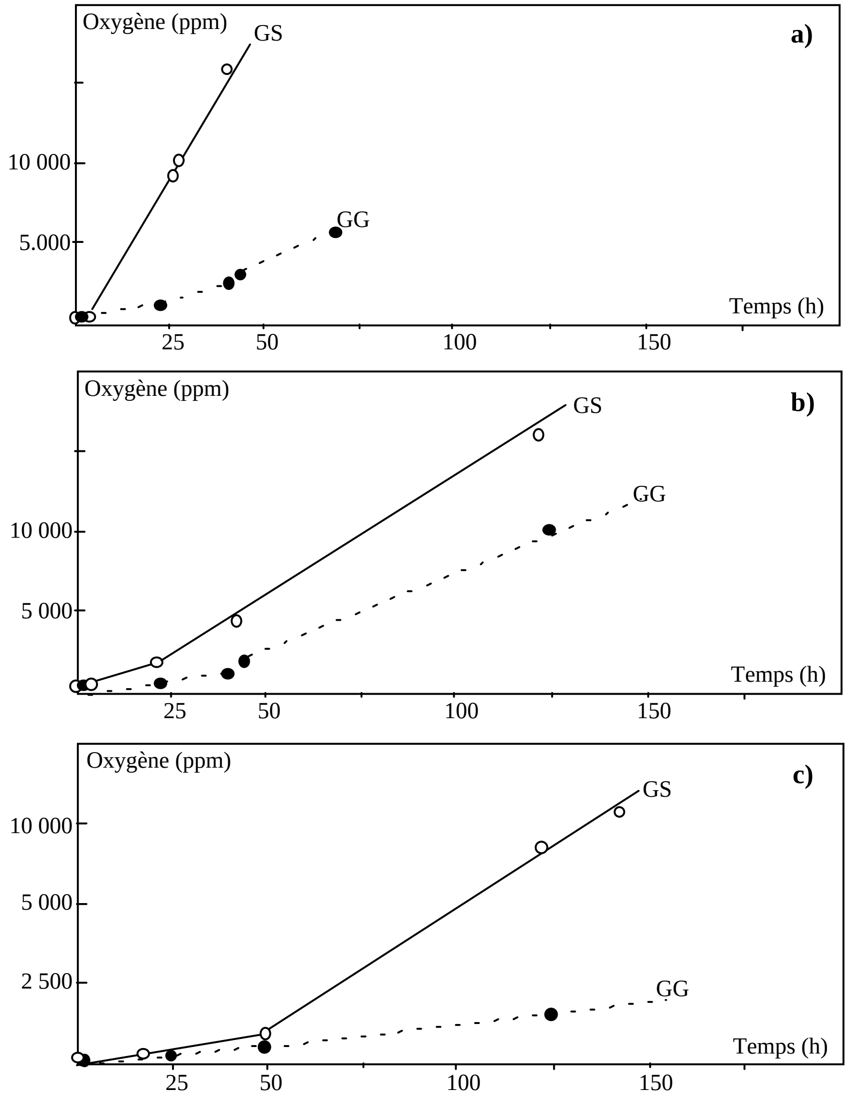


Figure 1: Effect of wood grain and thickness on oxygen transfer kinetics (GS, tight grain; GG coarse grain)
a) 2 mm; b) 4 mm; c) 8 mm

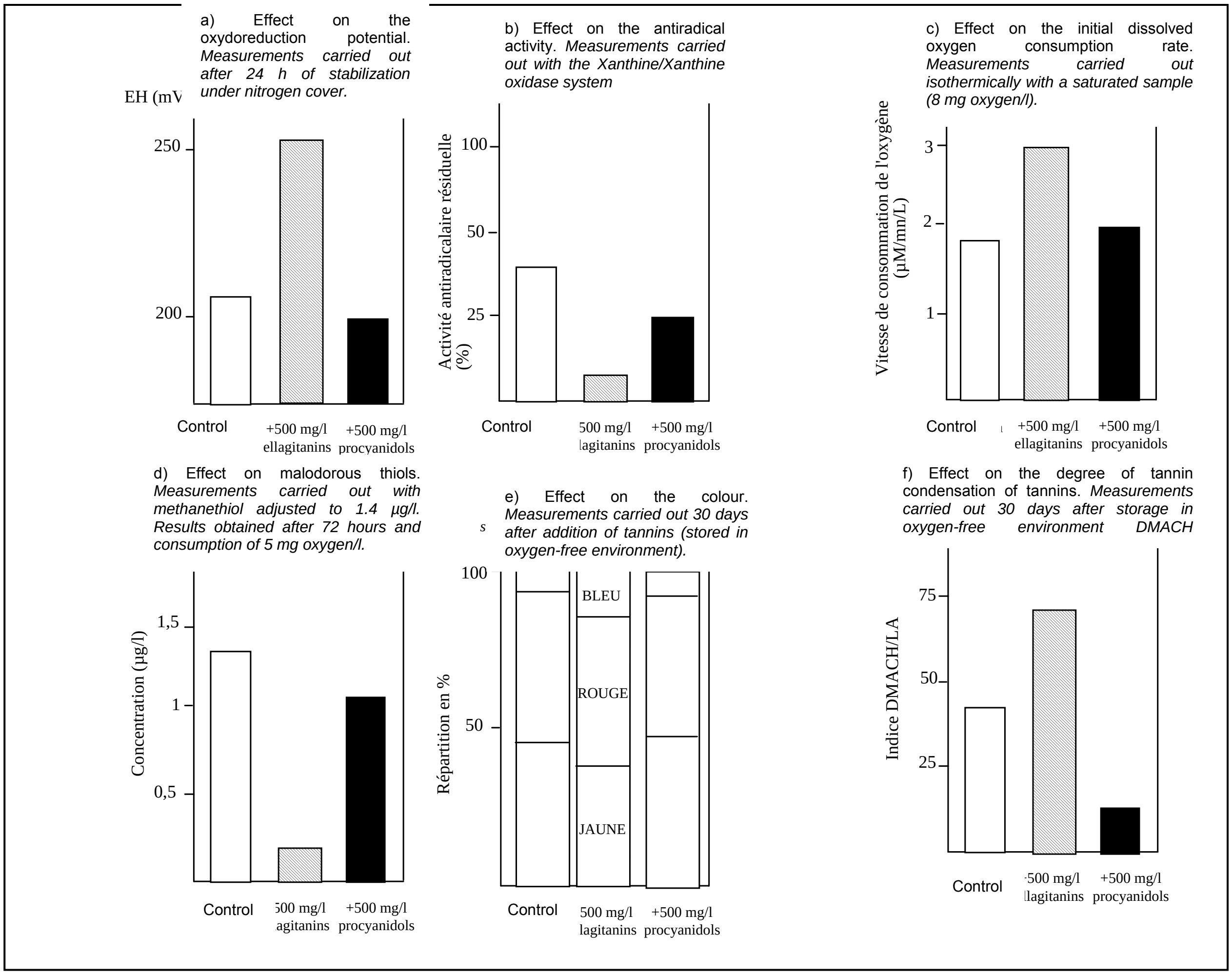
Ellagitannins are highly water soluble molecules and dissolve easily into wines during ageing in new barrels. Toasting does not decrease their concentration in wines since the depth of wine impregnation (8-12 mm) is greater than the one affected by toasting (<5 mm). These wood tannins are by far more oxidisable and have higher antiradical activity compared with grape tannins. These properties lead to very specific effects (Fig.2):

a) Ellagitannins alone increase the oxido-reduction potential without requiring dissolved oxygen. (Fig.2a). Thus, they render the medium more oxidisable and favour oxidation processes in general.

b) Ellagitannins effectively bind free radicals thus protecting wine compounds from the damaging effects of oxidations by radicals (Fig.2b).

c) Ellagitannins have the potential to bind oxygen and release it again in numerous reactions. They consume oxygen very rapidly, and faster than grape tannins do (Fig.2c).

Consequently, these diverse fundamental properties lead to the degradation of malodorous thiols and decrease reduced aromas (Fig. 2d). They also ensure a good colour development, by preserving the red and blue-mauve hues, giving the wine a more intense, dark and deep colour (Fig. 2e). Finally, thanks to the formation of acetaldehyde, which follows the consumption of oxygen, they increase the degree of grape tannin condensation thus decreasing their astringency (Fig.2f).



a) Effect on the oxydoreduction potential. *Measurements carried out after 24 h of stabilization under nitrogen cover.*

b) Effect on the antiradical activity. *Measurements carried out with the Xanthine/Xanthine oxidase system*

c) Effect on the initial dissolved oxygen consumption rate. *Measurements carried out isothermically with a saturated sample (8 mg oxygen/l).*

d) Effect on malodorous thiols. *Measurements carried out with methanethiol adjusted to 1.4 µg/l. Results obtained after 72 hours and consumption of 5 mg oxygen/l.*

e) Effect on the colour. *Measurements carried out 30 days after addition of tannins (stored in oxygen-free environment).*

f) Effect on the degree of tannin condensation of tannins. *Measurements carried out 30 days after storage in oxygen-free environment DMACH*

Figure 2: Significant properties of ellagitannins. Comparison with grape proanthocyanidols. Experiments carried out with a red wine of the vintage (Merlot)

Concerning the redox balances, it is easy to appreciate all the advantages of controlled oxidations. More specifically, they allow to reach higher oxido-reduction potential equilibria than those of big stainless steel or concrete tanks (Figure 3). Thanks to this method, the medium is constantly in a more oxidative physiochemical state and thus, the wine evolves noticeably. However, the most important aspect is the buffering of effects arising from strong oxidations. It is easily noticeable that under controlled oxidation conditions, the increase of the oxido-reduction potential after a racking step or any other oxygen addition is much more limited if compared with a strict reductive ageing. Thus, the EH variation between the initial stage (syn. state of equilibrium) and the final stage (over-oxidized) is smaller and always favours barrel ageing (Fig.3). The abundance of phenols in wines limits the EH increase and significantly decreases the ΔEH value by 20 to 30%. In wineries, when the EH varies strongly and repeatedly, important reductions of the varietal aromas generally are observed, as well as a premature development of the colour towards yellow hues and a thinning of the tannins. Thus, the quality of wine ageing relies on the regularity of oxidative phenomena. In this case, the positive effects of oxidations are favoured, i.e. increase in structure and stabilization, while limiting the damaging impact of oxidative damage.

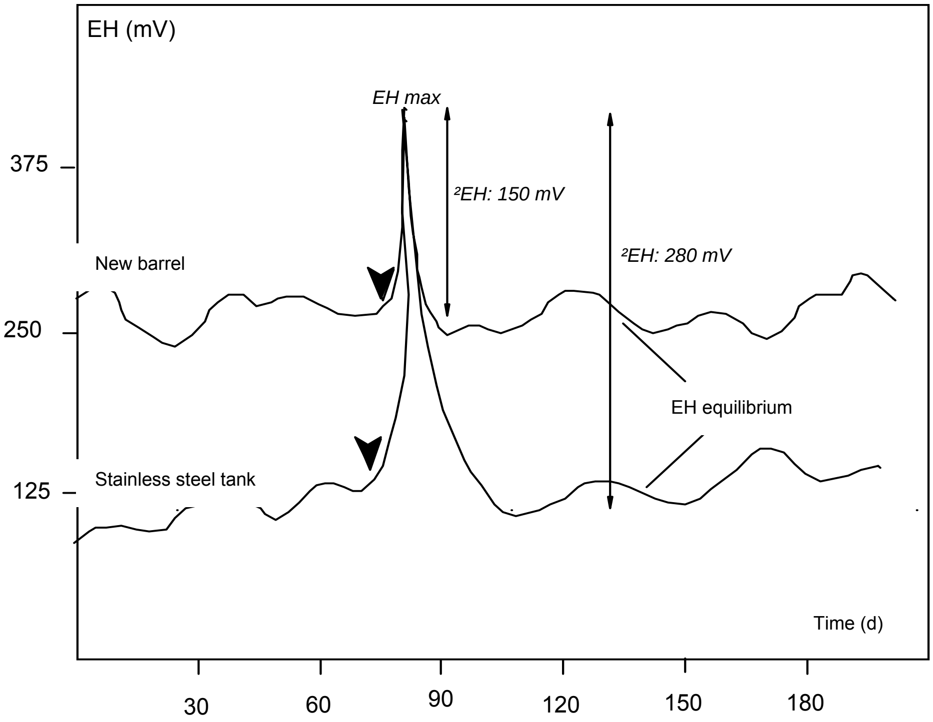


Figure 3: Effect of wine storage during ageing on the variation of the oxidoreduction potential (EH). Effect of one aerated racking step resulting in near oxygen saturation (arrow).

1.2.- The coupled oxidation

In practice, it is not the oxygen itself, which is responsible for wine development, but rather activated forms which act in cascades. First, oxygen diffuses into the wine and activates the redox systems and oxidation reactions by increasing the oxido-reduction potential. At this stage, phenolic compounds (redox couples) will auto-oxidise passing from their reduced to the oxidized form; these oxidised forms are quinones which polymerize and first increase yellow wine colours before precipitating. As a result, hydrogen peroxide (H_2O_2) is formed and can oxidise ethanol into acetaldehyde. If present in excess, hydrogen peroxide will form superoxide anions ($^{\cdot}OH$) through Fenton reactions, and these can then oxidise fragile substances such as the ones responsible for fruity aromas, or free anthocyans. Figure 4 summarizes the entire process. It is important to note that in this system, all strong oxidations and excessive activations of wine redox couples by EH increases, or the production of hydrogen peroxide to a degree exceeding the requirements of the wine in oxygen, will inevitably lead to oxidative damage.

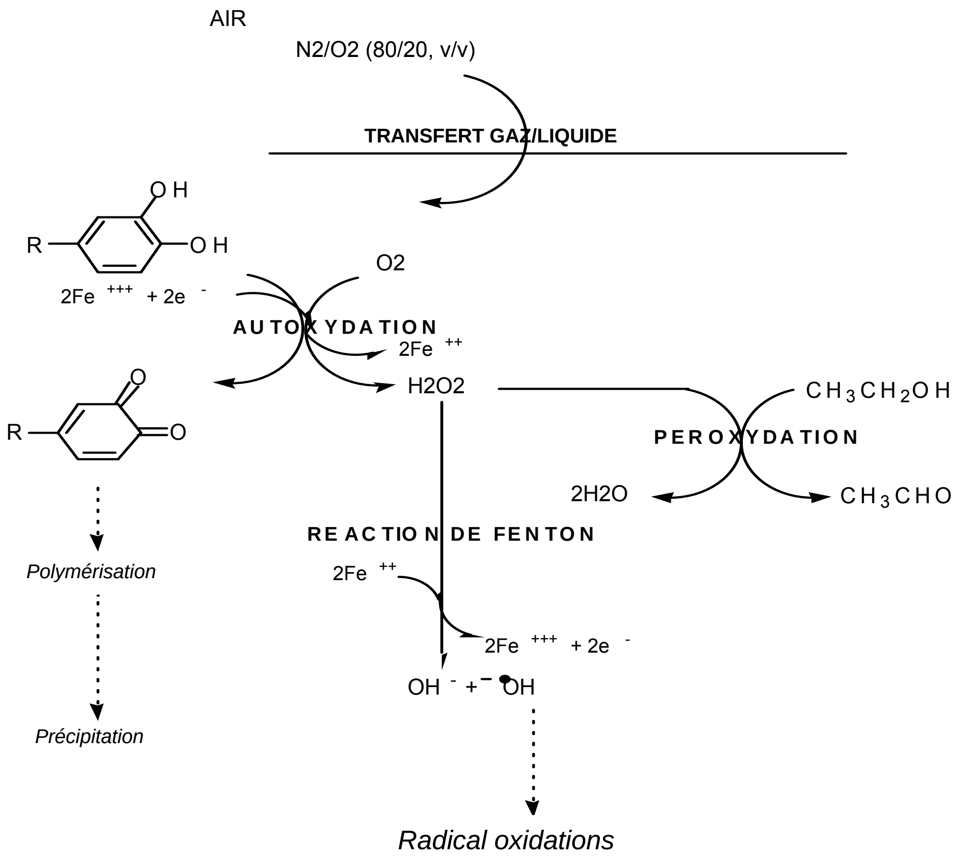
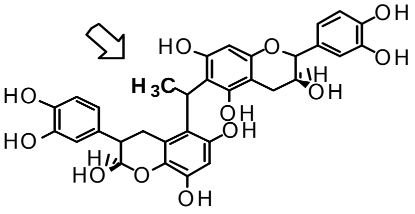
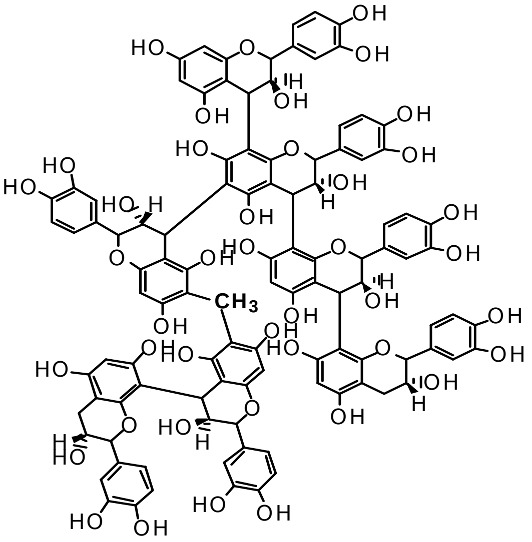


Figure 4: Simplified representation of coupled oxidations in wines in the presence of oxygen and cationic metal catalysts.

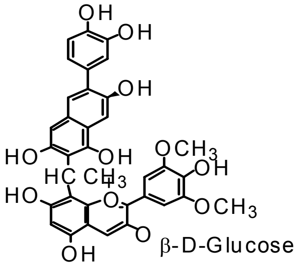
The acetaldehyde produced then plays an important role in tannin polymerization and colour stabilisation. On the molecular level, the changes caused by oxidative ageing lead to production of polymerized tannins and anthocyans, which involve acetaldehyde as condensation (ethyl-) bridge. In chemistry, this is called a nucleophilic addition. These forms are not found in grapes nor in fresh skin and pip extracts: It is only after the formation of acetaldehyde that they can be found, i.e. mainly during alcoholic fermentation and oxidative wine ageing. Please, refer to Figure 5 for examples of these structures.



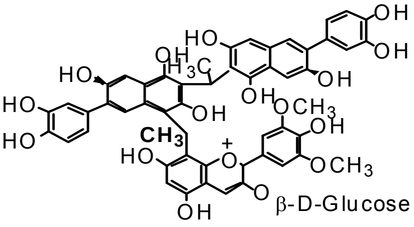
Exemple d'un catéchol-éthyl-catéchol



Exemple d'une combinaison procyanidol pentamère-éthyl-procyanidoldimère



Malvidine-3mG-ethyl-catéchol



Malvidine-3mG-ethyl-catéchol-ethyl-catéchol

From left to right, top to bottom:
- Example of a catechol-ethyl-catechol
- Example of a pentameric procyanidol-ethyl-procyanidol dimer combination
- Malvidin-3mG-ethyl-catechol
- Malvidin-3mG-ethyl-catechol-ethyl-catechol

Figure 5: Structure of polyphenolic polymers characteristic of wines after oxidative ageing: Examples of ethyl-flavanol, ethyl-proanthocyanidol and ethyl-anthocyan adducts.

In the laboratory, it is easy to simulate the main reactions taking place during oxidative ageing by adding variable quantities of acetaldehyde. In order to study the effect of acetaldehyde on the modification of red wine phenol composition and structure, we analyzed 4 wine samples adjusted to different acetaldehyde levels: 0, 50, 100, and 150 mg/l. The analyses were carried out after 3 months of storage in darkness at 20°C, and under nitrogen cover. The results are shown in Table 1.

	Acetaldehyde added (mg/l)			
	0	50	100	150

Acetaldehyde consumed (mg/l)	0	32	87	128
d*	45	43	36	32
CI'#	0.52	0.71	0.47	0.38
Hue†	0.66	0.7	0.88	0.87
Total Anthocyans (mg/l)	248	94	26	28
Total tannins (LA) (g/l)	3.1	2.8	1.9	2
Indices (%) :				
TA combinations	37	78	92	95
% of non-dialyzable polyphenols	14	26	36	34

*Total polyphenols (OD 280 nm)

Colour Intensity (∑420, 520, 620 nm)

† 420/520

Table 1: Effect of acetaldehyde addition on red wine phenolics (analyses carried out after 3 months).

The amount of total anthocyans (assessed by SO_2-decolourisation) decreased proportionally with the quantity of acetaldehyde added. Beyond 100 mg/l of acetaldehyde added, the amount of anthocyans reached a minimum of 30 mg/l. At the same time, an increase in tannin-anthocyan combinations, as determined by the PVPP index, could be observed; the wine colour changes from a clear red to a dark blue-mauve colour. Beyond 100 mg/l of acetaldehyde, all anthocyans are bound in complex and more stable structures. Acetaldehyde additions also lead to an important decrease of the tannins measured. This is partially due to the precipitation of some of the phenolic compounds and the decrease of the total phenols confirms this well. Tannin precipitation is caused by the increase of the condensation level. As soon as the non-dialyzable polyphenol index reaches a value close to 35, an important part of the tannins becomes insoluble in 12% (vol.) ethanol hydroalcoholic media. The increase of wine tannin polymerization level and the decrease of their astringency is normal during oxidative ageing. In fact, it was found that at similar polysaccharide levels, aged wines with a higher level of polymerized tannins obtained better tasting evaluations (Fig.6).

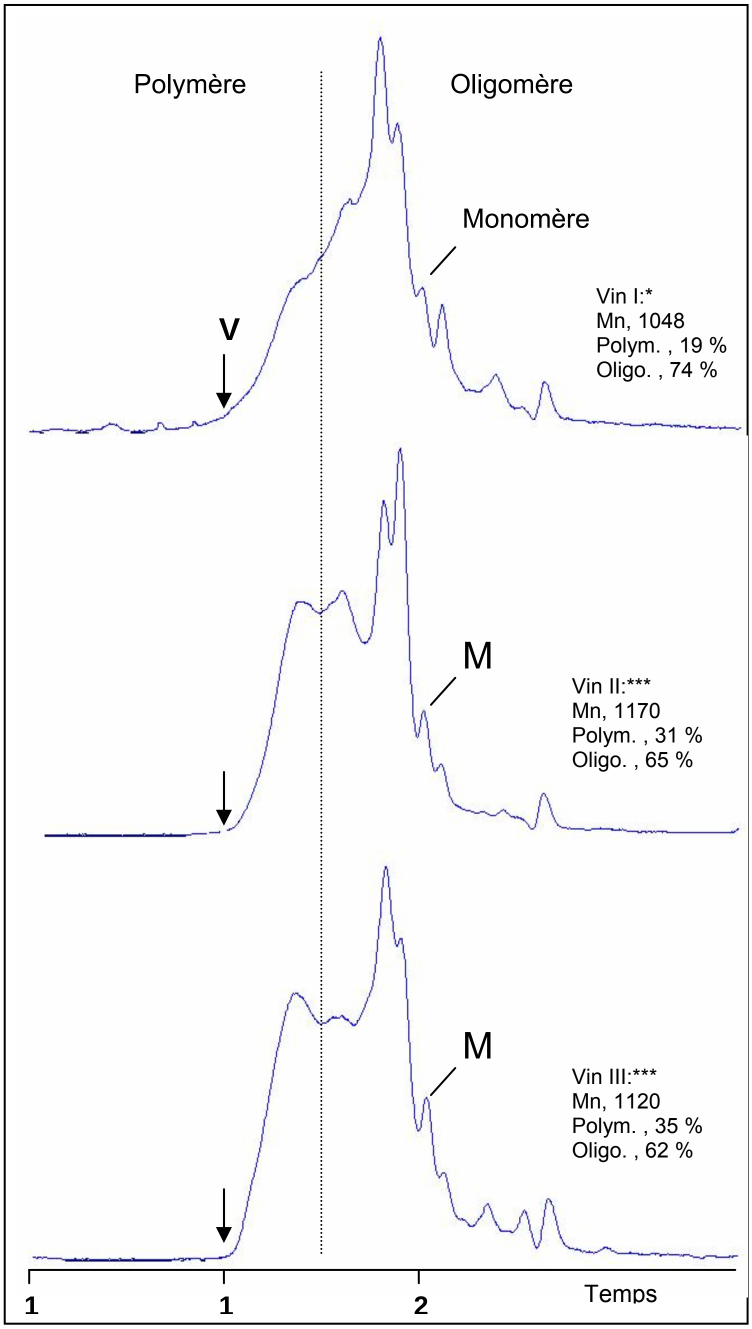


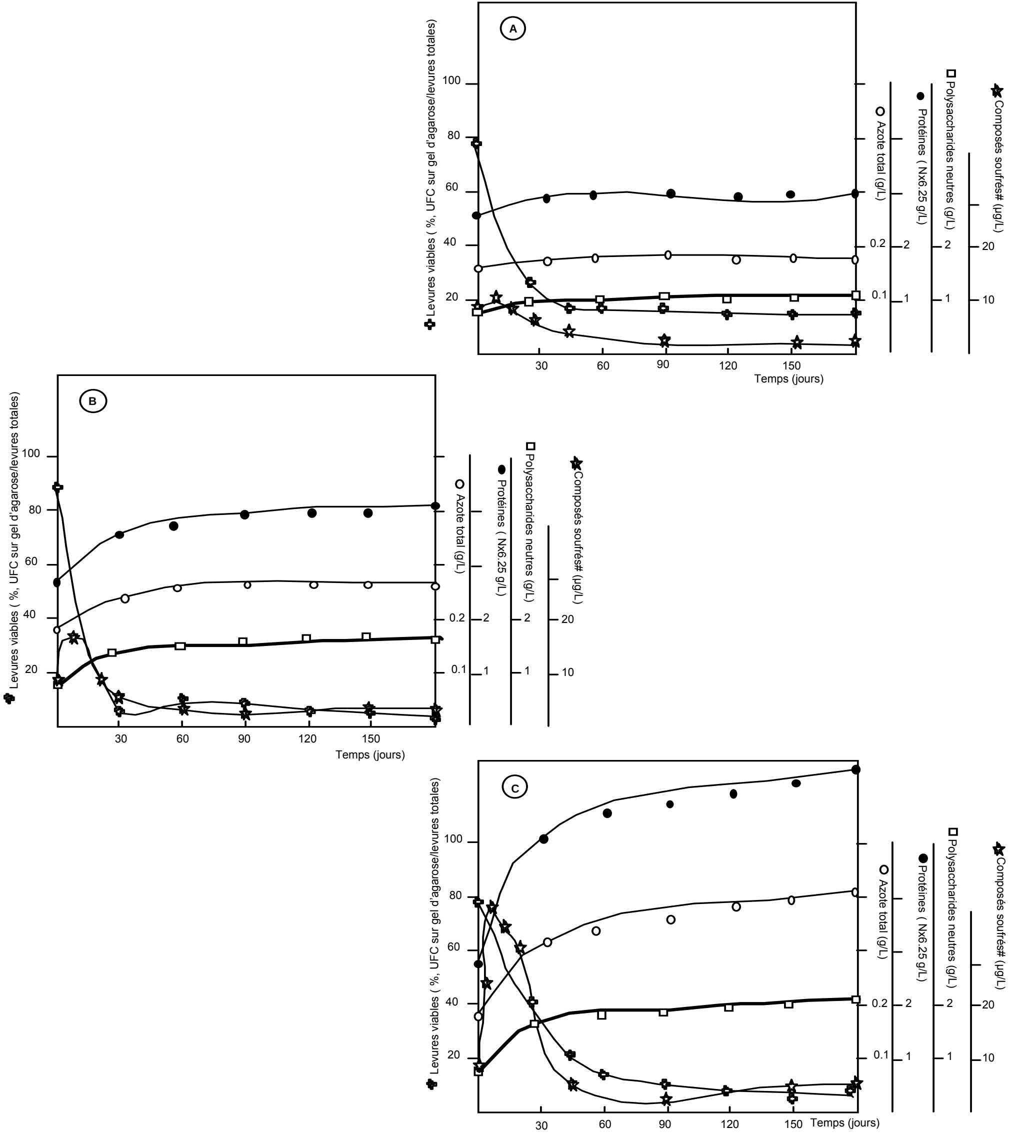
Figure 6: Size exclusion chromatograms of total proanthocyanidols (peracetylated) of different red wines (Mn, average molecular weight [Da], Vo, dead volume of the column). The stars indicate wine quality as assessed by a tasting panel (low, **medium,***excellent)*

1.2.- Oxidations under reductive conditions

As demonstrated above, the quality of an oxidation depends on favouring the stabilisation process, which relies on acetaldehyde production, and by limiting the quality degradation phenomena as much as possible. The latter are observed in two cases: Firstly, in the case of strong oxygenations, which contribute to an increased oxido-reduction potential (large ΔEH) and to the activation of redox systems. Under these conditions, the oxidation is fast and has negative effects. The second case occurs if the addition of oxygen is too high with regards to the phenolic content of the wine and its oxygen sensitivity. The excess hydrogen peroxide is then transformed into free radicals and affects the most fragile wine compounds. Thus, in order to preserve fruity aromas of wines and the freshness of their colour, it is important to favour gentle oxidations as much as possible, whose effects will be compensated by a proportional state of reduction. The definition and technical management of this state will be described in the next chapters.

2.- Advantages of red wine ageing on lees and their contribution on wine composition

An experiment was carried out in order to follow the time course of certain compounds during lees ageing: neutral polysaccharides and proteins from yeast autolysis, and the sulphur compounds produced by yeast reductase activities, which are responsible for sulphur notes. We compared the effect of different amounts of lees: a control without lees (but actually containing 0.5 g/l of dry lees), a wine with fine lees (containing 2 g/l of dry lees), and finally, a wine with a large amount of lees (7 g/l). The different batches were filled into new barrels at the same time and the results are shown in Figure 7.



A/B/C

Left Y-axis: Viable yeast counts (%, CFU on agarose gel/total yeast count)
X-axis: Time (days)
Right Y axis (left to right): Total nitrogen (g/l)
Proteins (N x 6.25 g/l)
Neutral polysaccharides (g/l)
Sulphur compounds# (µg/l)

Figure 7: Course of viable yeast counts, and total nitrogen, protein and neutral polysaccharide concentrations during barrel ageing of Merlot Noir wines. Time scale starts with end of malolactic fermentation in tanks. Wines were racked every 30 days for the first 3 months and then once after 6 months. A, Control wine without lees (0.5 g/l of dry lees); B, Wine on fine lees (2 g/l of dry lees); C, Wine on heavy lees (7 g/l of dry lees).
 # Sulphur compounds: H_2S *and methanethiol.*

Overall, most of the autolysis occurred in the first 3 months and then slowed down. The amount of lees was proportional to the concentration of yeast colloids in the wine (neutral polysaccharides and proteins). An interesting observation was made in relation to the sulphur compounds, which were formed at the beginning of ageing when the lees still contained viable yeast. The use of new barrels and the resulting solubilization of ellagitannins then allowed to eliminate these molecules and the sulphur notes they cause. This explains the strong decrease of their concentration and confirms our laboratory results.

Fine lees essentially composed of dead yeast cells, supply nitrogen compounds to the wine, which present a strong reductive effect, at least in laboratory trials. Thus, they act as protective “anti-oxidants” with regards to the negative effects of oxidations on fragile compounds, such as fruity wine aromas and the colour as represented by free anthocyans. Nitrogen compounds are mainly released by the young lees at the beginning of autolysis. This release can be increased by regular stirring or by addition of large amounts of specific enzymes. In fact, most of the commercial preparations used are inhibited by wine proanthocyanidols, but addition rates of 5-7 g/hl allow to compensate for this inhibition and ensure noticeable and measurable changes. Our first results allowed to observe a clear reduction of the colour evolution under oxidative conditions: the colour remained more red with a weaker hue compared with a control without autolysate (Table 2). Without further considering the consequences, we could observe a noticeable reduction of the oxido-reduction potential and the dissolved oxygen consumption rate (Table 3). Comparative sensory evaluations confirmed that the wines always preserved more fruity aromas. As an explanation, it has been suggested that lees compete with other oxidable wine compounds for oxygen (Fornairon *et al.,* 1999). However, trials carried out with protein fractions from autolysates produced results, which were relatively similar to prolonged ageing on fine yeast lees (Vivas *et al.,* 2004). Thus, it is very likely that autolysates contain strongly reducing compounds, and these issues are currently being considered by our research group. The objective is to study the effect of these compounds on the oxidation of polyphenols, and to determine the nature of the most active forms.

	Control wine			Wine + 10% autolysate	
	t0	t2months		t2months	
		O2	N2	O2	N2
Proanthocyanidols	3.4	3.2	3.5	2.8	3.1
Anthocyanidins	0.58	0.34	0.56	0.49	0.57
Colour intensity[1]	0.69	0.72	0.71	0.89	0.73
Hue[2]	0.65	0.84	0.67	0.64	0.62
Proteins	1.8	1.6	1.9	2.5	2.7
Neutral polysaccharides	0.75	0.72	0.77	0.82	0.84
DMACH Index[3]	65	73	68	47	60
Tannic potential (NTU/ml)[4]	129	156	114	58	62

[1] d420+d520+d620, [2] d420/d520, [3] Tannin polymerization index, [4] Tannin astringency index

Table 2: Effect of yeast autolysate addition on composition and quality of a red wine stored under oxidative conditions (O_2) or under inert gas cover (N_2). (results are given in g/l unless stated otherwise).

	Soluble proteins (g/l)	Vi (µmole O2/l/mn)	EH (mV)		
			Before aeration	after aeration	after 10 days
Control	2.4 ±0.2	32 ±7.4	125 ±20	360 ±27	185 ±38
After ageing on lees	13.6 ±2.3	36 ±6.8	48 ±16	174 ±32	97 ±23

Table 3: Effect of ageing wine on fresh yeast lees on the amount of soluble proteins, the initial rate of dissolved oxygen consumption (Vi) and the oxido-reduction potential (EH).

3.- Mode of action of soluble colloids from yeast autolysates

During ageing, yeast lees supply the wine with a certain amount of cell material at different stages of enzymatic degradation. The interesting compounds comprise mainly soluble neutral polysaccharides, amino acids, peptides and stable proteins. The composition of commercial products prepared by enzymatic or chemical hydrolysis of yeast propagated in industrial fermenters is variable but generally, these products contain all these compounds.

3.1.- Carbohydrate colloids

Figure 7 clearly shows that ageing wine on yeast lees led to a significant contribution of neutral polysaccharides. These polymers coat tannins and thus decrease their astringency. In a previous study we presented their effects on different groups of grape and wine tannins (Vivas *et al.,* 2004).

Using a standard protein (BSA), we could monitor the formation of tannin-protein complexes over time by nephelometry: the appearance of cloudiness is proportional to the reactivity of the polyphenol tested. According to this method, we made time course studies using a grape seed procyanidol extract with/without addition of varying amounts of total yeast polysaccharides produced in the laboratory (Figure 8). Similar conclusions were made in a study carried out in Burgundy with Pinot Noir (Feuillat *et al.,* 2001). Finally, and of general significance, similar results were obtained with other plant polysaccharides by Mateus *et al.* (2004).

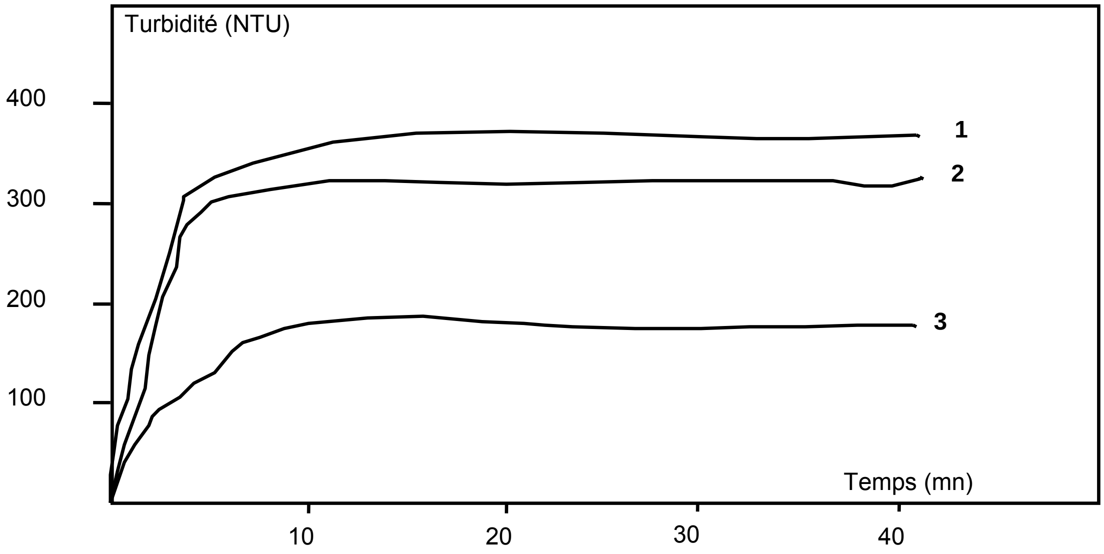


Figure 8: Effect of yeast polysaccharide additions on haze development of a hydro-alcoholic solution of grape seed procyanidols. The yeast polysaccharide fraction was obtained by ethanol precipitation (1/9 v/v) of medium produced from fermentation of Saccharomyces cerevisiae (EG8C) in synthetic medium.

1- control (0.1 g/L grape seed procyanidols and 0.08 g/L of BSA in a 12% vol. ethanol water mixture)

2- control solution with 150 mg/L yeast polysaccharides addition

3- control solution with 1 g/L yeast polysaccharides addition

3.2.- Nitrogenous compounds

During ageing on traditional lees, a steady increase in concentration of total wine nitrogen can be observed (Fig.7). Predominantly, amino acids can be found, such as cysteine and homocysteine (at concentrations above 10 mg/l, Lavigne-Cruège *et al.,* 2000)); glutathione, a tripeptide abundant in the yeast can be found at approximately 10 mg/l (Lavigne-Cruège *et al.,* 2003); peptides can amount to several hundreds of milligrams per litre (Alexandre *et al.,* 2003), and proteins can be found at up to 1 g/l. The increase in total nitrogen of a wine enriched with yeast autolysate produced in the laboratory allowed to regulate oxidative phenomena (Table 4). Compared with the control, the wine containing 10% of autolysate lost much less anthocyans in the presence of oxygen and the hue did not noticeably change. Thus, the colour remained red, and the astringency significantly decreased, probably because of the polysaccharide addition, but also because of the peptides, which conferred sweetness and roundness to the wine. However, the latter aspect has been studied only very recently in the oenological framework. Furthermore, it is known that certain peptides significantly affect the perception of food products. Thus, their role in wines should not be excluded.

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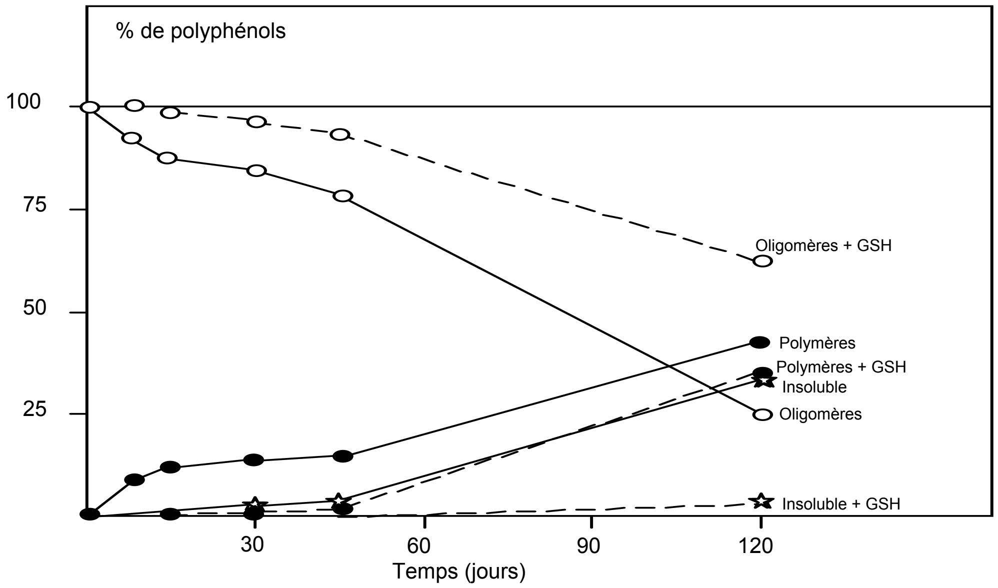
The amino-acids, specifically cysteine and homocysteine, have a strong antioxidative character since their effect is not pH dependent, and because they are largely unbound. Glutathione also regulates oxidative phenomena. We have carried out a series of laboratory experiments in order to study the effect of glutathione on red wine phenolics. First, we followed the evolution of oligomer and polymer forms in a sample of grape seed procyanidols during oxidation (Figure 9).



Y: percentage of polyphenols
X: Time (days)
Oligomers/Polymers/Insoluble/GSH

Figure 9: Time course study of the evolution of oligomer, polymer and insoluble forms of a grape seed procyanidol extract. Effect of glutathione addition at 150 mg/l. The experiment was carried out in a hydro-alcoholic model solution in the presence of copper (1 mg/l) and with weekly aerations (± 5 mg/l). The proportions of oligomers and polymers were measured by size exclusion chromatography.

It can be observed that high glutathione additions noticeably retarded oxidative polymerization reactions, and limited the formation of insoluble tannin sedimentation. Then, in an experiment with a light and oxygen sensitive red wine, a slight development of the yellow and blue colours could be observed compared with the control after 3 months of aerated storage in presence of glutathione concentrations characteristic of wines (20 mg/l). This indicates a decrease in the rate of the oxidative damage, but also a slower formation of tannin-anthocyan combinations absorbing in the blue range (620 nm) by way of acetaldehyde (Table 5). Eventually, in a final experiment, we compared the potentiometric titration curves of a wine aged in barrels with and without lees: 100 mg/l of glutathione were added to a sample of the treatment without lees before titration. The results (Figure 10) show a decrease of wine oxidability, which requires more oxidant after lees ageing or glutathione addition in order to obtain a complete titration curve. Thus, it would appear that glutathione reduces the rate of oxidation reactions and protects the wines from the risks of strong oxidations.

	Control			With glutathione		
	0	30	60 days	0	30	60 days
OD 420 nm	0.251	0.316	0.388	0.251	0.260	0.274
OD 520 nm	0.315	0.367	0.372	0.315	0.328	0.352
OD 620 nm	0.093	0.142	0.182	0.093	0.101	0.126
Hue 420/520	0.79	0.86	1.04	0.79	0.79	0.77

Colour intensity†	0.659	0.825	0.942	0.659	0.689	0.752
% OD 620 nm	14.1	17.2	19.0	14.1	14.6	16.7

† OD 420 + OD 520 + OD 620 nm

Table 5: Effect of glutathione addition (20 mg/l) on colour development of a light red wine (stored in darkness and exposed to air at 20°C, 30 mg/L of free SO2 and 150 mg/L of NaF)

Conclusions

Wines are exposed to oxygen throughout the winemaking process. The oxidative reactions following an aeration lead to changes in the structure of phenolic compounds, and affect wine colour and stability. Ellagitannins, which solubilize in red wines during barrel ageing, also participate in redox reactions. When added to the wine, these molecules increase the consumption of dissolved oxygen, and the production of hydroperoxide and acetaldehyde. Thanks to the release of nitrogenous compounds during the autolytic process, lees participate as reductors in the oxidative evolution of wines and limit the impact of oxygen. Generally, wines aged oxidatively, but under reductive conditions through abundant addition of lees or yeast derived products, are judged to be more aromatic and balanced, to have more roundness and body, but also more fruit: this currently represents the base criteria for assessing the commercial value of wines in the international market very well.

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