Müller-Späth showed in the 1970's that white grape winemaking practices in the presence of oxygen before the alcoholic fermentation were not necessary linked to negative results in the final wine and actually allowed for the wine to be less sensible to oxidation (Müller-Späth, 1977). Before the development of techniques that result in the extraction of a certain amount of polyphenols (mechanical harvest, maceration, sulphite addition during harvesting, inert pressing), several studies were published about treating grape musts with oxygen or «hyper-oxygenation» practices that permit for a reduction in polyphenol concentration in the pre-fermentation phases (Cheynier et al., 1989; Schneider, 1991; Cheynier et al., 1990; Cheynier et al., 1991; Cheynier et al., 1993; Ricardo da Silva et al., 1993).

The technique allows for the stabilization of white wines against oxidation (Müller-Späth, 1977; Cheynier et al., 1989; Schneider, 1991; Schneider, 1998; Immelé, 2011; Ribéreau Gayon et al., 1998), in particular for what concerns the colour and also the astringency and bitterness which is reduced (Schneider, 1998). On the other hand, the effect on the wine aromas is more controversial: since there can be a loss of intensity and varietal aromas in some (Singleton et al., 1980; Dubourdieu et al., 1990), no aroma degradation in others (Müller-Späth, 1977; Cheynier et al., 1989) and even increased intensity (Artajona et al., 1990) or aromatic profile changes in others (Schneider, 1998).

This technique is based on the enzymatic oxidation of polyphenols (Rigaud et al., 1988; Flanzy, 1988 thanks to tyrosinase found in the must (polyphenol oxidase, laccase in musts affected by botrytis). However the polyphenol concentration in the must is very variable (Cheynier et al., 1990; Du Toit, 2006) as is the amount of oxygen uptake between the harvest and the arrival in the sedimentation tank (Schneider, 1998; Immelé, 2011), the quantity of oxygen added to the must remains difficult to determine (Immelé, 2011), keeping in mind that an excessive addition can be useless (Cheynier et al., 1989) or even risky for the wine quality (Schneider, 1998). Many author have hence proposed that the uptake should be measured during the pressing and serve as an indicator (Cheynier et al., 1989; Schneider, 1998; Immelé, 2011).

In the following paragraphs, the development of a tool that will allow for the determination of the oxygen needs of a must as well as the knowledge developed over years of study on the oxygenation of musts from different grapes and different origins will be discussed.

**Determination of the oxygen needs of grape musts**

Vivelys developed a tool, Cilyo®, which allows for the residual consumption of oxygen of the must to be estimated before the sedimentation. The dose indicated by Cilyo® does not correspond to the total residual oxygen consumption of the must but has been optimized in order to avoid oxidation after the alcoholic fermentation and to improve the wine mouth feel without destroying the aromatic potential. This optimal dose was determined by comparing the oxygen consumption speed of the must and the sensory result obtained in the wine after treating the musts with different oxygen doses. For this reason a large number of microvinifications were done in experimental
wineries each of which systematically had a protected and sulphited control treatment as well as the various degrees of oxygenation treatments during the pre-fermentation stage.

Setting up the must oxygenation treatments

The measurement of the must oxygen needs by Cilyo® is done on a sample taken from the sedimentation tank; this has the advantage of being a representative measure of all that the must underwent before (sulphite addition, oxygen uptake during pressing and transfers). The risk of over or under evaluating the need is hence slim since the sample is taken in a perfectly inert manner and it is representative of the tank, notably in terms of turbidity.

Sulphite addition at levels superior to the enzyme inactivation limit makes it impossible to treat the musts with oxygenation. Only moderate sulphite addition should be done on the harvested grapes and juice in order to conserve all the enzymatic activity. Tyrosinase activity is reduced by 75-90% when 50 mg/L of SO₂ is added (Dubernet, 1974). The trials completed in our study show that a maximal sulphite addition of 3.5 g/hL is sufficient to maintain the tyrosinase activity while reducing the microbiological risk.

The oxygen supply dose determined by Cilyo® is added by using a micro-oxygenator. When adding the oxygen, sulphites can be added to the must in order to inactivate the tyrosinase and to remove the lees by flotation or static sedimentation. In this case no sedimentation delay was observed between the oxygenated tank in comparison to the control, as previously reported (Schneider, 1991). In any case, the sedimentation needs to yield a must with less than 70 NTU turbidity which guarantees the elimination of brown particles that form during the oxidation: these are not soluble in the must but can become soluble in the presence of ethanol and can bring a colour to the wine. If residual colour remains in the must, it will be eliminated by adsorption by the yeast during the fermentation (Schneider, 1998).

Results obtained

Vivelys has been applying must oxygenation practices for fifteen years. The knowledge described below was obtained through trials done in experimental wineries or in partner wineries on different grape varieties (Chardonnay, Sauvignon, Manseng, Chenin, Grenache, Marsanne, Viognier...) in different viticultural zones in the world (France, Spain, Italy, Portugal, Chile, Argentina, United States, Australia, South Africa).

The tasting were done by a selected and trained jury, made up of 6 to 12 people, with the help of the Syriel® (Vivelys) tasting tool from 2 months to 3 years after the vinifications.

Sensory effect of must oxygenation

Effects on the mouth feel quality of the wines obtained from oxygenated musts have already been reported in various studies: lower bitterness and astringency (Schneider, 1991; Schneider, 1998) but also an increase in «softness» (Cheynier et al., 1989). Our trials have confirmed these observations and furthermore showed that oxygen addition to the must modifies the viscosity sensation in the final wines: it increases with the amount of oxygen up until a certain point after which it begins to drop as indicated in Figure 1 which shows various trials on different grapes.
In an aromatic sense, oxygen addition to the must has an important effect, which explains the controversy expressed in the previously cited studies. It causes the appearance of new aromas that have not been previously described in literature as far as we know, which can modify the wine aromatic profile (Schneider, 1998). These compounds in general lead to an increase in the fruit ripeness perception, while masking its herbaceous aromas. If the oxygen dose is too high the aromas can also form an oxidative mask that causes a loss of varietal character. This is the case for example in thiol profile Sauvignon Blanc wines (Dubourdieu et al., 1990) that are exposed to high oxygen doses.

However, oxygen addition to the must does not cause a loss of thiol molecules, since these are only released during the alcoholic fermentation and their precursors are not sensitive to oxygen (Roland et al., 2010). Figure 2 shows that a Gros Manseng must treated with two different doses of oxygen, does not show significantly different varietal thiol (3-mercaptohexan-1-ol, 3MH and 3-mercaptohexyl acetate, 3MHA) concentrations than the control. The thiol perception between the treatments was also not significantly different upon tasting.
Figure 2: Analysis of wine thiols in a trial done on Gros Manseng grapes, Côtes de Gascogne 2010. The must was divided into lots, that were treated with increasing oxygen doses (0 mL/L for the control, and then 20 and 40 mL/L respectively). The vinification conditions of each lot were exactly the same. Analyses done by Nyseos.

An adapted oxygen supply can even allow for an increase in thiol character as perceived during tasting as shown in Figure 3. Roland et al. (Roland et al., 2010) showed that oxygen supplied to the must can allow for the production of thiol precursors, this could explain the increase in thiol character in the first oxygen treatment. On the other hand, the decrease in thiol character noted in the most highly oxygenated treatment would seem to be due to the appearance of an oxidative mask.
For white wines with terpene or fermentation aromatic characters, the oxygen given to the must is less risky aromatically speaking: the precursors and terpene aromas are not very sensitive to oxidation and the fermentation aromas cannot be oxidized since they have not yet been formed. However, in both cases, an excessive oxygen supply can lead to oxidative notes, which damage the final product profile. It is also essential to determine carefully the optimal dose of oxygen to give to the must.

It is also important to note that the difference brought about by the oxygen treatments are expressed more over time as described by Schneider (Schneider, 1998). The treated wines have a tendency to be «closed» in the first 6 months after the vinification, but later shown aromatic intensity greater than that found in the controls. This can be due to a more rapid oxidation in the controls, which have a higher polyphenol content. The timing of the tasting is hence very important for the aromatic profile evaluation and could explain the discrepancies found in certain previously cited publications.
Must oxygenation use in different vinification strategies

Must oxygenation techniques should not only be used for polyphenol rich musts. The first step to evaluate is the extraction step: it must be adapted to the initial must and it is especially important to avoid excessive extraction on oxygen sensitive musts (thiol). Adding sulphites to the harvest and must increases polyphenol extraction (Cheynier et al., 1989, Schneider, 1998, Singleton et al., 1980), as do all prefermentation macerations. The separation of the press juices is essential in order to segregate the musts that are initially poor in polyphenols and then the ones that are more concentrated such as the last press juices. The oxidation sensitivity of each fraction must be tested in order to oxygenate them accordingly. Oxygenation is applied only when necessary, free run juice is for example vinified without oxygen. The stabilized fractions can be assembled and this allows for:

- A maximum aromatic profile preservation in the wines by adapting the oxygenation.
- An addition of viscosity from the oxygenated juices without risking a premature oxidation of the blended juices.
- An increase in quality juice volume, which can be sold at a better price, by incorporating the press juices.

In reductive vinification practices that maintain a high phenolic content in the wines throughout the process, the risk of premature oxidation is very high (Schneider, 1998; Immelé, 2011). In this strategy which is often used for oxygen sensitive aromatic matrices (thiol potential harvests for example Sauvignon Blanc, Manseng, Colombard...), juice separation is critical: the free run juice and first press juices are protected from oxygen during the vinification if they are polyphenol poor and the press juices are oxygenated in a measured manner. If the free run and first press juices are polyphenol rich, then it is important to also treat them with some oxygen in order to prevent oxidation later. However, in this case, the herbaceous thiol profile wines (boxwood, tomato leaf) will undergo a change towards a more tropical thiol profile (grapefruit).

Oxidative vinification strategies are well suitable for all wines that have terpene and fermentation aroma profiles (white wines from Alsace or Burgundy...). The juice separation described earlier is applied; the two fractions are tested in terms of oxidation sensitivity and are subsequently treated accordingly, as described in other studies (Müller-Späth, 1977; Immelé, 2011). It is essential to avoid over oxygenation which can create a harmful oxidative mask.

To finish, must oxygenation can be used also in sulphite free vinifications (Immelé, 2011) since it can reduce early on the phenolic content and hence stabilize the wines against oxidation without the addition of sulphites.
Abstract

Grape must hyper-oxygenation was developed in the 1970’s in order to stabilize white wines and protect against oxidation. This technique was largely controversial considering the effects of this practice on the wine sensory profile, especially when used on musts such as Sauvignon Blanc which have thiol based aromatic profiles. This article presents the knowledge acquired after fifteen years of research about white grape must oxygenation practices on a number of white grape varieties from different countries (France, Spain, Italy, Portugal, Chile, Argentina, United States and Australia). These trials have demonstrated the efficacy of this practice in wine stabilization to protect against oxidation. However, it appeared necessary to adapt oxygen doses supplied in the must in order to optimize the effect on the mouth feel qualities and the wine aromas. A summary of the effects noted on the wine aromas is discussed with a particular focus on thiol type wines. In accordance with recent studies on this subject, it was shown that must oxygenation does not diminish the thiol concentration in the wines and can even cause an increase in the «thiol» characteristics identified during tastings. Finally, the value of this technique in different vinification strategies (reductive, oxidative, without sulphites) is discussed.

References