REDUCTIVE VINIFICATION OF WHITE AND ROSÉ WINES: THE QUESTION OF MUST EXTRACTION

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Carrying out reductive vinifications is a new trend, which is increasingly common in France and Europe for the production of white and rosé wines with an aromatic freshness that is respectful of the varietal characteristics. This type of wines, popular among today's consumers, is particularly suited to grape varieties rich in varietal aromas that are sensitive to oxidation such as Sauvignon, Colombard, Petit Manseng, Chenin, Gewürztraminer, or like Grenache, Cabernet Franc and Merlot with regards to rosé wines.
While the problem is well known, the measures applied today usually are either implemented too late, or their effects are not long-lasting enough to prevent oxidation reactions that have very fast reaction kinetics. The utilization of SO_2 with or without ascorbic acid, provides a partial solution only: the application rates are limited – which is a problem for highly SO_2-binding musts – its efficiency is low at high pH values, etc..

It is known that the natural grape antioxidants are very rapidly consumed in free-run juices, despite sufficient additions of SO_2 to the grapes in the hopper of the harvester and in the receiving bin (Delteil, 2001). This raises the question how grape compounds are to be protected against oxidation during crushing and/or the different steps of pressing, which often are the most critical with regards to must protection against oxygen. Hyper-reductive vinifications make use of new systems able to ensure the protection against oxidation, especially during must extraction, by using inert gases such as CO_2 and N_2 for example.

1.1 Principle of the oxidation phenomena

Oxygen is an essential element to life, providing its concentration remains under control. Indeed, high concentrations are extremely harmful, to such an extent that live organisms had to develop a series of protective measures against its active forms, which are responsible for oxidative damages. In addition, oxygen can induce the transformation and deterioration of beverages and foods. Thus, it is not surprising to see to what extent the contact of oxygen with wine during its production and ageing becomes a crucial factor regarding its final characteristics. The reduction of oxygen to water requires the progressive transfer of four electrons, leading to three highly reactive intermediate forms: superoxide anion radicals ($^{\cdot}O_2^-$), hydrogen peroxide (H_2O_2) and hydroxide radicals ($^{\cdot}OH$).

The management of oxygen contacts in red wine production can be rather flexible as red wines are protected by the presence of high levels of polyphenol antioxidants, which are able to neutralize the negative effects of free oxygen radicals (Rigo et al, 2000).
However, poor control of oxygen levels can be particularly problematic in white and rosé vinifications, because of the low antioxidant levels. Consequences of oxidation are the browning of juices because of enzymatic oxidation of the grape phenols (mainly phenols and flavanols). This leads to the formation of quinones, and then addition products between oxidized and non-oxidized phenols (Rigaud et al., 1990, 1991).
Without sulfiting, these oxidized juices produce heavy wines with little varietal character, specifically with Sauvignon (Dubourdieu and Lavigne, 1990). These enzymatic oxidation mechanisms of polyphenols can only be partially reversed by sulfiting the juice.
Thus, the choice of protection against oxygen is one of the main factors, which will define wine style (Figure 1).

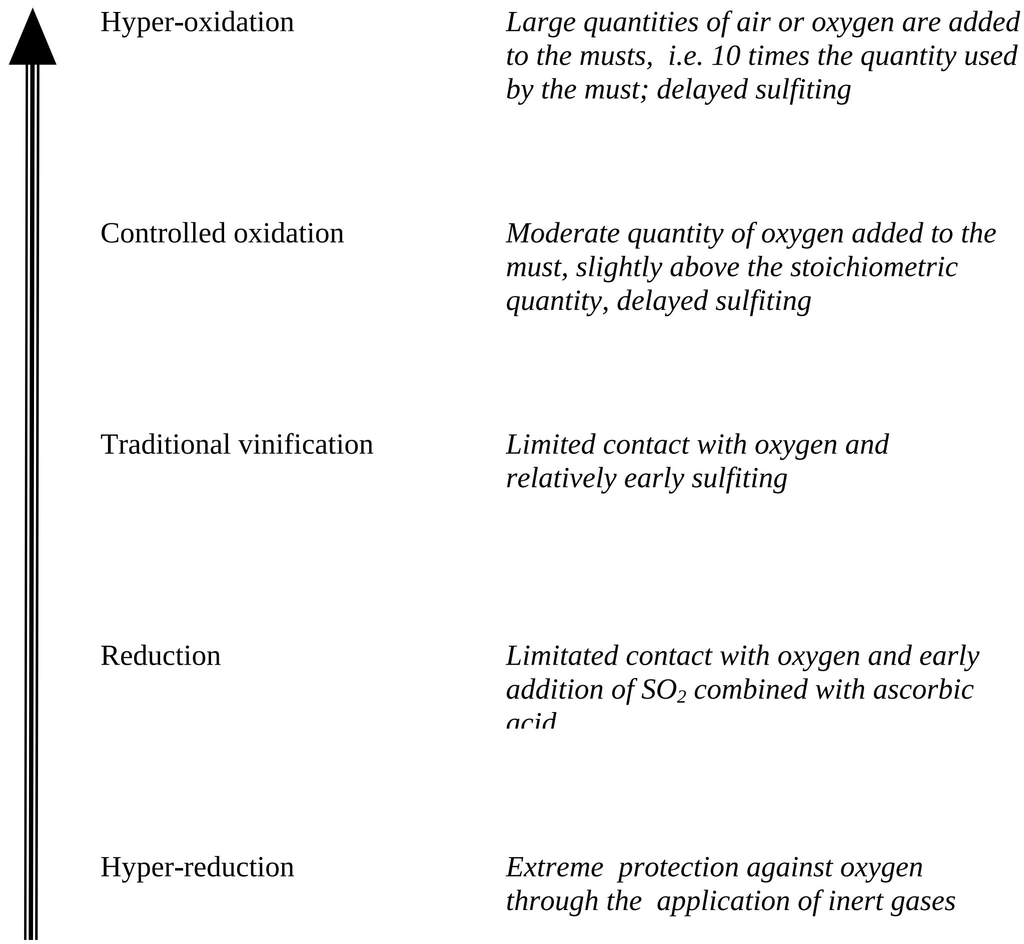

Figure 1: Interaction with oxygen during vinification of white wines

Over the years, winemaking practices have allowed the utilization of extremely different processes, from techniques achieving stabilization by oxidation, such as hyper-oxygenation, to reductive vinification methods. The choice of the initial vinification technique determines the suitability of the product to undergo successive treatments (barrel ageing, on-lees ageing, secondary fermentations, etc.), which have to be compatible with the initial choices, specifically for wines produced under reductive conditions as they have to be kept protected from oxygen during ageing.

1.2 The limits of current technologies

The techniques currently available are largely proven and allow to produce different styles of quality wines. Of course, there is no standard recipe for all the situations as each technique has specific limitations, which will be briefly presented in this chapter.
Oxidative techniques deplete wines because they remove the most oxidizable compounds of the grapes from the must at an early stage. Thus, they favour stability by removing compounds responsible for instabilities, this however, to the detriment of the varietal quality of the wines.

The various forms of traditional white wine vinifications allow to produce good quality wines, but not to fully express all varieties. In addition, the oxidative reactions invariably lead to the degradation of sulphur dioxide either through its oxidation, or indirectly through the formation of binding compounds. Thus, important sulphur dioxide additions are required for traditional vinifications in order to maintain active SO_2 levels, even if it were preferable to decrease its usage for health reasons.
Reductive vinifications with ascorbic acid only are not recommended since, initially, ascorbic acid decreases the redox potential, but then acts as oxidizer in the wine (Scarpa et al., 1983; Rigo et al., 1985 ; Halliwell, 1996).Thus, in addition to ascorbic acid, reductive vinifications effectively require the presence of sulphur dioxide, which is able to neutralize the reactive forms of oxygen.
However, the polyphenols present in wines can compete with sulphur dioxide since they can also react with superoxide anions, and then produce semiquinone radicals, which can lead to oligomerization reactions with a total antioxidant effect in the presence of oxygen (Bors, 2000). They can also react through a series of radical chain reactions (Singleton, 1987) producing hydrogen peroxide and an oxidizing effect. The hydrogen peroxide thus produced reacts as easily with polyphenols as with other wine compounds, for example by forming acetaldehyde through ethanol oxidation.
These polyphenol dependent auto-oxidation reactions can be detrimental during wine conservation and occur more rapidly at high pH values.

Consequently, it is common to control the concentration of polyphenols in white wines, particularly at high pH: high concentrations can be a potentially destabilizing factor, which can lead to browning and over-oxidation during conservation. No efficient remedy has been found for this phenomenon. In fact, while reductive vinifications allow to ensure a good protection against enzymatic oxidations during the prefermentary stage, it was observed that the simultaneous presence of ascorbic acid and sulphur dioxide does not guarantee a lasting total protection against oxidation. On the contrary, the presence of ascorbic acid may favour the transition from a protective to an oxidative phase during wine ageing (Peng et al. 1998).
Thus, the utilization of ascorbic acid is not recommended for wines intended for mid or long-term ageing.

1.3 The utilization of inert gases: the first steps

Because of the rapid degradation of grape anti-oxidants in free-run juices, the reductive vinification technique does not address the protection of grape compounds against oxidation during crushing and the different pressing stages, either. The hyper-reductive vinification technique applies new systems to ensure the continuing protection against oxidation through the application of inert gases such as $CO_{2,}$, N_2 among others, specifically during must extraction.

This allows to obtain individual pressings of high quality, and to ensure a satisfactory extraction of desirable organoleptic compounds, which are mainly located in the skin. The development of this technique and the evaluation of its application and utility in winemaking have not been studied extensively to date.

From the sixties and onwards, experimental presses had been developed in order to obtain non oxidized juices from entire harvests under a CO_2 cover (Martinière and Sapis, 1967).
However, the technological plans for white wine production had not been optimized yet and the pressing experiments under inert gas had not been pursued. During the nineties, the application of inert gases during harvest (mainly CO_2 in dry ice form) combined with pre-fermentation macerations have allowed to obtain non oxidized free-run juices characterized by a green colour. The varietal aroma expression of wines produced from these juices that are protected from enzymatic oxidation and sulphited, presents more elegance and often a better stability over time. Even if the free-run juice and first pressings are protected from enzymatic oxidation thanks to the preliminary application of inert gas by addition of dry ice to the grapes, the following pressings will rapidly show brown hues because of the massive exposure to oxygen.
Studies on the nature of Sauvignon blanc and other white varietal aromas (Petit and Gros Manseng, Chenin, Gewürztraminer, Arvine, Colombard) have contributed to understand the loss of typical aromas observed with wines produced from brown juices obtained from oxidized grapes. The typical aroma of wines produced from these varieties is due to highly odorous, volatile thiols found at trace levels. In grapes, the thiols are present as precursors conjugated to cysteine, and the metabolism of the yeast *S. cerevisiae* leads to the release of thiols during alcoholic fermentation (Ribéreau-Gayon et al., 2004). It has been well established that thiols, in particular, are highly reactive with regards to quinones of oxidized musts (Cheynier et al., 1986; Montero-Rodil, 2003). Thus, it is important to emphasize the benefits of reducing enzymatic oxidations of grape polyphenols by pressing with inert gases on the composition of musts and wines.

2 Pressing under inert gas cover: evaluation of an innovative technique

VASLIN BUCHER has developed pneumatic presses using inert gas cover systems for juices. This patented process, called INERTYS®, is the only one allowing to transfer gases entering or exiting the press without slowing down the operation. The inert gas is stored in a flexible reserve having the same volume than the press, and thus can be returned to the press regardless of the volume or the required flow rate. Consequently, this system offers the added advantage of allowing gas recycling.

The study presented below (Sartron C., 2004), investigated the efficiency of Sauvignon juice protection against oxidation during pressing with an experimental model. An analysis of the chemical composition of the grape juices, and a preliminary estimation of the quality of wines produced from the same grapes pressed either under nitrogen cover or without inert gas cover was performed.

2.1 – Principle of the INERTYS® pneumatic press

The tank of the press is connected to a flexible gas reserve through the juice reception tank. The “tank and juice reception tank” and the “juice reception tank and flexible reserve” components of the system are either connected or disconnected depending on the pressing stage. During pressing, there is a transfer of gas (nitrogen) between the press tank and the flexible reserve. The flexible reserve volume is equivalent to the tank capacity. This reserve, made of flexible PVC membranes, is installed next to the press. The juice, which is released during pressing, is automatically pumped into a juice reception tank. The crucial stage of the pressing procedure is the removal of all the oxygen in the press and the piping. This is the actual phase of inert gas flushing. The control of this phase ensures a very low level of oxygen, which reduces the rate of accumulation of oxygen in the inert gas of the reserve (increase of O_2 level is estimated to be 0.1% per pressing cycle). This allows to perform successive pressings without having to purge the reserve.

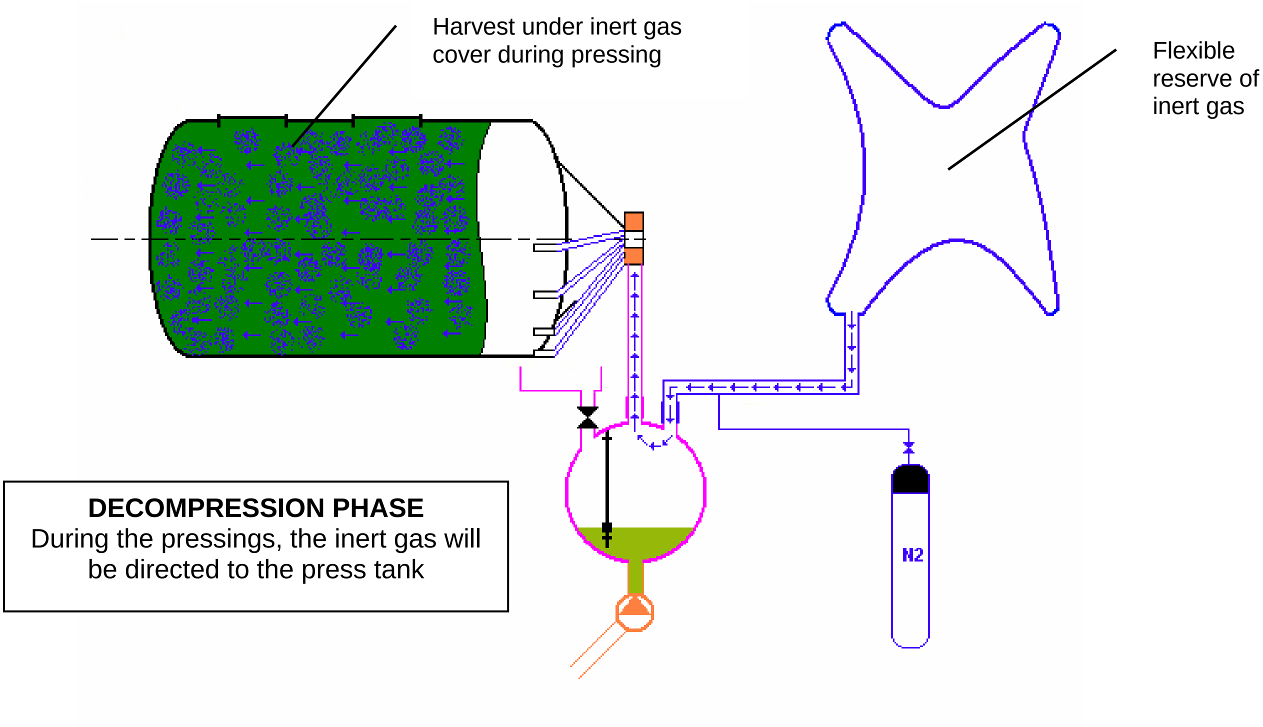

Figure 2: Illustration of the INERTYS® inert gas cover press system, Vaslin Bucher - 2006

2.2 Phenolic composition of juices and wines, levels of glutathione: experimental results

2.2.1 Bordeaux 2004

The study (Darriet et al., 2004) was carried out at the Domaine de Chevalier (Appellation Pessac- Léognan) with Sauvignon grapes during the 2004 vintage

The dissolved oxygen concentration (optical detection) in the musts was measured during the pressing cycles carried out either under nitrogen cover or exposed to the atmosphere. The measurements were carried out with juices treated with sulphur dioxide (5 g/hl) sampled at the outflow of the press. The measurements show that the levels of dissolved oxygen were consistently below 1 mg/l for juices pressed under nitrogen cover, whereas the levels found in traditionally produced juices were at least 3 mg/l. These measurements were probably afflicted by a downward bias with regards to the juices produced by traditional pressing since the grape polyphenol oxidase (PPO) rapidly degrades the oxygen present in pressed juice that have not been sulphited yet. However, in the first 100 litres of juice obtained with the press under nitrogen gas cover, dissolved oxygen levels of around 3 mg/l were also measured. This result seems to be due to the difficulty of removing all of the oxygen present in the entire harvest. Such complications should not occur with grapes that were destemmed and covered with inert gas before the transfer to the press.
The colour of the juices obtained from sulphited grapes pressed either under inert gas cover or exposed to the atmosphere confirmed the dissolved oxygen levels measured. The level of enzymatic juice oxidation was highly limited after pressing under inert gas cover. These juices kept a colour dominated by green and yellow, whereas the juices from traditional pressing had brown hues.

The levels of glutathione (a reductive grape peptide) were determined in the grape juices obtained with the different pressing treatments (Lavigne et al., 2003). Other measurements (phenols, optical density at 420 nm) were carried out by spectrophotometry (Figure 3).
The OD420 provides a measure for the intensity of the yellow colour of musts and thus the degree of oxidation of their phenolic compounds. In oxidized juices, glutathione was depleted since it had reacted with the quinones of the oxidized juices. On the other hand, in juices produced under nitrogen cover, the levels of glutathione were found at concentrations up to 40 mg/l in musts pressed under nitrogen cover and then sulphited.
At the same time, the amounts of phenols were higher in musts obtained from pressing under nitrogen cover because these compounds had not been oxidized and the OD at 420 nm confirmed these observations with higher values in oxidized musts.

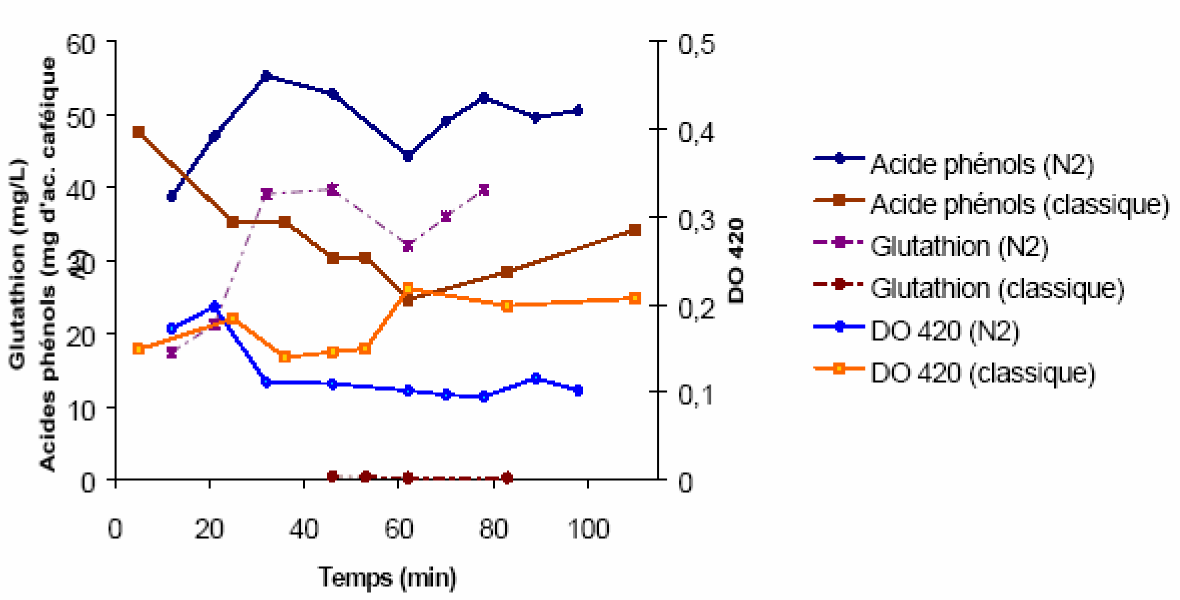

Figure 3 – Levels of phenols, glutathione and OD420 according to the pressing treatments, Darriet et al., 2004

Conclusion
All these determinations confirm the limited level of oxidation of the grape juices from pressing under nitrogen cover. They suggest a reduced reactivity of the grape polyphenols with the aromatic thiol compounds expressed during the alcoholic fermentation of the musts.
The future aromatic potential of the wines, which relies on the thiol concentrations, is thus protected.

2.2.2 – Trentin (Italy) - 2002

The following study (Vrhovsek et al., 2004) was carried out in Faedo (Trento) with Müller-Thurgau, during the 2004 vintage.

Its objective was the phenol composition of musts, perfect indicators of oxidative reactions. In fact, according to previous studies, it is known that:

- Caftaric and p-coutaric acids are the best indicators for the protection against enzymatic oxidation by the PPO, whose preferred substrates they are.
- p-Coutaric acid is mainly found in the skin and is the best indicator for skin extraction in order to prove the efficient protection against the possible oxidations of successive pressings.

The free run-juices were identical in the two studies (Figure 4), while the overall levels of caftaric acid derivatives were 2 to 4 times higher in the pressings protected from oxidation as compared with the control. The levels of p-coumaric acid derivatives were 2 to 5 times higher. Since free run-juices represent the major part of the total must, the latter as well as the wine had levels that were 25 and 60% higher as compared with the control, respectively.

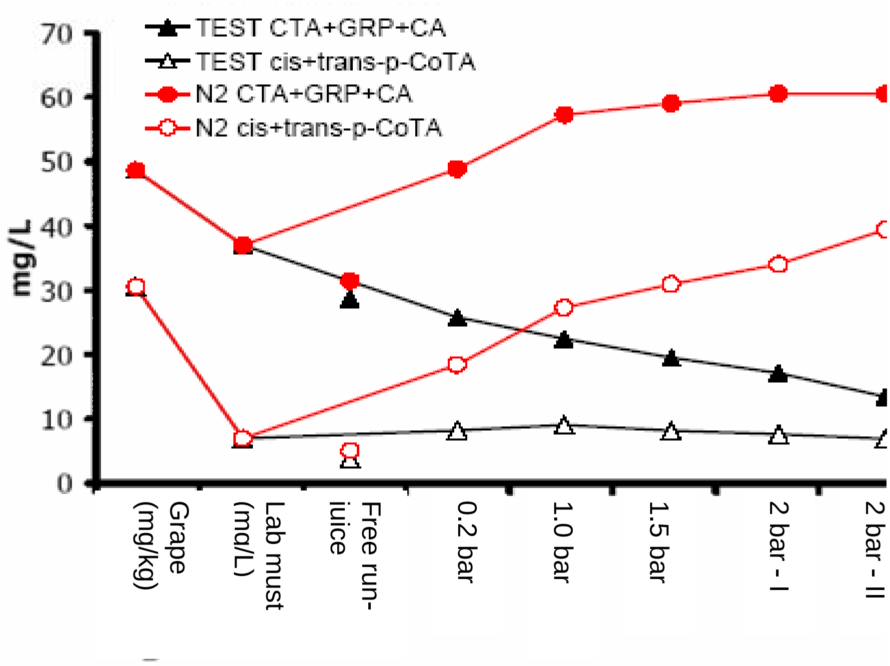

Figure 4: Total values of caffeic acid derivatives (trans-caftaric acid, CTA; 2-S-glutathionyl-caftaric acid, GRP; trans-caffeic acid, CA) and p-coumaric acid derivatives (cis+trans-p-coutaric, p-CoTA) in grapes and musts obtained in the laboratory under complete protection against oxidation, during the different phases of pressing – Vhrovsek et al., 2004

Conclusion

These analytical results thus confirm that pressing under inert gas cover not only allows to protect the oxidizable compounds of the must, but also to better extract these compounds. The tasting results demonstrated a significant difference between the wines produced with 15% of juice obtained from the final pressing under nitrogen cover (slightly oxidized juice), compared with those wines made with 15% of juice obtained from the final pressing of traditionally pressed grapes (more heavily oxidized juices).

Perspectives

According to the data available, a significant advantage of this technology is the decrease of SO_2 required to stabilize the wines since the sensitive compounds are not degraded through enzymatic oxidation during the pre-fermentation phase. These findings will be confirmed in additional studies. Besides, wines thus produced have a composition, which more closely resembles the composition of the grapes they are made of. Specifically, the wines are richer in antioxidant compounds whose health benefits are well known.

Current consumer demands increasingly prompt producers to adopt an industrial approach to vinifications, even at small scale. Applied in addition to sulfiting, must extraction under inert gas cover allows to avoid interrupting the "reductive" chain, in analogy to the cold chain essential for fresh agrifood products. Sulfiting is a precious ally, but it cannot provide a total control of white and rosé vinifications. In order to reach established product objectives, a particular attention should be paid to the pressing step.

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