Must and Wine Oxidation: Mechanisms and Biological Solutions

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Oxidation is a natural process where contact with oxygen, whether desired or not, plays a crucial role. This reaction can significantly influence the character and quality of wine. When managed with precision, oxidation can enhance the complexity and depth of a wine, contributing to its unique bouquet and flavor profile. However, if not carefully controlled and limited, oxidation can lead to undesirable aromas and flavors that detract from the wine's intended expression.

It is generally agreed that a slow input of oxygen during ageing helps the wine to develop and reveal its aromatic potential, but at some point, the oxygen becomes detrimental and starts to overwhelm the wine with the appearance of "oxidative notes" described as bruised apple, buttery, curry spice or hazelnut.[1]

Understanding oxidation mechanisms helps to manage the phenomenon and use appropriate solutions to prevent oxidative damage. Traditionally, chemicals such as sulfites (SO_2) and ascorbic acid are used to prevent oxidation in winemaking, but the consumers preference to have less chemicals used in their wines motivates researchers to explore new natural strategies such as selected active non-*Saccharomyces* yeast or specific inactivated yeast derivatives. Indeed, these oenological tools help limit the oxidative risk.

This paper provides an updated state of the art on wine oxidation and how biological solutions reduce the oxidative risk in the early stages of winemaking as well as preserve wine quality after fermentation until bottling (including ageing, storage, transfers, stabilization operation, transportation...).

1. UNDERSTANDING OXIDATION

Oxidation occurs when oxygen is present in the grape juice in quantities that are detrimental to wines. Some wines are more sensitive to oxidation such as aromatic whites like Riesling, as well as thiolic varieties such as Albariño, Verdejo and Sauvignon blanc. Red wines are less sensitive to oxidation because of the higher abundance of phenolic compounds, which are natural antioxidants. The presence of antioxidants in the juice and the wine, whether naturally present or added, is one of the key determinants for protection against this phenomenon.

1.1 Types of antioxidants

Antioxidants in oenology are compounds that contribute to limit the production of oxidants. Some technical processes help to limit oxidation, for example the use of inert gas or some subtractive methods which limit the presence of easily oxidisable polyphenols or metals in the juice/must or the wine. Chemical antioxidants such as SO_2 and ascorbic acid are also widely used. Biological antioxidants (e.g. specific or selected active or inactivated yeast), are also used to increase juice or wine antioxidant capacity.

There are two distinct families of compounds known as antioxidants:

> **Reductant compounds**. Chemically, a reductant is a compound transferring one or several electrons to an oxidant (which lacks electrons). This reaction is called the reduction of the oxidant. The reduction of one compound leads to the oxidation of the other compound (Figure 1). Once reduced, the compounds can again undergo an oxidation reaction with other oxidants present in the medium. Depending on the redox potential, defined as the dynamic equilibrium between oxidants and reductants in a given wine, a specific compound can be either an oxidant or a reductant. One of the well known chemical reductants used in winemaking are ascorbic acid and sulfites.

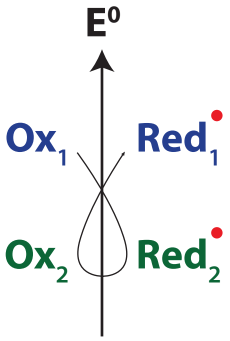


Figure 1: Coupled reaction of Ox_1/Red_1 with Ox_2/Red_2 according to the electrochemical potential (also called redox potential, E^0) of each species.

Nucleophilic compounds. Nucleophilic compounds (or compounds with nucleophilic property, or nucleophiles) have two electrons available to share with an electrophilic compound (which lacks of electrons). This reaction leads to the production of a new compound with a moiety from the electrophilic compound and another moiety from nucleophile and they are bound by a covalent bond (Figure 2). These compounds stay in the juice/wine and are less reactive than the initial electrophile, which contribute to stabilize the solution. One of the most well-known chemical nucleophilic compounds in winemaking is glutathione (GSH) which can trap oxidized caftaric acid (the electrophile) to form grape reaction product (GRP).

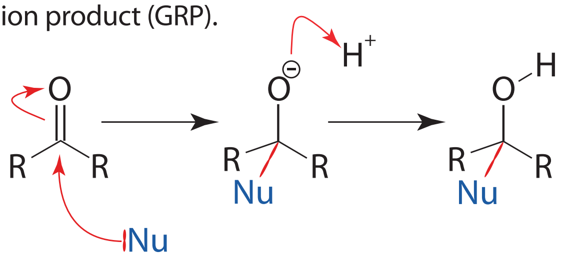


Figure 2: One step nucleophilic addition of a model nucleophile (Nu) on carbonyl function in acid condition

These two families of compounds, reductants and nucleophiles, have the capacity to limit the impact of oxidants by different and complementary mechanisms. Depending on their concentration in juice and wine, it will influence their oxidation sensitivity.

1.2 Oxidation risk and impact

The oxidative risk appears when the juice, or the wine, becomes unbalanced with more oxidant compounds compared to antioxidants. The most readily oxidisable compounds are polyphenols. When there is an accumulation of oxidized polyphenols, (also called quinones) it impacts wine quality in three ways, which all are related to the electrophilic / oxidant activity of quinones towards other wine components (Figure 3).

Browning. When quinones are formed, they can react with other grape polyphenols. Reaction of quinones with these polyphenols could appear successively in several cycles and form a large polymer resulting in a brown tint to the wine. This defect is especially visible in white and rosé wine but also occurs in red wine.[2]

Off-flavor production. Free amino acids from the juice can react with quinones to form the corresponding aldehydes. The accumulation of certain aldehydes contribute to the appearance of aged off flavors such as bruised apple or hazelnut.[3]

Loss of varietal aromas. Some families of aromas, such as thiols, are sensitive to oxidation since their free sulfhydryl function (-SH group) possesses nucleophilic properties (see section 1.1).Thus, these compounds can quickly react with quinones (electrophilic compounds) forming a compound without aromatic properties leading to a decrease in wine aromatics.[4]

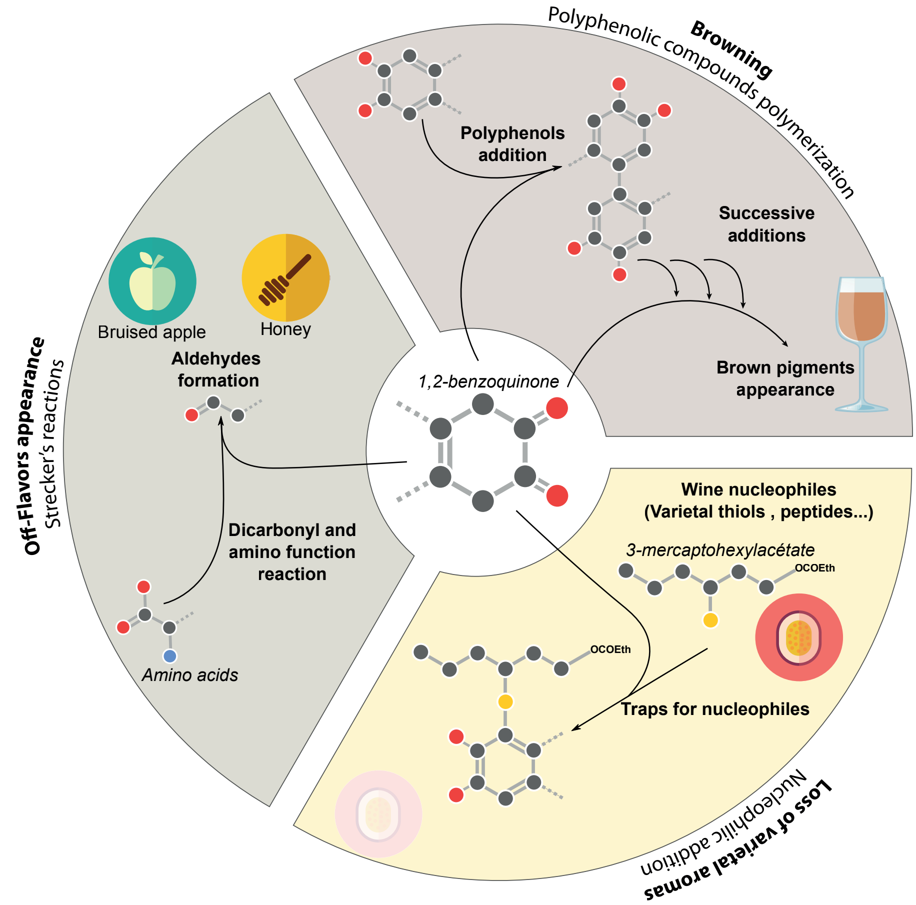


Figure 3: Main reaction of 1,2-benzoquinones (= quinone, oxidized polyphenols) on wine components leading to appearance of oxidative defects.

2. PRE-FERMENTATION OXIDATION

Must oxidation is the result of the production of quinones arising from the oxidation of polyphenols with oxygen, catalyzed by enzymatic activity.

2.1 Oxidation from enzymatic activity

Enzymes are naturally present in healthy grapes and in the juice. The generic group of enzymes responsible for oxidation are the polyphenol oxidases which convert monophenol or diphenol function of polyphenols into carbonyl (active function of the quinones). In grapes contaminated with *Botrytis*, in addition to the polyphenol conversion, *Botrytis* also produce some specific laccase enzymes which can oxidize polyphenols (Figure 4) increasing the risk of over production of quinones, in the presence of oxygen.

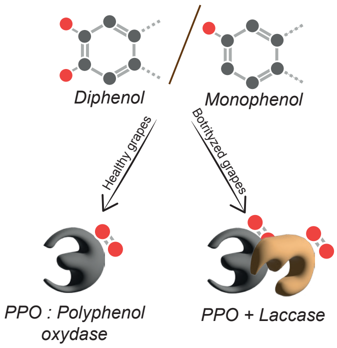


Figure 4: Both monophenol or diphenol function from polyphenol can be oxidized by enzymes polyphenol oxidase or laccase in the presence of oxygen.

Once oxidized, the quinones can polymerize with polyphenols, this latter can then go through another cycle of enzymatic oxidation. This cycle reaction leads to the production of highly polymerized polyphenols which could produce brown pigments or precipitate (Figure 5).[5]

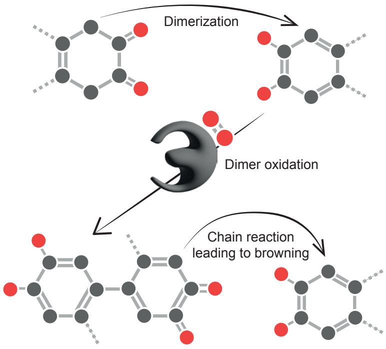


Figure 5: Quinones reacting with polyphenols and become a substrate to enzymes for another cycle of oxidation. This cycling reaction leads to polymerization of polyphenols.

2.2 Strategies to protect from enzymatic oxidation

Several strategies are possible to reduce juice oxidation due to enzymatic action in the presence of oxygen. The most common strategies are to reduce the concentration or the activity of the enzymes, quickly remove the oxygen, or to trap the quinones early to interrupt the oxidation cycle and before leading to the appearance of brown pigments. We will mainly focus on the biotechnological solutions efficiently applied to consume the oxygen and trap the quinones in the juice.

2.2.1 Active oxygen consumption

Limitation of oxygen in the juice can be achieved by flushing out the air during the winemaking process with inert gas. Another solution is to quickly consume the oxygen dissolved in the juice after pressing. After pressing or during settling, the use of an active microorganisms specifically selected for their fast oxygen consumption rate and non fermentative properties (LEVEL2 INITIA™) is very effective as it was selected for its high capacity to consume oxygen in addition to its microbial bioprotection. It results in a limitation of the polyphenol oxidation since the dissolved oxygen is decreased in the juice, leading to a dimished oxidative damage such as browning (Figure 6)[6]. *Metschnikowia pulcherrima* yeasts do not have the ability to efficiently absorb lipids (polyunsaturated fatty acids and phytosterols) from grapes and must therefore synthesize these lipid compounds. Incorporation of lipids into the membrane is essential for their survival. The synthesis of fatty acids, such as linoleic and linolenic acid require substantial consumption of oxygen, which explains this unique feature. Further more, we characterized different *Metschnikowia pulcherrima* strains which showed that there is a large variability in regard to their dissolved oxygen consumption capacities (quantity and rate) within the different strains of this specie and LEVEL2 INITIA™ was the most effective.

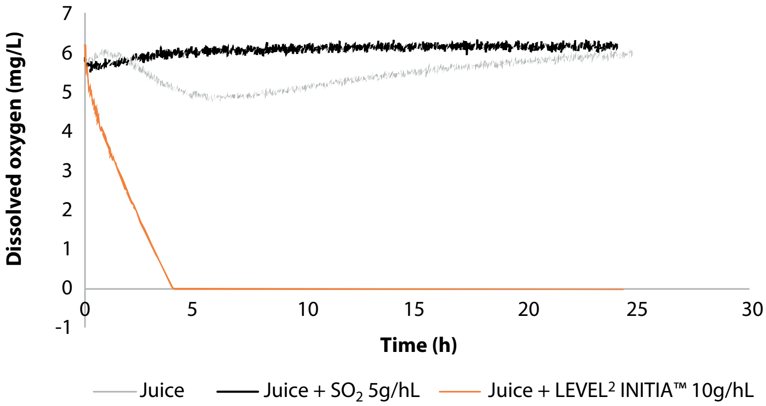


Figure 6: Evolution of dissolved oxygen in juice during the first 24 hours before fermentation with or without addition of sulfites (chemical protection) or LEVEL² INITIA™ (biological protection).

2.2.2 Quinones trapping

The use of inactivated yeast with guaranteed glutathione level (G-IY) helps winemakers to enrich the juice in nucleophiles in the early stage of the winemaking and limits the use of chemical antioxidants such as sulfites. Our specific G-IY, GLUTASTAR™, has been specifically developed to accumulate and release in the juice, the highest concentration of glutathione. But at the same time, the uniqueness of this inactivated yeast strain is its richness in other accumulated nucleophilic compounds which contribute to protecting the juice as well as the glutathione itself. Nucleophiles limit the browning of the juice by limiting the polymerization of the polyphenols by reacting with quinones (Figure 7). Finally, the nucleophiles present in GLUTASTAR™ improve and/or preserve the natural antioxidant capacity of the wine, which leads to a better preservation of sensitive compounds (such as varietal thiols) (Figure 8).[7]

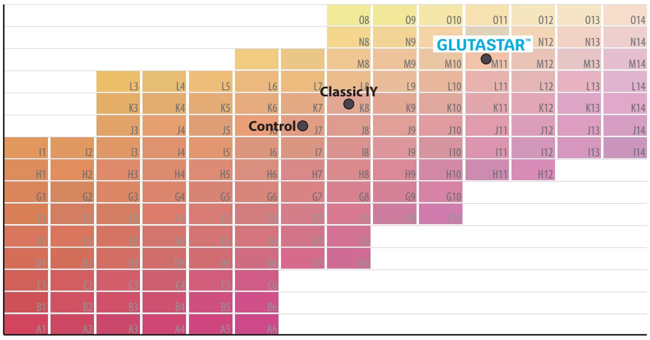


Figure 7: High nucleophilic GLUTASTAR™ better preserves a light rosé wine color compared to a classic inactivated yeast or a control. (Figure adapted from the Centre du Rosé, France)

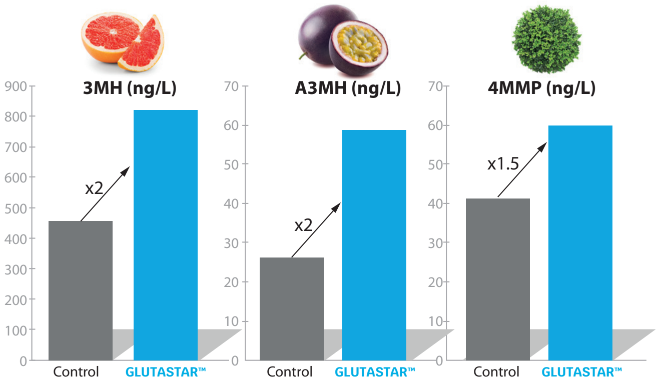


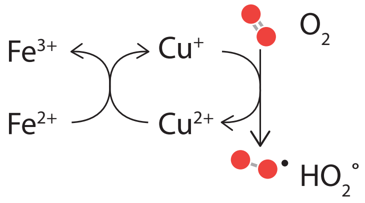
Figure 8: Varietal thiols concentration in Sauvignon blanc from Val de Loire (FR) with the use of Glutastar™ in prefermentative stages compared to a control. 3MH, 3 mercaptohexanol; A3MH, acetate 3-mercaptohexanol; 4MMP, 4-mercaptopentan-2-one.

3. POST-FERMENTATION OXIDATION

3.1 Iron and copper catalyzed oxidation

Once the fermentation is finished, most of the enzymes are inactive. In this case, the oxidation becomes mainly driven by the presence of oxygen and metals. Oxygen does not react directly when in solution in wine, however, iron and copper act as electron donors to reduce the oxygen into other species, called reactive oxygen species (ROS) (Figure 9).

Figure 9: Electron transfer from metal to oxygen to form hydroperoxyl radical (ROS).



The ROS generated during the reduction of the oxygen can act as electron donor to polyphenols, leading to the production of oxidized polyphenols with the transfer of 2 electrons from ROS and the metal moiety, resulting in a semi-quinone then a quinone, and a coupled reduction of oxygen to hydroxyl radical. The hydroxyl radical being highly instable, reacts quickly with the ethanol to form ethanal (acetaldehyde) and water, which is the last step of the reduction of the oxygen (Figure 10).[8]

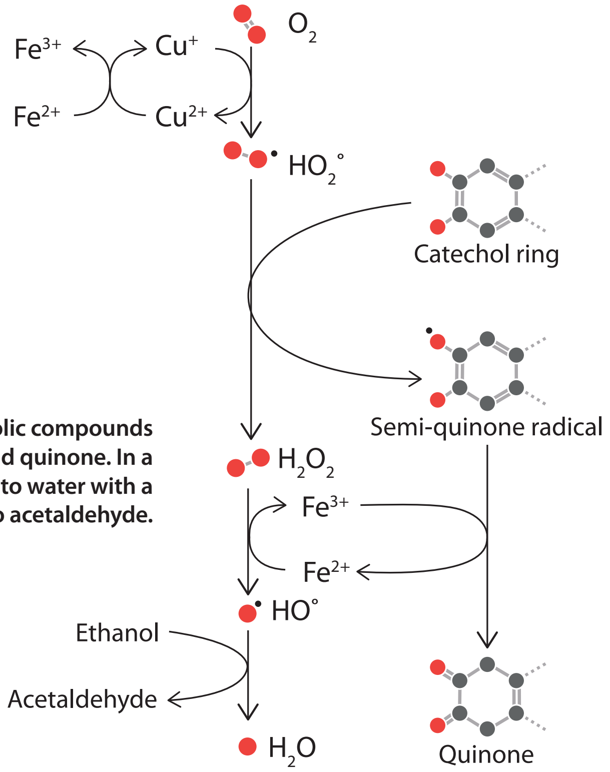


Figure 10: Electrons are transferred to phenolic compounds to produce successively semi-quinone and quinone. In a parallel reaction, oxygen is fully reduced to water with a last step involving oxidation of ethanol to acetaldehyde.

3.2 Strategies to protect wine from chemical oxidation

In wine, the strategies to manage oxidation are different than in juice because it involves different mechanisms, and as there are minimal enzyme activities associated with the reactions, we have to look beyond the enzyme control.

3.2.1 Managing metals

Decreasing the concentration of metals (copper and iron) can be of first importance to limit the browning and aromas loss, by limiting the first step of the oxidative cascade. The trapping of copper and iron at early stages of winemaking by selected non-*Saccharomyces* wine yeast such as LEVEL^2 INITIA™ or LEVEL^2 GUARDIA™, slows down the appearance of ROS and thus oxidation defect in the wine (Figure 11). These 2 different strains of *Metschnikowia pulcherrima* have been selected for their ability to scavenge copper (LEVEL^2 INITIA™), or iron (LEVEL^2 GUARDIA™).

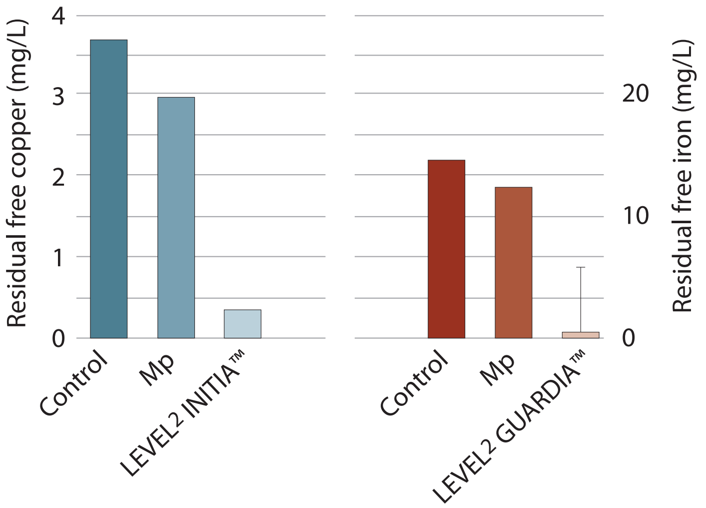


Figure 11: Trapping capacity of LEVEL^2 INITIA™ or LEVEL^2 GUARDIA™ to trap metals compared to another strain of *Metschnikowia pulcherrima* (Mp).

3.2.2 Progressive oxygen consumption

Ageing wine on lees preserves the wine from oxidative damage. One of the postulates is the capacity of yeast lees to consume oxygen actively (from residual enzymatic activity) or passively with specific cellular structures. In collaboration with INRAE, we developped a specific inactived yeast (PURE-LEES LONGEVITY™), highly efficient to consume 1 mg/L dissolved oxygen when 20 g/hL of PURE-LEES LONGEVITY™ is added (Figure 12)[9]. The consumption of oxygen decreases the production rate of radical oxygen species and thus limits the appearance of oxidative damage on the wine during ageing.

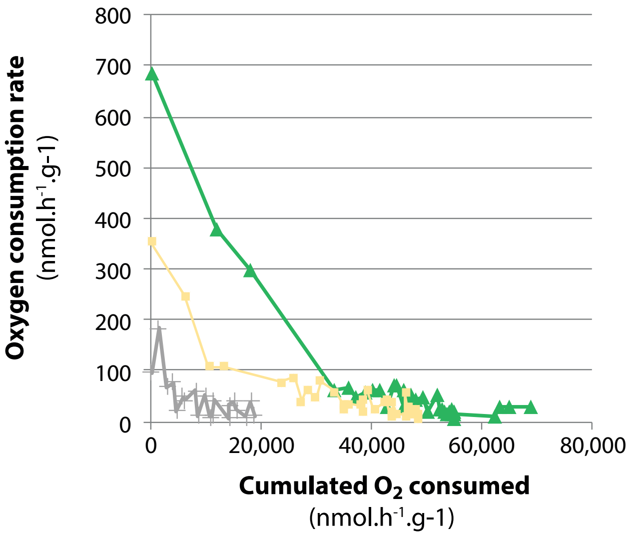


Figure 12: Oxygen consumption rate of different yeast derivatives, one classical inactivated yeast (yellow squares), one cell walls (grey cross) and PURE-LEES™ LONGEVITY (green triangle).

3.2.3 Quinones trapping

As well as in juice, the quinones can be trapped by nucleophilic compounds present in the wine. These nucleophiles are naturally present in the wine (result of the fermentation of the juice, autolysis, ...) or can be added by using specific inactivated yeast either in juice or directly in wine. You can then enrich your wine with yeast nucleophiles to improve the wine antioxidant capacity. The mechanism of action in that case is the same as in juice, nucleophiles trap the quinones and form a new nucleophile-quinone product which stays in the wine. These new compounds can (or not) react with another quinones or can be a substrate of oxidation by radical oxygen species. In this way, the nucleophiles you add to your wine will protect the compounds such as thiols.

4. FUTURE TRENDS

Consumers are more and more aware of the impact of the food on their health, which drives the needs for professionals to decrease the amount of chemical input and offer biological solutions.

Several research projects have focused on selected non-*Saccharomyces* with biocontrol properties including natural antioxidant capacities for protecting juice against oxidation. Our projects also focus on yeast fractions from specific inactivated *Saccharomyces* yeast, as they have shown that they can control oxidation phenomena. The uniqueness of non-*Saccharomyces* yeast leads to investigate new fractions from those microorganisms for specific winemaking applications[10], recently approved for use by OIV. Future research is directed in the exploration of new yeast biodiversity, from different environments to understand their specificity and how winemakers can take advantage of these microorganisms in active or inactivated form, that can be considered as processing aids, to potentially replace additives that must be labelled.

5. CONCLUSION

The antioxidant capacity of a wine is determined well before the end product is ready for bottling, from the vineyard and during the winemaking process. Lack of oxidation management could induce defect appearance early in the wine's life and reduce perceived wine quality by consumers (color and aromatic quality loss)

Oxidized polyphenols (quinones) are at the centre of many pathways leading to oxidative defects. The management of these quinones, before or after their production, is the major lever to protect wine from oxidation. Limiting the production of quinones can be firstly achieved by depleting the medium in metals with LEVEL^2 INITIA™ or LEVEL^2 GUARDIA™, another possibility is by consuming oxygen actively (selected microorganisms LEVEL^2 INITIA™) or passively (oxygen consumption by specific inactivated yeast such PURE-LEES LONGEVITY™), or finally by trapping the quinones with nucleophiles added by specific inactivated yeast with high nucleophilic content (GLUTASTAR™). All these biotechnological solutions can work in synergy and it is possible to combine the early consumption of oxygen (LEVEL^2 INITIA™) with the release of nucleophiles (GLUTASTAR™) to control oxidation (Figure 13).

The efficiency of our natural solutions is due to the combination of the extensive characterization we do on our inactivated yeast's fractions or our active non-*Saccharomyces* yeast and the understanding of their mechanisms of action during winemaking.

Today, with our expertise in juice and wine oxidation, our R&D team develops efficient biological solutions to support the natural antioxidant capacity of the white or rosé wines, to improve the overall quality of the wine and increase freshness of the final product. These tools are powerful opportunities for winemakers to reduce or replace traditional chemical input.

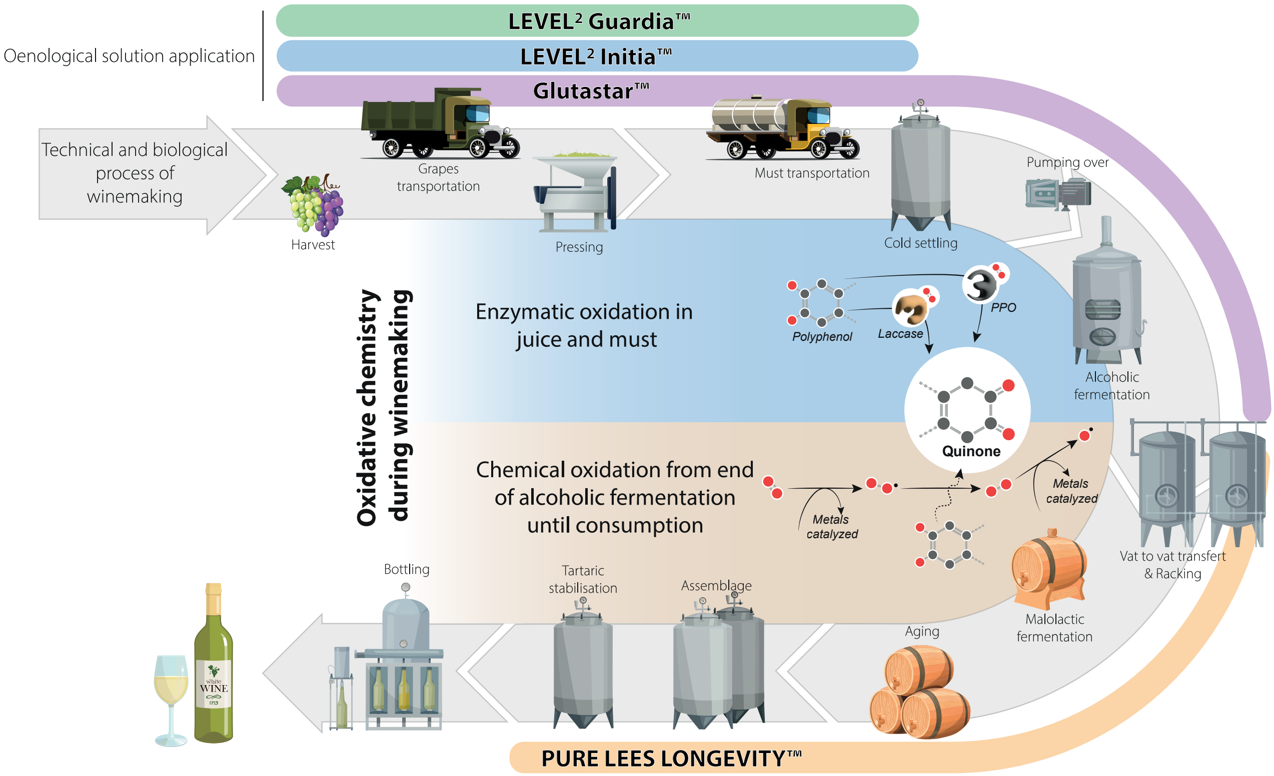


Figure 13: Summary of the winemaking process with the main mechanism of oxidation (i.e. enzymatic or chemical) respectively before and after the completion of the alcoholic fermentation.

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