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OXYGEN INTAKE AND COLOR EVOLUTION IN SAUVIGNON BLANC AND MUSCAT OTTONEL WINES TREATED WITH ASCORBIC ACID AND GLUTATHIONE

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Abstract

Understanding of antioxidants role in the wine matrix is an important aspect when it comes to sensory quality and conformity of the product. This study aims to compare the effects of sulphur dioxide (SO_2) as a conventional antioxidant, alone or in combination with ascorbic acid (AA), in the presence or not of reduced glutathione (GSH) in white wines from Sauvignon blanc and Muscat Ottonel grape varieties. The results demonstrate that consumption of oxygen occurs differently, mostly according to the phenolic composition of the variety rather than the antioxidants added fortheir protection. Sauvignon blanc wines are believed, and our results proved it, to be more sensitive to chemical oxidation due to certain polyphenols that may act as a good substrate for oxidation. On the other hand, Muscat Ottonel wines seem to have more resistant polyphenols to oxidation, while the additional presence of higher concentrations of terpenes may improve this resistance due to their ability to act as antioxidant substances as well. Regarding color intensity values, which are desirable to be low in white wines, we observed that the smallest values were achieved in wine samples treated with sulphur dioxide with or without glutathione addition. Conversely, wines treated with ascorbic acid had the most intense color, due to the oxidation of polyphenols, irrespective of grape variety. The samples treated with combinations of sulphur dioxide, glutathione and ascorbic acid showed increased color intensity as compared to samples treated only with glutathione, but not to the same extent as in the case of samples treated with ascorbic acid alone. Knowing that the main tool of the winemaker for the protection of aroma compounds and polyphenols remains the use of antioxidant supplementation, a better choice was proven to be the addition of glutathione in the presence of a moderate concentration of SO_2. Precautions should be taken when using ascorbic acid as antioxidant, because the depletion of SO_2 is fast, and then ascorbic acid acts as pro-oxidant, releasing hydrogen peroxide.

Key words*: glutathione, ascorbic acid, chemical oxidation.*

INTRODUCTION

The use of antioxidants in wine industry is crucial for the quality of products, especially when it comes to the preservation of aroma and colorcompounds. Many antioxidants have been tried, but no one yet was able to fulfill all functions sulphur dioxide exhibit in wine aside that of antioxidant, such as antiseptic, antioxidasic, clarifying agent, acidifying agent, good solvent for maceration and capacity of carbonyl compound binding (Waterhouse et Laurie, 2014; Danilewicz et al., 2008; Ribéreau-Gayon et al., 2000). World Health Organization recommendsan intake of maximum of 0.7 mg/kg body weight/day of sulphur dioxide. Due to concernsregarding its toxicity, the researchers are looking for alternative ways to preserve the aroma compounds andpolyphenols. An alternative is ascorbic acid (AA), but it was found out that in the absence of sulphur dioxide it actually acts as pro-oxidant, leading to the formation of hydrogen peroxide (Bradshaw et al., 2003; Scollary, 2002). Recently, studies shifted toward a new antioxidant molecule, the reduced glutathione (GSH), which is a powerful cellular antioxidant and metabolic regulator, found in most living organisms, including grapes (Cheynier et al., 1989; Badea et Antoce, 2015). Lately, experiments performed with both ascorbic acid and glutathione have revealed that under some circumstances they may act as pro-oxidants in the wine matrix, but even so there are some expectations regarding the usage of glutathione (Wegmann-Herr et al., 2015).

As already mentioned, ascorbic acid alone is a powerful antioxidant, but in order to be used

efficiently it is important for wine to be protected with sufficiently high concentrations of sulphur dioxide, to prevent the oxidative damage due to the formation of degradation products of dehydroascorbic acid (DHA) in acidic mediumand hydrogen peroxide release (Bradshaw et al., 2003; Scollary, 2002).When sufficiently high doses of sulphur dioxide are present, hydrogen peroxide is neutralized to sulphate anions, protons and water, while dehydroascorbic acid (DHA) binds with sulphur dioxide on one ketone group to form an adduct (Addams, 1997). Other authors noted too that ascorbic acid degradation products are weak binders of sulphur dioxide under winemaking conditions (Barril et al., 2012).

In the cases of low sulphur dioxide, none of thesechemical mechanisms work and even if the hydrogen peroxide is completely neutralized, DHA will undergo multiple steps degradation to by-products that modify the wine color and aroma compounds. The resulted DHA degradation products lead to the yellow pigment formation (Scollary, 2002). Recent observations showed that wine treatment with ascorbic acid under oxidative conditions could lead to the formation of xanthylium cations, when flavan-3-ols are linked together through glyoxylic acid or by-products resulted from dehydroascorbic acid degradation (Wegmann-Herr et al., 2015). Glyoxylic acid is a compound derived in wine from tartaric acid oxidation, catalyzed by transition metals, and is involved in flavan-3-ols condensation (Fulcrand et al., 1997). It was later demonstrated that flavan-3-ols condensation through carboxy-methine bridges occurs faster in the presence of copper (II) cations (Clark et al., 2003).

However, it was revealed that glutathione could inhibit to some extent the condensation of flavan-3-ols mediated by glyoxylic acid or dehydroascorbic acid degradation products, but, in the same time, it was also found it increased the levels of acetaldehyde in wines (Wegmann-Herr et al., 2015). Acetaldehyde behaves similarly regarding the condensation of flavan-3-ol molecules, but the reaction is faster with glyoxylic acid than with acetaldehyde, while when both are present, the reaction takes place even faster (Drinkine et al., 2005). Thus, contradictory results obtained by different authors may be explained by the competitive addition of both acetaldehyde and glyoxylic acid molecules on C8 position to flavan-3-ol, the result being dependent on their ratio and concentration.

The result of acetaldehyde mediated polymerization of flavan-3-ols is a colorless adduct, methyl-methine-linked-flavan-3-ols (Oliveira et al., 2011). Methyl-methine linkages are sensitive to acid-catalyzed cleavage and generate vinyl-flavan-3-ols (Oliveira et al., 2011; Cruz et al., 2008; Asenstorfer et al., 2001; Es-Safi et al., 1999), which are also reactive.

In wines treated with ascorbic acid a dramatically increased consumption of SO_2 was observed, accompanied by further degradation of dehydroascorbic acid, which led to yellow pigment formation, while no pigment could be detected in treated wines only with SO_2 (Scollary, 2002).

Another important aspect regarding wine oxidation is the speed of consumption of dissolved oxygen in the presence of specific antioxidant combination. Some studies showed that SO_2 is the most active in consumption of oxygen, but ascorbic acid is effective as well, while glutathione proved to be less effective in oxygen scavenging processes (Comuzzo et al., 2015). However, the reaction of molecular oxygen with antioxidants and organic molecules is ‘spin forbidden’ due to the arrangement of electrons in molecule and before any reaction occurs, a conversion from the lowest energy oxygen (ground state) to a higher energy (excited state) is absolutely necessary (Scollary, 2002). In chemical oxidation of wine, activation of molecular oxygen is done by transition metals even if their concentration is in trace amounts (Scollary, 2002).

MATERIALS AND METHODS

Sauvignon blanc and Muscat Ottonel wines were produced in 2014 harvest from Dealu Mare region of Romania. Both wines were produced with a short maceration on skins of about twelve hours at 14°C and the resulted musts were decanted at 10°C using 4 g/hl pectolytic enzymes. Acidity of both musts was corrected with 2 g/l tartaric acid. Alcoholic

fermentation was carried out at controlled temperature of 12-14°C. Protein stability was acquired through fining with 100 g/hl Na-bentonite. Potassium hydrogen tartrate stabilization was carried out through cold and static contact with seedling crystals at -4°C two weeks with daily agitation.

Bottling process of experimental samples was carried out under oxidative conditions with a small gravitational filler. Temperature of wines during filling was about 4˚C to enhance the absorption of oxygen to saturation. Both wines were oxygen saturated to ~8.5 mg/l O_2 during filling and due to the bottle headspace. Control samples were made by simply filling 0.75 liters bottles with wines described above, while the other samples were prepared after filling, by adding various doses of GSH and AA using freshly prepared solutions applied with micropipettes.

Prepared experimental samples description and codification are summarized in Table 1.

Table 1. Description and codification of experimental samples

*Groups	Experimental samples	Treatments	
		Glutathione (GSH), mg/l	Ascorbic acid (AA), mg/l
CT	CT	0	0
GSH	GSH10	10	0
	GSH20	20	0
	GSH30	30	0
	GSH50	50	0
	GSH100	100	0
GSH: AA	AA30GSH10	10	30
	AA30GSH20	20	30
	AA30GSH30	30	30
	AA30GSH50	50	30
	AA30GSH100	100	30
AA	AA30	0	30
	AA40	0	40
	AA50	0	50
	AA60	0	60
	AA70	0	70

*CT - control samples; GSH - glutathione treated samples; AA - ascorbic acid treated wines; GSH: AA - glutathione and ascorbic acid treated wines.

After a period of storage of 4 months, samples were analyzed by measuring color intensity and residual dissolved oxygen to better understand the oxidation mechanisms and assess the chemical behavior of GSH and AA in wines treated with moderate SO_2 under oxidative filling conditions.

Spectrophotometric measurements were performed in 10 mm optical path quartz cuvettes with a UV–VIS double beam spectrophotometer Specord 250 from Analytik Jena running WinAspect software, version 2.2.7. Data analysis wasperformed using ANOVA functions in Origin 10.0 software.

Dissolved oxygen was measured with a NOMA*Sense*O_2 Prime analyzer based on the principle of luminescence technology, using an invasive Pre*Sens* oxygen dipping probe.

Control wine physico-chemical analyses before filling are included in Table 2.

Table 2. Physico-chemical analyses of control wines

Physico-chemical analyses	*Variety		Method
	SB	MO	
Free SO_2, ppm	35	42	OIV-MA-AS323-04A
Total SO_2, ppm	86	80	OIV-MA-AS323-04A
Total acidity, meq/l	80.0	80.0	OIV-MA-AS313-01
Volatile acidity, meq/l	4,80	4.40	OIV-MA-AS313-02
% vol. alc.	14.5	13.1	OIV-MA-AS312-01A
Reducing sugars, g/l	1.20	1.23	OIV-MA-AS311-01A
Specific gravity 20°C	0.9912	0.9902	OIV-MA-AS2-01A
Non-reducing extract, g/l	19.80	20.20	OIV-MA-AS2-03B
Total dry extract, g/l	21.20	21.43	OIV-MA-AS2-03B
ITP, AU (10 mm)	2.1555	2.0920	ITP = D_{280}
Dissolved O_2, ppm	8.13	8.75	Noma*Sense* - App. note

*SB - Sauvignon blanc; MO – Muscat Ottonel.

RESULTS AND DISCUSSIONS

After 4 months from bottling samples were opened and the remaining dissolved oxygen determined.

The means of oxygen determinations for groups of samples treated with AA, with GSH and with a combination of AA and GSH show significant statistical differences in the case of Sauvignon blanc wines (Figure 1), but insignificant statistical differences for Muscat Ottonel wines (Figure 2).

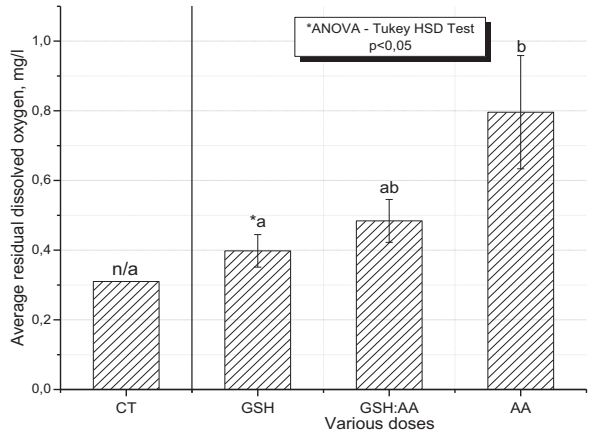

Figure 1. Average residual dissolved oxygen in Sauvignon blanc wines treated with various doses of reduced glutathione (GSH), ascorbic acid (AA) and mixtures of GSH and AA

This situation can be explained by the differences in the polyphenols type of the grape

variety, Sauvignon blancbeing more sensitive to oxidation. Muscat Ottonel wines appear to be more resistant to chemical oxidation, probably due to a lower concentration of polyphenols sensitive to oxidation. This rate of oxygen consumption after 4 months in bottle correlates well with color intensity developed in wines (Figures 3 and 4), which show that Sauvignon blanc has an increased overall color as compared to Muscat Ottonel wines, irrespective of antioxidant combination used for treatment.

Antioxidant molecules present modify the rate of oxygen consumption in Sauvignon blanc wines. The highest oxygen consumption was observed in control Sauvignon blanc wines, which have the smallest average value of dissolved oxygen left after 4 month of aging (Figure 1). When glutathione is used, residual dissolved oxygen measured is a little higher than in control samples of Sauvignon blanc, suggesting that some changes occur due to the presence of glutathione, and sulphited wines treated with GSH may be better protected than those treated with sulphur dioxide alone. The result observed sensorially also indicated a better protection of aroma compounds and of certain polyphenols susceptible to oxidation. In samples of sulphited Sauvignon blanc wines treated with ascorbic acid, the situation is different and the average value of residual dissolved oxygen is higher than in all groups of samples, suggesting that the rate of oxygen consumption was reduced, but this does not necessarily mean that those wineswere sufficiently protected from oxidation and color change. It can be observed from the color intensity determinations that Sauvignon blanc wines treated with sulphur dioxide and ascorbic acid are the most oxidized, having higher values for color intensity compared with control sample or glutathione treated samples and even compared with glutathione and ascorbic acid treated samples (Figure 3).

It is well known that ascorbic acid (AA) when oxidized by dissolved oxygen forms hydrogen peroxide and dehydroascorbic acid (DHA). The presence of high enough SO_2 levels can block all hydrogen peroxide, but the reaction reduces the available active sulphur dioxide. Later on,under low SO_2 levels, DHA can decay in wine conditions relatively fast and yellow pigments are formed via nucleophile attack and cyclization of L-xylosone on 8 or 6 position (+)-catechin (Barril, 2008). However, an even worse situation may occur, when SO_2 level is not enough to react with all generated hydrogen peroxide and, through Fenton reaction, hydroxyl radical is formed. This is a powerful oxidant that starts a chain reaction that leads to tartaric acid oxidation to glyoxylic acid and alcohols oxidation to aldehydes. Glyoxylic acid isan important product derived from successive oxidation of tartaric acid, which will react with two molecules of (+)-catechin to form xanthylium cations (Fulcrand et al., 1997).

Also, the degradation products of ascorbic acid are binders of sulphur dioxide which reduce the free SO_2 once they are formed.

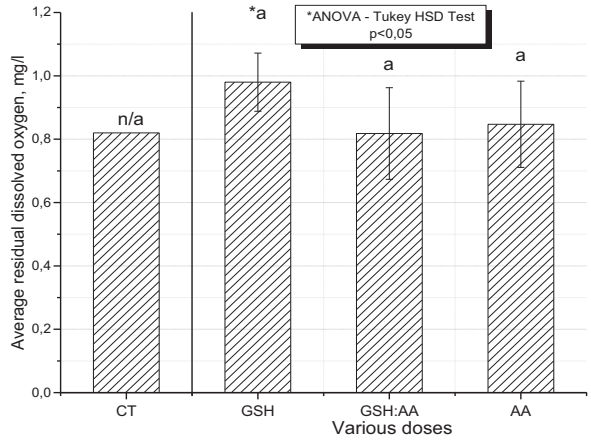

Figure 2. Average residual dissolved oxygen in Muscat Ottonel wines treated with various doses of reduced glutathione (GSH), ascorbic acid (AA) and mixtures of the GSH and AA

In Muscat Ottonel wines the situation is different as regards the average residual oxygen measured in groups treated with different antioxidants.Due to the better resistance of this variety to chemical oxidation the oxygen consumption is not significantly different from one group to another (Figure 2). The antioxidant doses and combinations are less important, the polyphenol composition of wines leading to a similar behavior regarding the oxygen consumption rate. Another possible explanation would be the presence of much more terpene compounds in Muscat as compared to Sauvignon, which are also known as good antioxidants (González-Burgos et Gómez-Serranillos, 2012). The presence of terpenes may improve to some extent the

resistance of Muscat Ottonel wines against dissolved oxygen.

When the color intensity of groups of Sauvignon blanc wines is compared taking into account the type of antioxidants added, the control wines and glutathione treated wines proved to behave similarly,displaying the smallest color intensity, while, when only ascorbic acid is introduced into wines, the color intensity increases dramatically.

When glutathione and ascorbic acid are both present in sulphited wines, intermediary results regarding color intensity can be observed (Figure 3). However, here too, degradation of ascorbic acid occurred, due to a low to moderate concentration of SO_2 present in the treated wines. In our experiment, the concentration of free SO_2 proved insufficient to ensure protection under the oxygen saturated conditions present during bottling wines.

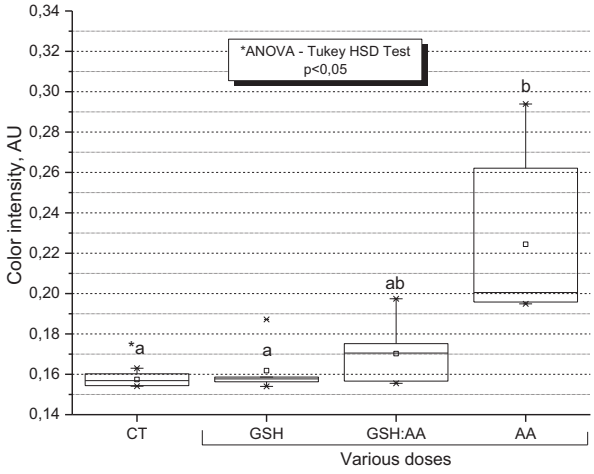

Figure 3. Average color intensity in Sauvignon blanc wine treated with various doses of reduced glutathione (GSH), ascorbic acid (AA) and mixtures of GSH and AA

Color intensity of Muscat Ottonel wines is overall smaller than in Sauvignon blanc, irrespective of the antioxidant type of treatment used (Figure 4).This fact confirms the better resistance to oxidation of this variety, but even so, when observing the groups of wines treated with various antioxidants, a similar pattern with Sauvignon blanc wines is displayed (Figure 4).

For this variety too the least oxidized samples were control samples and glutathione treated samples. When ascorbic acid is present, color intensity increases and this behavior is most likely due to the depletion of sulphur dioxide and degradation products of ascorbic acid.

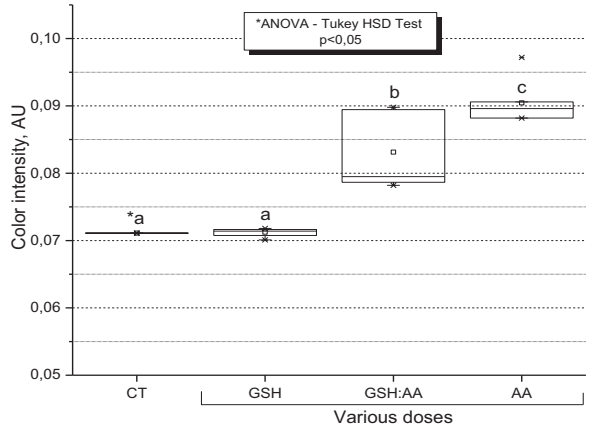

Figure 4. Average color intensity in Muscat Ottonel wine treated with various doses of reduced glutathione (GSH), ascorbic acid (AA) and mixtures GSH and AA.

A mechanism of oxygen consumption and antioxidants interaction in wine is suggested in Figure 5, which can explain the consumption of free SO_2 observedin ascorbic acid treated wines. This mechanism suggests a molar ratio of O_2:AA:SO_2 of 1:1:(1.7 to 2),which means that 1 mg/l dissolved O_2 will consume 5.5 mg/l ascorbic acid and 3.5 to 4 mg/l free SO_2. A good protection in wines is ensured by a molar ratio between O_2 and SO_2 of 1:1.7 and hasbeen already suggested by Danilewicz et al. in 2008 forred wines, while a molar ratio between SO_2 and ascorbic acid of 2:1 hasbeen suggested by Barril in 2011 for white wines.

However, in our experiments the free SO_2 level proved to be under the optimum protection dose: Sauvignon blanc samples had 35 mg/l free SO_2 while, in accordance to our proposed calculation method, a level of 55 mg/l free SO_2 should ensure a good protection when the wine is also treated with AA. These calculations are based on a molar consumption ratio of 1:1:1.7 for O_2:AA:SO_2 plus a minimum of 25 mg/l free SO_2. Similarly, Muscat Ottonel samples had 42 mg/l free SO_2, while the concentration of free SO_2 calculated based on the proposed mechanism and the amount of oxygen present should be above 56 mg/l in wines treated with AA.

Thus, for a good prediction of the necessary free SO_2 for bottling, the winemaker should take into account the oxygen and ascorbic acid levels in the wine. If the proposed mechanism of wine oxidation in the presence of AA, GSH and SO_2 (Figure 5) holds true and the soluble oxygen in wine is known, then the required dose of sulphur dioxide can be calculated. Our

experimental conditions described a worst case scenario, with a level of around 8 mg/l oxygen present at bottling time, but the exposure of wine to oxygen can be limited to 1-2 mg/l total oxygen, including the one available in the headspace (O'Brien et al., 2009).

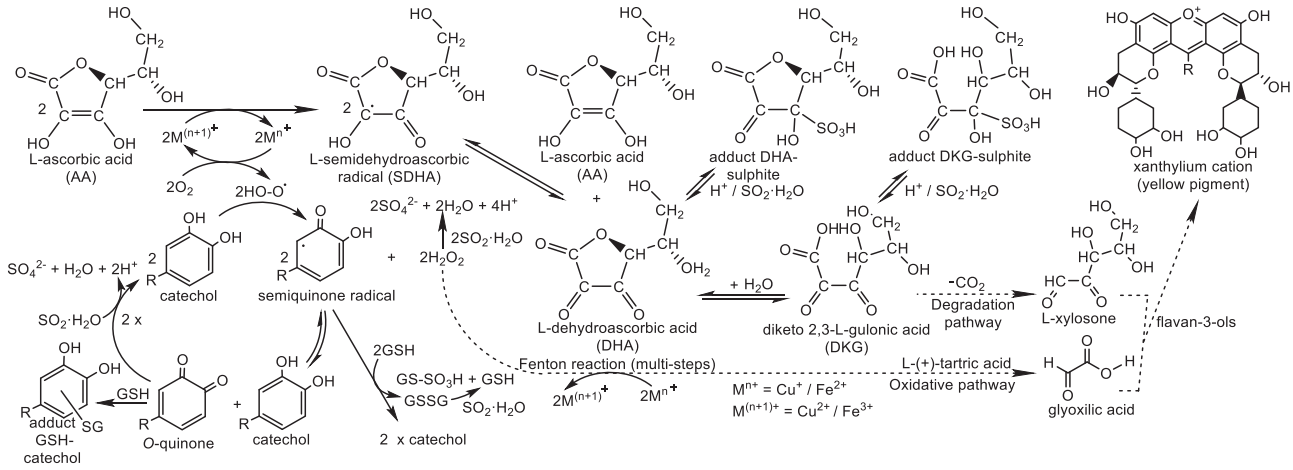

Figure 5. Proposed mechanism of wine oxidation under specific antioxidant combination (SO_2, AA and/or GSH)

CONCLUSIONS

A combination of SO_2, GSH and AA added to wine at bottling time could improve the antioxidant protection as compared with the already classic treatments with SO_2 and AA, but the success of this operation depends highly on ensuring a sufficient level of $SO_2$to prevent the formation of oxidation and degradation products which may lead to color change.

The consumption of oxygen during bottle aging is affected more by the grape varietyand differences in polyphenol composition of wines, rather than by the type of antioxidant used for the protection.More important differences on residual oxygen in samples can be observed in the case of the groups of Sauvignon blanc wines treated with various types of antioxidants, leading to the conclusionthat the polyphenols of Sauvignon are more sensitive to oxidation. The better resistance to oxidation of Muscat Ottonel wines is probably due to its polyphenols structure, but also to the presence of more terpenes, which act as antioxidants in wine.

Even though treatments with GSH with or without AA lead in the beginning to more fruitier wines compared with only SO_2 treated wines (results not shown), after a period of storage, a decay of product is observed, with the color and smell being firstly affected. These undesirable effects are most likely due to an underestimation of the SO_2 concentration, which allowed the occurrence of a complex phenolic compounds oxidation mechanism, involving AA and/or GSH. The main reactions occur through the addition of glyoxylic acid or L-xylosone with (+)-catechin which act as nucleophiles. As the available SO_2 is combined, hydrogen peroxide resulted from oxidation of catechols or ascorbic acid, under iron or copper mediated catalysis, is reduced to hydroxyl radical, which has a stronger reactivity towards organic molecules.

When GSH or AA additions are desired, a comprehensive study of the polyphenol matrix behavior should be done and the dissolved oxygen uptake rate evaluated, in order to calculate and adjust SO_2 levels in the treated wine. As opposed to the general belief, the SO_2 levels should be higher when the wine is also treated with AA than in the case $SO_2$is used alone, otherwise, the AA degradation products formed could negatively modify the color and overall sensory properties of product. Likewise, when GSH treatment is desired, the levels of sulphur dioxide should be high enough to react with all hydrogen peroxide to prevent hydroxyl radical occurrence.

Unexpected results of a relatively simple experiment show us that a reduction of SO_2 concentration in wines, when it is used along with GSH and AA, does not ensure protection

to dissolved oxygen consumption and color oxidation.

Often, GSH with SO_2 can offer good results regarding the preservation of aromatic complexity of young fruity wines, but only when wines are well protected from oxygen contact or sufficient SO_2 concentration is present. If free $SO_2$is quickly depleted, GSH and/or AA may act as a pro-oxidants and negatively affect the sensory characteristics of wine.

Further investigations are needed to establish for each variety the optimum doses for GSH and AA combination, along with a protective SO_2 concentration.

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