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EFFECTS OF TREATMENTS WITH ASCORBIC ACID AND GLUTATHIONE ON THE SAUVIGNON BLANC WINE COLOUR DURING BOTTLE AGING

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Abstract

The colour of wine is an important characteristic which contributes to the construction of the quality profile of a wine. This parameter is extremely important especially for white wines, a close connection being present in the consumer's perception between the colour of the wine and its quality level. The appearance of brown shades due to oxidation reactions is equivalent to a decrease in the quality level of the white wine and may indicate even an irreversible degradation of the wine.

The present study evaluates the colour evolution of a Sauvignon Blanc wine during storage in bottles after being treated just before bottling with different combinations and doses of sulphur dioxide (SO_2), ascorbic acid (AA) and reduced glutathione (GSH).The use of various antioxidants aims to reduce and prevent the browning evolution of white wine colour, and many hopes are expressed regarding the newly OIV approved reduced glutathione. However, this preliminary study results indicate that, in certain conditions, these antioxidants may not confer the expected protection and could even damage the final colour of white wine.

Key words*: White wine colour, glutathione, ascorbic acid, browning of white wine, Sauvignon Blanc.*

INTRODUCTION

As the white wine oxidation during aging is an important quality issue in winemaking, finding new methods for protecting the colour, along with the preservation of varietal aromas during the period of storage in bottle, is a priority. Even though a multitude of choices are available at present for improving the white wine stability against oxygen exposure, a complete and easy to apply antioxidant treatment of the white wine, able to ensure long time protection for both aroma and colour, is not yet available. Therefore, several studies have been conducted in the last decades in order to find the best combination and the optimal dose of some antioxidant agents, classical or newly discovered, to be added in wine to prolong its shelf life. One of the most well-known methods used to protect wine from oxidation and to prevent the formation of the secondary characteristics specific to wine aging, is to use agents with special antioxidant properties such as SO_2, ascorbic acid (AA), tannins and last, but not least, reduced glutathione (GSH) (Nikolantonaki et al., 2014). These materials are usually added to wine during the various stages of the production chain, but especially prior to bottling, and act either by reacting with oxygen or by removing or stabilizing the substrates sensitive to oxidation found in the wine, such as polyphenols (Oliveira et al., 2002), aromatic compounds etc.

While many studies already showed the beneficial action of these preservatives on the aromatic characteristics of the wine, their effect on colour conservation was not considered so much of a concern. It is, however, well-known that the colour of the wine is influenced especially by the grape variety, the pH values, the storage temperature, the winemaking protocols and the conditioning treatments applied before the wine bottling (Antoce, 2002).

The above mentioned antioxidant agents, considered essential for the prevention of oxidative processes in wine during aging (Brajkovich et al., 2005; Lavigne Cruège et al., 2003; Ugliano et al., 2011), including the GSH, are able to block quinones forming non-coloured polymers (Singleton et al., 1985; Antoce, 2007).

Previous studies have shown that in combination with small doses of sulphur dioxide, GSH slows down the oxidation rate of aromatic compounds such as volatile thiols, mono-terpenes and esters, and the formation of yellow xanthylium pigments specific for the browning reactions of the white wines (Lavigne and Dubourdieu, 2002; Bouzanquet et al., 2012; Roussis et al., 2007; Sonni et al., 2011). The presence in wine of xanthylium pigments originating from epicatechin determines a major change of its colour as they are two times more coloured than the pigments formed from catechins (Labrouche et al., 2005).

Similarly, although the effect of ascorbic acid as antioxidant agent is generally recognized, its addition in the wines without sufficient sulphur dioxide leads to detrimental reactions of its degradation products and catechins, forming also yellow xanthylium pigments (Barril et al., 2009; Barril et al., 2012).

Despite the existence of numerous studies regarding the role of GSH in protecting the varietal aromas of wines (Papadopoulou and Roussis, 2001, 2008; Roussis et al., 2009), especially regarding volatile thiols, its effect in combination with other antioxidants agents and particularly on the evolution of the white wine colour, has not been equally researched (Kritzinger et al., 2012; Badea and Antoce, 2015). As in July 2015 the OIV included among the allowed oenological practices for must and wine the addition of a maximum 20 mg/l of GSH (Resolutions OENO-TECHNO 10-445 and 10-446/July 2015) the research regarding these treatments has also intensified. This dosage has been approved based on some previous studies. Lavigne-Cruège and Dubourdieu (2002) have proposed a smaller dosage, of only10 mg/L of GSH, for the prevention of the browning phenomenon, while other scientists (Papadopoulou and Roussis, 2001, 2008; Ugliano et al.,2011) proposed the addition of 20 mg/L GSH for protection against wine aging defects.

In this work, the evolution of the colour during 4 months of aging in bottles of a Sauvignon Blanc was studied aiming to test the effect of different combinations and doses of these main wine antioxidants added prior to wine bottling.

MATERIALS AND METHODS

The wine samples have been prepared from the same Sauvignon Blanc base wine with a given level of free SO_2 in which various dosages of GSH, ascorbic acid and combinations of the two were added at bottling time.

The base wine was produced in September 2014 in the Dealu Mare vineyard, Romania, from Sauvignon Blanc grapes harvested from a plantation founded in 2007. The wines were treated and bottled in February 2015, the base wine parameters being included in Table 1 and those of the prepared samples shown in Table 2. The reduced glutathione (GSH) and the ascorbic acid (AA) were provided by Enologica Vason Italy.

Table 1.Physico-chemical parameters of Sauvignon Blanc base wine

Physico-chemical analysis report of the basis wine	
Identification data of the wine	
Cultivar / Product range	SAUVIGNON BLANC
Vintage year	2014
Quality category	CDO
Producer	DOMENIILE DEALU MARE URLATI
Wine physico-chemical parameters	
Free sulfur dioxide (mg/l)	35
Total sulfur dioxide (mg/l)	86
Total acidity (g/l tartaric acid)	6
Volatile acidity (g/l)	0.36
alcoholic concentration (%)	14.5
Sugar (g/l)	1.2
Relative density at 20 °C	0.9912
Nonreducing dry extract (g/l)	19.82

The bottled wine samples were analyzed 4 months after the treatments, by measuring their colour parameters with a computer-controlled double beam spectrophotometer Specord 250 from Analytik Jena AG running the software WinAspect version 2.2.7 and applying the CIELab system.

The computer automatically calculates the trichromatic components (CIE XYZ tristimulus values), *xy* chromaticity coordinates and CIELab colour parameters of the wine samples.Triplicate measurements of the colour parameters for each wine sample were performed by using 1 mm glass cuvettes.

Table 2.Variants of Sauvignon Blanc wines treated with different doses of antioxidants

Wine sample Code	Dosage
SBControl	Control
SBGSH10	10 mg/l Glutathione
SBGSH20	20 mg/l Glutathione
SBGSH30	30 mg/l Glutathione
SBGSH40	50 mg/l Glutathione
SBGSH50	100 mg/l Glutathione
SBAA30	30 mg/l Ascorbic Acid
SBAA40	40 mg/l Ascorbic Acid
SBAA50	50 mg/l Ascorbic Acid
SBAA60	60 mg/l Ascorbic Acid
SBAA70	70 mg/l Ascorbic Acid
SBAA30GSH10	30 mg/l Ascorbic Acid and 10 mg/l Glutathione
SBAA30GSH20	30 mg/l Ascorbic Acid and 20 mg/l Glutathione
SBAA30GSH30	30 mg/l Ascorbic Acid and 30 mg/l Glutathione
SBAA30GSH50	30 mg/l Ascorbic Acid and 50 mg/l Glutathione
SBAA30GSH100	30 mg/l Ascorbic Acid and 100 mg/l Glutathione

The transmittance of wine was recorded every 1 nm over the visible spectrum of 400-700 nm and colour parameters calculated for a D65 standard illuminant and 2° observer angle.

The results have been analysed by using the software Microsoft Excel and the package Chroma Ver. 2.0.

The software automatically calculates the trichromatic components (X, Y, Z) and the basic colour percentages (*x*– the percentage of red and *y*- the percentage of green), as well as the CIELab space system parameters (the colorimetric coordinates L*=clarity, *a**= red/green colour component, *b** =yellow/blue colour component, C* = chroma, H* = hue).

In the CIELab system, an uniform colour space can be very clearly expressed through three dimensions, the Cartesian parameters L*, *a** and *b**. L* represents the luminosity (lightness), *a** defines the place on the red-green axis, while*b** represents the value on the yellow-blue axis.

With the aim of assessing the modifications induced by the treatments, the white wine colour differences were calculated for the CIELab parameters (ΔL^*,Δa^*, Δb^*, $\Delta C_{ab}^*$and ΔH_{ab}^*), as well as the overall colour difference (ΔE_{ab}^*), against the non-treated control sample (SBControl), using the OIV recommended method (OIV- Resolution OENO 1/2006, OIV-MA-AS2-11: R2006).

RESULTS AND DISCUSSIONS

The Sauvignon Blanc variants treated with different doses of antioxidants as presented in the Table 2, were analysed after 4 months from bottling. The values of trichromatic parameters (X, Y, Z) and of the chromaticity coordinates of colour, x-the luminance and y-the chromaticity are presented in the Table 3.

Table 3.Trichromatic parameters and basic colour percentages of Sauvignon Blanc wines after 4 months of bottle aging

	Trichromatic parameters and basic colour percentages				
Wine sample Code	X	Y	Z	x % of red	y % of green
SBControl	88.3994	92.2442	93.5300	0.3224	0.3364
SBGSH10	88.1638	91.6632	92.8239	0.3234	0.3362
SBGSH20	88.0719	91.2673	93.0589	0.3233	0.3350
SBGSH30	86.6174	89.7314	90.9998	0.3240	0.3356
SBGSH50	82.3637	85.4208	87.0727	0.3232	0.3352
SBGSH100	85.8873	88.8166	89.6845	0.3249	0.3359
SBAA30	84.4163	88.5165	89.7646	0.3214	0.3370
SBAA40	91.1027	96.0821	97.4189	0.3201	0.3376
SBAA50	92.0580	97.2069	98.9560	0.3194	0.3373
SBAA60	91.3393	96.4066	97.6347	0.3201	0.3378
SBAA70	92.4338	97.6716	99.3375	0.3194	0.3374
SBAA30GSH10	89.6307	93.8582	95.3269	0.3215	0.3366
SBAA30GSH20	88.8788	93.1425	94.2912	0.3217	0.3372
SBAA30GSH30	89.4165	93.6848	95.8051	0.3206	0.3359
SBAA30GSH50	90.0345	94.5040	95.8355	0.3211	0.3370
SBAA30GSH100	89.1820	94.0326	95.8042	0.3196	0.3370

It can be observed that there are differences between the colour of the samples, even if not very big, as the influence of the base wine characteristics was more important than the treatments themselves.

The parameter X values are high (above 91) for all the samples containing only ascorbic acid, except the case of sample SBAA30, the one containing only 30g/l AA, for which it is only 84.4. All the other samples containing AA and GSH also had high values of X – between 89 and 90, while the samples prepared only with GSH had lower values, especially at higher GSH doses. This means that the main influence was brought about by the treatment with AA. It can also be observed that the control sample SBControl and the wines treated with 10 and 20 mg/l GSH have shown similar colour parameters.

The parameter Y, defining the wine samples luminosity (Table 3) is lower for all the samples treated with GSH alone and for the sample SBAA30 with only 30mg/l AA, which means that they are less transparent than the control

sample or the wines treated with AA or AA-GSH combinations. Regarding the luminosity too, treatments with AA have a more important influence than GSH. Similar conclusions can be drawn with the CIELab method (Table 4), when the values for the clarity L* are taken into account (Figure 1).

Table 4.Chromatic characteristics values of Sauvignon Blanc wines after 4 months of bottle aging

Wine sample Code	L*	*a* *	*b* *	C_{ab} *	H_{ab} *
SBControl	**96.9189**	**1.3322**	**4.1080**	**4.3224**	**1.2599**
SBGSH10	96.6825	1.9211	4.1775	4.5981	1.1398
SBGSH20	96.5199	2.4519	3.7370	4.4698	0.9901
SBGSH30	95.8844	2.4895	4.0573	4.7607	1.0201
SBGSH50	94.0561	2.2711	3.6709	4.3183	1.0160
SBGSH100	95.5037	2.7680	4.3130	5.1254	1.0001
SBAA30	95.2456	0.5411	4.0529	4.0902	1.4380
SBAA40	98.4649	-0.4025	4.1641	4.1836	-1.4744
SBAA50	98.9098	-0.6032	3.9205	3.9667	-1.4181
SBAA60	98.5935	-0.5310	4.2435	4.2766	-1.4464
SBAA70	99.0926	-0.7190	3.9865	4.0508	-1.3924
SBAA30GSH10	97.5748	0.7642	4.0225	4.0945	1.3831
SBAA30GSH20	97.2783	0.6350	4.2308	4.2821	1.4170
SBAA30GSH30	97.5047	0.6749	3.5815	3.6445	1.3844
SBAA30GSH50	97.8347	0.3793	4.1300	4.1474	1.4792
SBAA30GSH100	97.6428	-0.3579	3.8212	3.8380	-1.4770

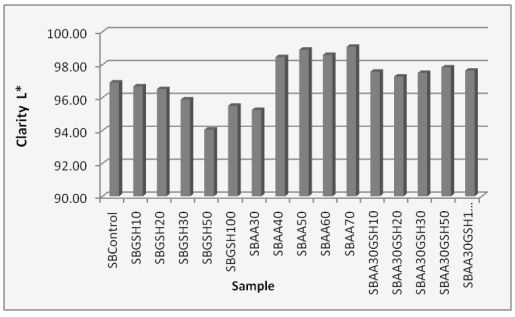

Figure 1. CIELab coordinate L* of Sauvignon Blanc wines after 4 months of bottle aging

As it is already known, when the parameters *a** and *b** are positive, the colour will be in the range of red-orange-yellow and when*a** is negative and *b** is positive, the colour will be in the range of yellow-green. Therefore, as we can observe in Table 4, the wine samples treated only with ascorbic acid (except SBAA30) and the sample with 100 m/l GSH and 30 mg/l AA (SBAA30GSH100) are in the colour range of yellow - green, while the control sample and all the other samples treated with GSH with or without AA, reveal a more or less accentuated tendency towards red-orange-yellow.

From the representation of these values in an*ab* diagram (Figure 2) we can observe that three groups and the control sample are clearly individualized in the space, in direct correlation with the applied treatment: the group treated with different doses of GSH alone, the group treated with a combination of different doses of GSH and 30 mg/l ascorbic acid and the group treated with different doses of AA alone.

While the first two groups containing GSH and the control sample SBControl can be very clearly differentiated in the red-orange-yellow colour space, the group of samples treated only with AA is clearly positioned in the yellow-green space. Thus, it can be concluded that the presence of GSH makes the colour of wine shift toward orange/brown shades.

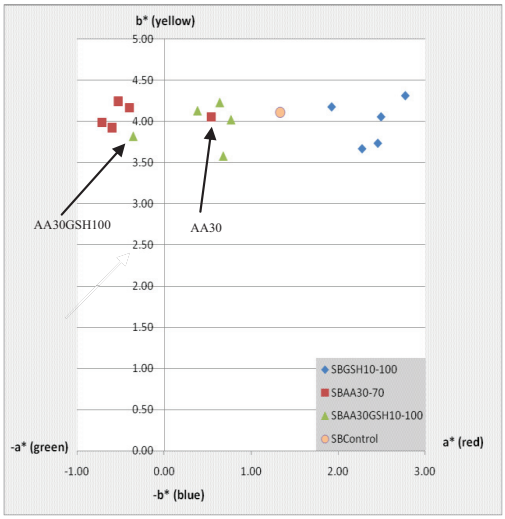

Figure 2.The *ab* diagram describing the position of the colour of Sauvignon Blanc wines treated with ascorbic acid and/or glutathione after 4 months of bottle aging

However, samples treated only with GSH are shifted more toward orange/brown than are the samples treated with the combination of various doses of GSH and 30mg/l ascorbic acid, with the control sample lying in between these groups. This led us to the conclusion that the presence of ascorbic acid in the wine samples has the main positive impact when it comes to the preservation of their yellow-green colour in time.

Another observation is that, although distinct groups are formed in accordance to the type of treatment, in each group an outlier is present: the sample SBAA30 located in the group of samples treated with GSH and AA combinations and sample SBAA30GSH100 located in the group of samples treated only with AA.

The presence of AA30 in the more oxidized GSH-AA group may suggest that the dose of 30 mg/l AA may not be sufficient, only the samples with 40-70 mg/l AA being not oxidized. Conversely, the presence of the sample with 30 mg/l AA and 100 mg/l GSH in the group of not-oxidized samples treated with AA, suggests that a higher dose of GSH, of at least 100 mg/l, is required for protection of colour in Sauvignon Blanc.

After the calculation of the differences in luminosity ΔL_{ab}*, saturation ΔC_{ab}*, position on the red-green Δa* and yellow-blue Δb* space, it was confirmed that the wine samples treated with ascorbic acid had a distinct behavior as compared to the samples treated with GSH or the control sample.

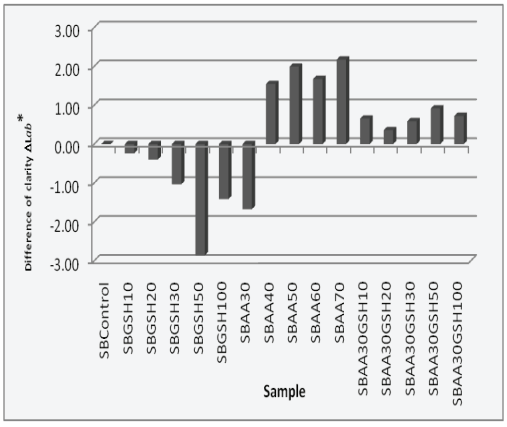

Figure 3.Graphic representation of the difference of luminosity ΔL_{ab}* of Sauvignon Blanc wines after 4 months of bottle aging

As represented in the Figure 3, while the wine samples SBGSH10-100 treated only with GSH and SBAA30 were less transparent than the control SBControl, all other samples containing different doses of AA, with or without GSH, were lighter than the control.

Analysing the variation of the parameter C_{ab}* (chroma), it is easy to observe that the ΔC_{ab}* values are negative for all the samples treated with AA, as such or in combination with GSH, therefore they have a smaller saturation than the control sample SBControl (Figure 4).

The samples treated only with GSH have mainly positive ΔC_{ab}* values, so they are more chromatic (colorful) than the control sample.

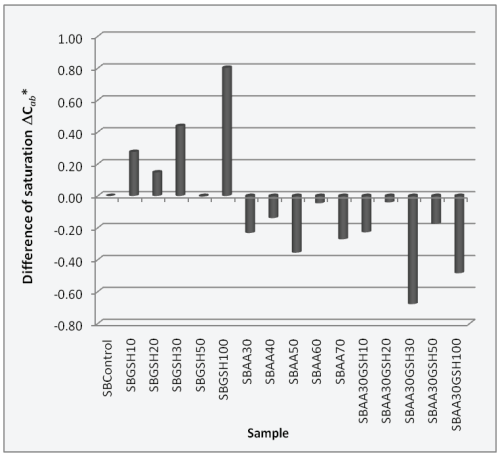

Figure 4. Graphic representation of the difference of saturation ΔC_{ab}* of Sauvignon Blanc wines after 4 months of bottle aging

In order to determine the overall colour difference of the samples against the control, the parameter ΔE_{ab}* was calculated.

For the assessment, it is considered that when the overall colour difference ΔE_{ab}* between two samples is under 0.5 units, the difference is not perceptible, when the difference is situated between 0.5-1 units it is slightly perceptible, while in the case of values between 1.5-3 units the difference is perceptible and above 3 units the colour difference is clearly perceptible.

The values of the colour difference ΔE_{ab}* between the samples treated only with GSH and the control sample are in the range of 0.5-3 units, which means there are perceptible differences between these wines colours and the control sample, the difference mainly increasing with the GSH dose applied (Figure 5).

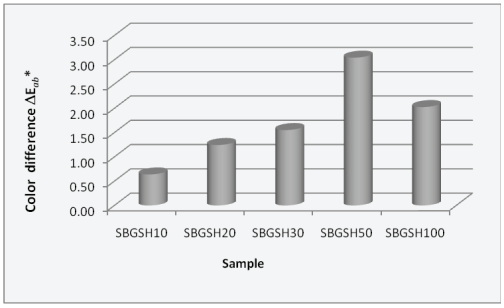

Figure 5. Graphic representation of the overall colour difference ΔE_{ab}* of Sauvignon Blanc wines treated with various doses of GSH against the control sample measured after 4 months of bottle aging

Similarly, the values of the colour difference ΔE_{ab}* between the samples treated only with AA and the control sample (Figure 6)are in the range of 1.5-3 units, all being perceptible by the human eye.

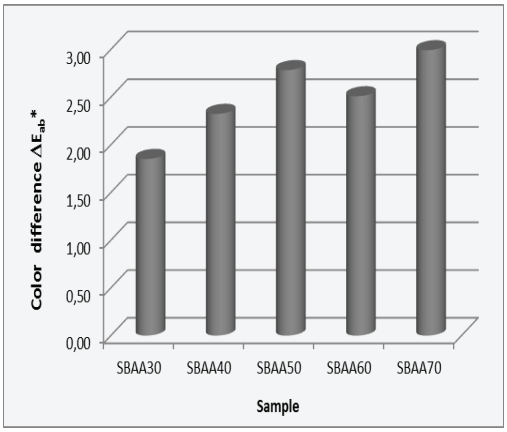

Figure 6. Graphic representation of the colour difference ΔE_{ab}* of Sauvignon Blanc wines treated with various doses of ascorbic acid against the control sample measured after 4 months of bottle aging

In the Figure 7, the differences for samples treated both with GSH and 30 mg/l AA were plotted by taking as control not only the wine without any treatment (SBControl), but also the wine treated with 30 mg/l ascorbic acid (SBAA30Control).

As it can be seen, the colour differences induced by GSH addition are higher, when the effect of those 30 mg/l AA is eliminated (comparison with SBAA30Control), showing that the GSH in the absence of AA produces in time perceptible colour changes.

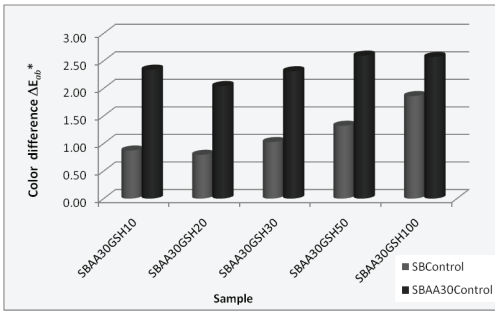

Figure 7. Graphic representation of the colour difference ΔE_{ab}* of Sauvignon Blanc wines treated with 30mg/l ascorbic acid and various doses of GSH against the control samples measured after 4 months of bottle aging

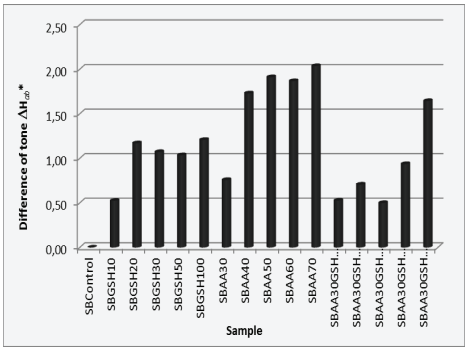

Figure 8. Graphic representation of the difference of hue ΔH_{ab}* between samples of Sauvignon Blanc after 4 months of bottle aging

As for the variation of hue, all the calculated ΔH_{ab}* values are positive (Figure8), therefore, after comparing the wine samples values with the yellow hue value recorded for control sample (SBControl), we can conclude that all the treated wine samples, irrespective of the dose and type of antioxidant added, are greener, which proves they are less oxidised than the untreated base wine.

CONCLUSIONS

The results obtained in this research show that the colour of wine can be clearly influenced by the type and dose of the antioxidants used for its protection.

The addition of ascorbic acid as such in the wine determined obvious changes of the wines colour.

The samples treated with AA are more transparent than the control sample and as

compared to the samples treated only with GSH. All the wine samples treated with ascorbic acid, with or without GSH, are brighter than the untreated wine.

The samples treated only with GSH are darker than the untreated wine.

The wine samples treated only with ascorbic acid (with the exception of SBAA30 and SBAA30GSH100) are located in the *ab* space in the range of yellow-green colour. All the other samples treated with GSH, irrespective if in combination or not with AA, have showed a more or less important tendency to place towards orange/brown tones.

Three groups of treated wines and the control sample are clearly individualized in the colour space, in direct connection with the treatment used: the group treated with increasing doses of GSH, the group treated with the combination of various doses of GSH and the fixed amount of 30 mg/l AA and the group treated with different doses of AA.

The group treated with GSH is placed more toward orange colour than the control sample, while the other two groups, both containing AA with or without GSH are placed more toward green colour.

From green to red, the groups are placed in *ab* space as follows: AA group, AA30-GSH group, untreated wine, GSH group. No major differences were recorded among the samples on the yellow-blue axis, all being in the same range of the yellow space.

Addition of more than 40mg/l of AA proved more efficient in protecting the green component of colour, but using a higher dose of GSH (100 mg/l) in combination with the usually recommended 30mg/l of ascorbic acid (sample SBAA30GSH100) provided the same level of protection of the colour as the wines treated with 40-70 mg/l AA alone.

All the treatments containing AA, irrespective of the dosage or the presence of GSH, determined less oxidised wines compared to the untreated wine.

We can conclude that the usage of ascorbic acid alone or in combination with glutathione ensured a better antioxidant protection to the wines than the treatment with GSH alone. These additions should be made only when a sufficient level of sulphur dioxide is provided.

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