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COLOR CHANGES DURING THE STORAGE IN BOTTLES OF MUSCAT OTTONEL WINE TREATED WITH ASCORBIC ACID AND GLUTATHIONE

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Abstract

The present work aimed to determine the colour evolution after aging in bottle of a white wine from Muscat Ottonel cultivar and the effect of the addition of different antioxidants at bottling time, such as SO_2, reduced glutathione (GSH) and ascorbic acid. Wine colour is an important indicator of its degree of oxidation, therefore the evolution toward a brownish colour is equivalent with some quality loss or even degradation. Considering that colour is also a key parameter that contributes to the perception of the quality profile of a white wine, the study attempted to determine the effect of certain doses and combinations of some anti-oxidants, which are known to ensure the best wine quality preservation during aging of wine in bottles. Our preliminary study shows that, in some cases and conditions, the anti-oxidants used to protect the aroma and the colour of wines end up affecting the colour.

Key words*: Glutathione, ascorbic acid, white wine colour, browning of white wine, oxidation of white wines.*

INTRODUCTION

The protection of white wines from oxidation is a condition for preserving their quality and prolonging their shelf life. The loss of aroma compounds, the browning phenomenon and the precipitation of phenolic substances are characteristics of the white wines oxidation (Kilmartin, 2010).As the wine colour is an important indicator of its degree of oxidation, the occurrence of brownish colour in white wine is equivalent with a decrease in quality, because when such oxidation is apparent, it also means that the aromatic compounds in wine were already affected (Singleton, 1987).

The addition of different conditioning agents, particularly prior to bottling, is the classic method employed to keep the quality of wine, by eliminating or stabilizing the substrates susceptible to oxidation, such as polyphenols, volatile compounds, etc.

Antioxidants such as SO_2, ascorbic acid, reduced glutathione (GSH) have the capacity to reduce and eliminate quinones and are essential for the management of oxidative aging processes of wine (Brajkovich et al., 2005; Lavigne Cruège et al., 2003; Ugliano et al., 2011). It is already known that using SO_2 and ascorbic acid combined in various ratios slows down the oxidation of polyphenols in wine in different proportions (Oliveira et al., 2002). But, as the ascorbic acid is a highly unpredictable molecule, its addition in white wines involves some risk taking. Its degradation products may not be captured by $SO_2$and could react further with any catechins found in wine, determining the occurrence of yellow xanthylium pigments(Barril et al.,2009; Barril et al., 2012)or the appearance of sotolon, which affects the dry white wine aroma (Pons et al.,2010).

The tripeptide glutathione (GSH),a natural antioxidant found in grapes, alsodrew the researchers' attention in the last decade for potential use in wine production or during storage. In July 2015 OIV approved the addition of GSH in must and wine up to a concentration of 20 mg/l(Resolutions OENO-TECHNO 10-445 and 10-446/July 2015).

However, although results of numerous studies on the effect of adding glutathione to wine were recently published, the role of this antioxidant and its complementary action with the most used wine preservatives like sulfur

dioxide and ascorbic acid have to be further examined (Kritzinger et al., 2012; Badea and Antoce, 2015).

The researches have showed that in combination with SO_2, GSH reduces the degradation of aromatic compounds like monoterpenes and esters and slows down the browning reaction of white wines, in particular the formation of yellow pigments of xanthylium (Bouzanquet et al., 2012; Roussis et al.,2007; Sonni et al., 2011).

While a lot of researches have proved that glutathione has an important contribution to the protection of varietal aroma during the aging of wines (Papadopoulou and Roussis, 2001, 2008; Roussis et al., 2009), its effect on the wine colour is not clearly established. Various doses of exogenous GSH that should be added to wine to provide antioxidant protection were proposed by researchers. For muscat-type aroma, the terpineol and linalool protection in wines kept in contact with the air at 20°C for 3 days has been assured by the addition of 20 mg/lGSH (Papadopoulou and Roussis, 2001, 2008).A smaller quantityof only 10 mg/l of GSH has been proposed by Lavigne-Cruège and Dubourdieu (2002) who demonstrated that the formation of aging defects, such as yellow pigments and the loss of the varietal characteristics of wine, can be prevented with this low dosage. However, the effects of glutathione in wines depend on the wine composition and conditions, so that contradictory results have been reported. While it was proved that GSH protects against oxidation, it was reported that it can also contribute to oxidation (Vaimakis and Roussis, 1996) or that glutathione addition may determine the occurrence of higher contents of H_2S during the storage of a wine (Ugliano et al., 2011).

The wine colour is influenced by some factors like the grape variety, the pH values, the storage temperature, the winemaking technologies and the stability treatments applied before the bottling (Antoce, 2002). Numerous studies have demonstrated during last decades a direct correlation between the white wines oxidative stability during aging in bottle, the values of pH, total phenol and total SO_2 contents and the GSH or ascorbic acid dosage added to wines.

In this study we have investigated the color changes after 4 months of bottle storage of a Muscat Ottonel wine treated at bottling time with GSH and/or ascorbic acid in various dosages and conditions.

The tested Muscat Ottonel wines were prepared from a base wine with a given level of sulfur dioxide treated with GSH or/and ascorbic acid in different dosages and combinations, aiming to determine an optimal treatment for this type of wine.

MATERIALS AND METHODS

The studied wine was industrially produced in September 2014 in the Domeniile Dealu Mare Urlati Wine cellar from Prahova County, Romania, from Muscat Ottonel grapes cultivated in their own vineyard. The wine samples have been prepared in February 2015, by bottling the same base wine after treatment with different combinations and dosages of glutathione or/and ascorbic acid, both supplied by Enologica Vason Italy, as represented in the Table 1.

Table 1 - Variants of Muscat Ottonel wines produced in 2014 and treated with different doses of antioxidants

Wine sample Code	Dosage
MOControl	Control
MOGSH10	10 mg/l Glutathione
MOGSH20	20 mg/l Glutathione
MOGSH30	30 mg/l Glutathione
MOGSH40	50 mg/l Glutathione
MOGSH50	100 mg/l Glutathione
MOAA30	30 mg/l Ascorbic Acid
MOAA40	40 mg/l Ascorbic Acid
MOAA50	50 mg/l Ascorbic Acid
MOAA60	60 mg/l Ascorbic Acid
MOAA70	70 mg/l Ascorbic Acid
MOAA30GSH10	30 mg/l Ascorbic Acid and 10 mg/l Glutathione
MOAA30GSH20	30 mg/l Ascorbic Acid and 20 mg/l Glutathione
MOAA30GSH30	30 mg/l Ascorbic Acid and 30 mg/l Glutathione
MOAA30GSH50	30 mg/l Ascorbic Acid and 50 mg/l Glutathione
MOAA30GSH100	30 mg/l Ascorbic Acid and 100 mg/l Glutathione

The physico-chemical parameters of the base wine used are shown in Table 2.

Table 2 - Physico-chemical parameters of Muscat Ottonel base wine

Physico-chemical analysis report of the basis wine	
Identification data of the wine	
Cultivar / Product range	MUSCAT OTTONEL
Vintage year	2014
Quality category	CDO
Producer	DOMENIILE DEALU MARE URLATI
Wine physico-chemical parameters	
Free sulfur dioxide (mg/l)	42
Total sulfur dioxide (mg/l)	80
Total acidity (g/l tartaric acid)	6
Volatile acidity (g/l)	0.33
Alcoholic concentration (%)	13.1
Sugar (g/l)	1.23
Relative density at 20 °C	0.9902
Nonreducing dry extract (g/l)	20.2

The bottled wines were analyzed 4 months after the treatment with the above mentioned doses of antioxidants, by measuring the colour parameters with a computer-controlled double beam spectrophotometer Specord 250 from Analytik Jena AG, running the software WinAspect version 2.2.7, which automatically calculates the trichromatic components (CIE XYZ tristimulus values), the *xy* chromaticity coordinates and the CIELab colour parameters. The obtained results have been analysed by using the software Microsoft Excel as well as the package Chroma Ver. 2.0. For measuring the colour parameters 1 mm glass cuvettes were used, while the standard illuminant was D65and the angle of observer 2°. The transmittance of wine was measured every 1 nm over the visible spectrum between 400-700nm.

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 their transformation into the CIELab space system parameters (the colorimetric coordinates L*=clarity, *a**= red/green colour component, *b** =yellow/blue colour component, C* = chroma, H* = hue) were automatically calculated by the software. In order to evaluate the changes of colour induced by the treatments, differences of these parameters were calculated against the untreated control (MOControl).The differences of CIELab parameters ΔL*,Δa*, Δb*, ΔC_{ab}* and ΔH_{ab}*, as well as the overall colour difference against the control sample, ΔE_{ab}*, have been calculated in Excel in accordance with the OIV method (OIV- Resolution OENO 1/2006, OIV-MA-AS2-11: R2006).

RESULTS AND DISCUSSIONS

The variants prepared were assessed after 4 months of storage in bottles and the parameters recorded in accordance to the CIEXYZ and CIELab methods. The measured values of trichromatic parameters (X, Y, Z) and of the chromaticity coordinates of colour, luminance *x* and chromaticity *y*are included in Table 3.

We can see that the colour parameters determined are rather close for all the samples, as the base wine imposes its characteristics on all. From these results we can see, however, that the luminosity (parameter Y in Table 3) is lower for all the samples with GSH and the control sample, while the samples with ascorbic acid, irrespective of the dosage, are brighter.

Table 3 - Trichromatic parameters and basic colour percentages of Muscat Ottonel wines determined after 4 months of bottle aging

Wine sample Code	Trichromatic parameters and basic colour percentages				
	X	Y	Z	*x* % of red	*y* % of green
MOControl	**92.7895**	**97.6440**	**101.1128**	**0.3183**	**0.3349**
MOGSH10	91.6838	96.4309	99.3223	0.3190	0.3355
MOGSH20	92.5881	97.4078	100.9868	0.3182	0.3348
MOGSH30	91.5139	96.2079	99.2848	0.3189	0.3352
MOGSH40	91.4817	96.1447	99.3360	0.3188	0.3350
MOGSH50	92.0272	96.7019	100.4661	0.3182	0.3344
MOAA30	92.6896	97.8371	101.0445	0.3179	0.3356
MOAA40	93.7348	99.0493	102.4415	0.3175	0.3355
MOAA50	92.4725	97.6794	100.4128	0.3183	0.3362
MOAA60	94.0322	99.4636	102.5979	0.3176	0.3359
MOAA70	92.6805	97.9860	100.5360	0.3183	0.3365
MOAA30GSH10	92.8468	98.0147	100.9786	0.3181	0.3359
MOAA30GSH20	93.5649	98.8449	102.3002	0.3175	0.3354
MOAA30GSH30	92.2983	97.5105	100.1315	0.3183	0.3363
MOAA30GSH50	93.6449	98.9087	102.4180	0.3175	0.3353
MOAA30GSH100	92.9785	98.2005	101.0512	0.3182	0.3360

The parameter X is smaller for the GSH samples and for the control than in all the other samples, showing that indeed the addition of GSH correlates with some changes in colour.

In the CIELab system, the color can be very precisely defined through the Cartesian parameters L*, *a**, *b**. By definition, if the

parameters *a** and *b** are positive, the colour of the analysed sample will be in the range of red-orange-yellow and if the parameters *a** and *b** have different signs (*a** is negative and *b** is positive)the colour of the analysed sample will be in the range of yellow-green.

Table 4 - Values of chromatic characteristics of Muscat Ottonel wines after 4 months of bottle aging

Wine sample Code	L*	*a**	*b**	C_{ab}*	H_{ab}*
MOControl	**99.0818**	**-0.0377**	**2.8162**	**2.8165**	**-1.5574**
MOGSH10	98.6032	0.0471	3.1526	3.1529	1.5559
MOGSH20	98.9889	0.0037	2.7374	2.7374	1.5694
MOGSH30	98.5148	0.1229	3.0246	3.0271	1.5302
MOGSH40	98.4897	0.1731	2.9480	2.9531	1.5122
MOGSH50	98.7105	0.2009	2.5940	2.6017	1.4935
MOAA30	99.1565	-0.5421	2.9919	3.0416	-1.3907
MOAA40	99.6312	-0.7272	2.9105	3.0000	-1.3260
MOAA50	99.0957	-0.6632	3.2927	3.3588	-1.3720
MOAA60	99.7922	-0.8956	3.0882	3.2154	-1.2885
MOAA70	99.2160	-0.8104	3.4203	3.5150	-1.3381
MOAA30GSH10	99.2272	-0.5625	3.1536	3.2034	-1.3943
MOAA30GSH20	99.5516	-0.6851	2.8637	2.9445	-1.3360
MOAA30GSH30	99.0293	-0.6883	3.3606	3.4304	-1.3688
MOAA30GSH50	99.5765	-0.6505	2.8312	2.9050	-1.3449
MOAA30GSH100	99.3000	-0.6416	3.2321	3.2952	-1.3748

As it can be observed in the Figure 1, the wine samples treated only with glutathione are darker compared to control sample(they have higher L* values), while the rest of samples with ascorbic acid, with or without GSH, resulted lighter than the control sample.

By analyzing the resulted values of the CIELab space parameters, we can observe that all the wines treated with ascorbic acid, with or without GSH, are in the color range of yellow - green, while the samples treated only with GSH showed a slight tendency toward red - orange - yellow.

If we plot these samples on an *ab* diagram (Figure 2) we can easily see that they form three groups, which correlate with the type of treatment: the group treated only with various doses of GSH, the group treated only with various doses of ascorbic acid and the group treated with 30 mg/l ascorbic acid and various doses of GSH.

The first group is clearly differentiated in the color space, while the other two overlap, showing that the main influence on the color is due to the presence of ascorbic acid.

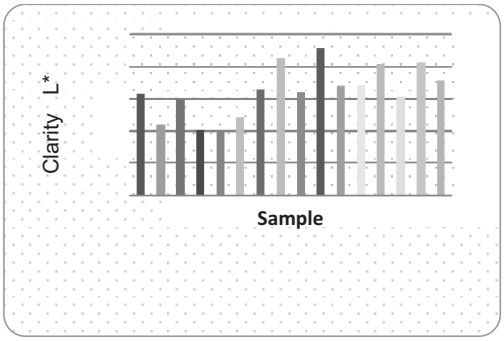

Figure 1.CIELab coordinate L* of Muscat Ottonel wines after 4 months of bottle aging

The samples produced only with GSH acquired a shade of orange, while the rest of samples, with ascorbic acid and with or without GSH did not show the same trend.

If we analyze the differences in brightness ΔL* and saturation ΔC_{ab}*, and the variations of red-green Δa* and yellow-blue Δb* shades, we observe that the wine samples treated with various doses of GSH and/or ascorbic acid, behaved differently from the control sample.

As regarding the variation of the parameter *a** (red-green), most of the Δa* values are negative (Table 5), which means that the samples are greener than the control sample, except for the wines treated only with glutathione, which are redder than the control.

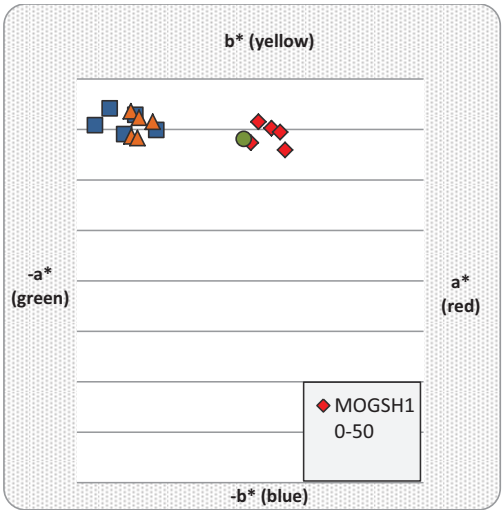

Figure 2. The *ab* diagram describing the position of the colour of Muscat Ottonel wines treated with ascorbic acid and/or GSH determined after 4 months of bottle aging

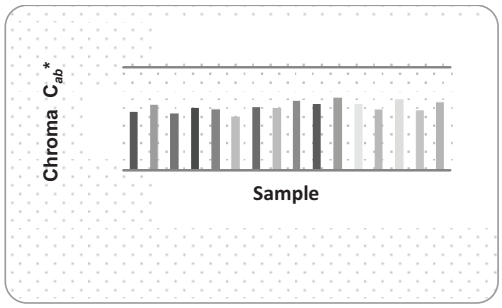

Figure 3. CIELab coordinate C_{ab}* of Muscat Ottonel wines after 4 months of bottle aging

This indicates that the usage of ascorbic acid provided a better antioxidant protection even in combination with glutathione, compared with using only GSH.

The comparison of samples containing ascorbic acid, whether they were treated only with increasing amounts of ascorbic acid or the fixed amount of 30mg/l ascorbic acid and increasing doses of glutathione, with the control sample MOControl showed that the samples treated only with ascorbic acid are predominantly greener than those treated with both antioxidants (Figure 2) and the variations of the parameter *b** (yellow-blue), the Δb* values, are mostly positive, so they were yellower than the control sample. This demonstrates that in the presence of large quantities of ascorbic acid the oxidation process is slowed, while glutathione has antioxidant effect only with the concomitant use of ascorbic acid.

Table 5 - Variations of CIELab coordinates of Muscat Ottonel wines after 4 months of bottle aging

Wine sample Code	ΔL*	Δa*	Δb*	ΔC_{ab}*	ΔE_{ab}*	ΔH_{ab}*
MOControl						
MOGSH10	-0.4786	0.0848	0.3364	0.3364	0.5911	0.0848
MOGSH20	-0.0929	0.0414	-0.0788	-0.0791	0.1287	0.0408
MOGSH30	-0.5670	0.1606	0.2084	0.2106	0.6251	0.1577
MOGSH40	-0.5921	0.2108	0.1318	0.1366	0.6422	0.2077
MOGSH50	-0.3713	0.2386	-0.2222	-0.2148	0.4941	0.2453
MOAA30	0.0747	-0.5044	0.1757	0.2251	0.5393	0.4844
MOAA40	0.5494	-0.6895	0.0943	0.1835	0.8866	0.6713
MOAA50	0.0139	-0.6255	0.4765	0.5423	0.7864	0.5694
MOAA60	0.7104	-0.8579	0.2720	0.3989	1.1466	0.8068
MOAA70	0.1342	-0.7727	0.6041	0.6985	0.9900	0.6885
MOAA30GSH10	0.1454	-0.5248	0.3374	0.3869	0.6406	0.4895
MOAA30GSH20	0.4698	-0.6474	0.0475	0.1280	0.8013	0.6364
MOAA30GSH30	-0.0525	-0.6506	0.5444	0.6139	0.8499	0.5855
MOAA30GSH50	0.4947	-0.6128	0.0150	0.0885	0.7877	0.6066
MOAA30GSH100	0.2182	-0.6039	0.4159	0.4787	0.7650	0.5554

Analysis of the variation of the parameter C_{ab}* (chroma) showed that the ΔC_{ab}* values are mostly positive (except for the samples MOGSH20 and MOGSH50), which means that they have a higher saturation than the control sample (Figure 4), so they have a higher brightness than the control sample.

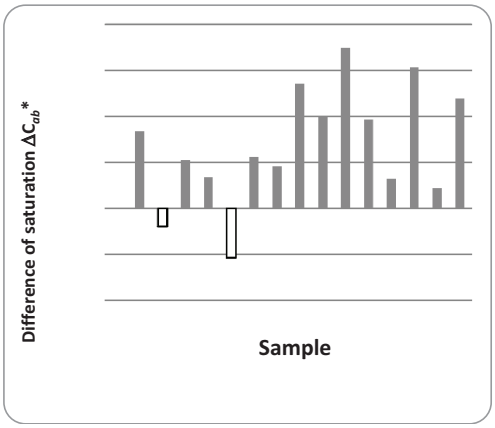

Figure 4. Graphic representation of the difference of saturation ΔC_{ab}* of Muscat Ottonel wines after 4 months of bottle aging

In order to determine whether there are global colour differences between different wine samples, the calculated values of the overall colorimetric difference parameter ΔE_{ab}* (Table 5) have been analysed. In accordance with the use of grey scale to compare two colours, if the difference between those two colours is under 0.5 units, it is considered barely perceptible; if the difference between the two colours is in the range of 0.5-1 units, it is considered slightly perceptible.

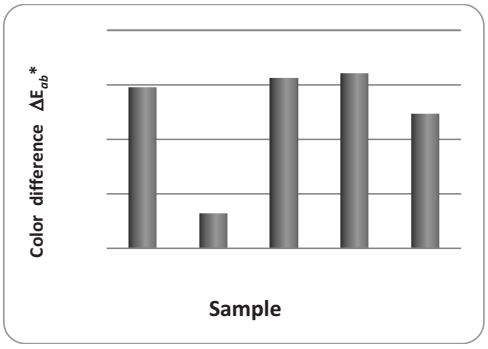

Figure 5. Graphic representation of the overall colour difference ΔE_{ab}* of Muscat Ottonel wines treated with various doses of GSH after 4 months of bottle aging

Overall, the values of the colorimetric difference ΔE_{ab}* between the samples with GSH and the control sample MOControl are situated over 0.5 units, meaning that the difference in colour between the sample wines and the control MOControl is slightly perceptible, except for some samples - namely MOGSH20 and MOGSH50, for which the difference is barely perceptible (Figure 5). The same conclusion can be drawn if we analyse the samples containing only ascorbic acid: the difference in colour between the wines and the control sample MOControl is also slightly perceptible (Figure 6).

There are some slightly perceptible differences between the control sample MOControl and the samples containing increasing doses of ascorbic acid, except for the sample treated with 30 mg/l, which did not show the same behaviour, the difference between this sample and the control sample being barely perceptible.

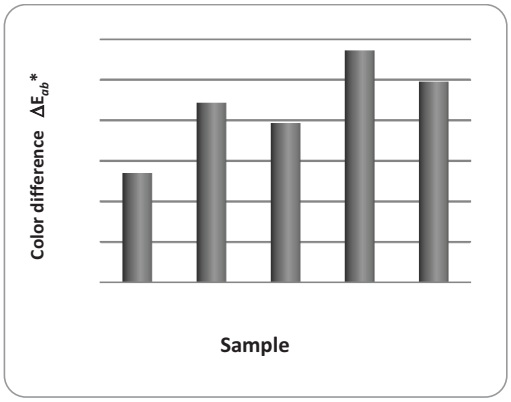

Figure 6. Graphic representation of the colour difference ΔE_{ab}* of Muscat Ottonel wines treated with various doses of ascorbic acid after 4 months of bottle aging

As we can observe in the Figure 7, the differences between samples containing 30 mg/l ascorbic acid combined with ascending doses of glutathione and MOAA30 containing only 30 mg/l ascorbic acid, considered as the control sample MOAA30Control, are barely perceptible, while comparing the same samples with the control sample without ascorbic acid, MOControl the differences are easier to perceive.

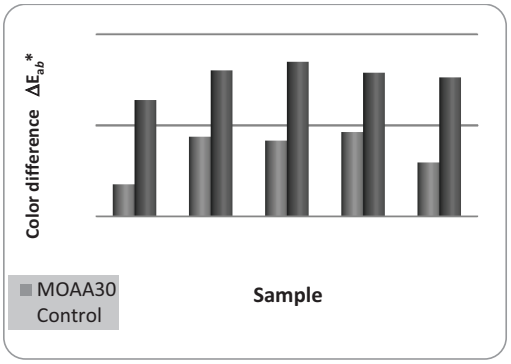

Figure 7. Graphic representation of the colour difference ΔE_{ab}* of Muscat Ottonel wines treated with 30mg/l ascorbic acid and various doses of GSH after 4 months of bottle aging

As regarding the variation of the parameter H_{ab}* (the hue), all the ΔH_{ab}* values are positive, which means that comparing with the control sample MOControl colour (considered yellow as the wine is white) all the wine samples, whatever the treatment applied, were greener, so less oxidised (Figure 8).

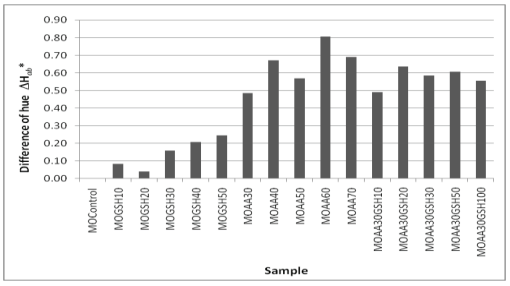

Figure 8. Graphic representation of the difference of hue ΔH_{ab}* between wine samples of Muscat Ottonel after 4 months of bottle aging

With the purpose to summarize the obtained results, we can make the following remarks:

-Regarding the wine samples treated only with GSH, we have noticed that the samples treated with higher doses than 30mg/l GSH showed a pinking effect, developing orange shades.

- Regardingthe overall color differences ΔE_{ab}* of the treated wines and the control sample MOControl, the values were not significantly different, but positive values recorded for the differences of tone ΔH_{ab}* have showed that all the samples treated with glutathione were greener than the control sample, which means that GSH has ensured some antioxidant

protection to the treated wines, even if not very advanced.

-The samples treated only with ascorbic acid in variable quantities evolved differently: all treated samples were greener than the control sample MOControl, which can be interpreted as a slowdown of the polyphenol oxidation, as previous studies demonstrated too. Regarding the variation of the yellow-blue parameter *b**, the most yellow sample, compared to the controlone, was MOAA5, the sample treated with 70mg/l ascorbic acid; thus we can conclude that the use of ascorbic acid had an obvious antioxidant effect.

-Analyzing the determined values of the color components *a** and *b** in the case of wine samples treated with a constant amount of 30mg/l ascorbic acid and varying amounts of glutathione, we found that all samples treated with GSH and ascorbic acid were greener than the control sample MOControl, as the Δa* has negative values, but in the same time the Δb*values are positive, so all the samples were yellower than the control. The overall colorimetric differences ΔE_{ab}* were in all cases barely perceptible (Figure 7), but the difference of hue ΔH_{ab}* had positive values (Figure 8), so the samples were greener than the control, which means that the combination of GSH and ascorbic acid assured a better antioxidant protection to the wines treated in this way, than the use of glutathione alone or in combinations with small quantities of ascorbic acid. This led to the conclusion that low quantities of glutathione, used alone or in combination with small dosage of ascorbic acid have no important effect on the yellow color of the wine, but in higher quantities these antioxidants showed a cumulative effect leading to the intensification of the yellow shades of wine samples. We must keep in mind that although negative values of the difference Δa* between the values of the colour parameter *a**- the red-green component of the color, can mean a slowing down of the polyphenols oxidation, while increasingvalues of the parameter *b**- the yellow-blue component of the colour with the increasing of the ascorbic acid quantity may indicate the formation of yellow xanthylium cation pigments, equivalent with a certain wine oxidation and degradation, as we mentioned previously.

CONCLUSIONS

The measured data revealed that treating wine with varying doses of glutathione alone could not assure an effective conservation of the color characteristics of the wine, which showed a tendency to develop shades of orange specific to browning reactions. In the case of wines treated with varying doses of ascorbic acid, higher doses of ascorbic acid were more appropriate for the conservation of the wine colour, and in the case of variable doses of glutathione together with a fixed dose of 30 mg/l ascorbic acid, the combination of these two antioxidants, (in the presence of small quantities of sulfur dioxide, of course), was found to have a synergistic antioxidant action and to be more effective for the conservation of the wine color characteristics. The glutathione and ascorbic acid in combination with the sulfur dioxide can improve wine stability and prevent the formation of atypical oxidative character during wine aging, but the most appropriate dosage of these antioxidants was not clearly determined until now. It is therefore important to continue the research to determine the minimum/optimal amounts of free sulfur dioxide, as well as the doses of GSH and ascorbic acid necessary to ensure the resistance of wine to oxidation and to extend its shelf life.

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