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SENSORY PROFILE CHANGES INDUCED BY THE ANTIOXIDANT TREATMENTS OF WHITE WINES - THE CASE OF GLUTATHIONE, ASCORBIC ACID AND TANNIN TREATMENTS ON FETEASCA REGALA WINES PRODUCED IN NORMAL CELLAR CONDITIONS

Arina Oana ANTOCE, George Adrian COJOCARU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems, 59 Mărăşti Blvd., District 1, 011464 Bucharest, Romania

Corresponding author emails: arina.antoce@horticultura-bucuresti.ro; aantoce@yahoo.com

Abstract

The present work aims to determine in which way the sensory profiles of the Feteasca regala white wines are affected by treatments with various antioxidants under normal wine cellar conditions and whether the changes induced are perceivable by tasters. The tested antioxidants were the reduced glutathione (GSH) and ascorbic acid (AA), both added during the fermentation of Feteasca regala musts, and also catechinic tannin and carbon dioxide added in the young wines during bottling.

The GSH in doses of 0, 20 and 40 mg/l was added in the must just before fermentation, with or without an addition of 50 mg/l AA. The following 50 l variants were thus obtained: G0, G20, G20A50, G40 and G40A50. The young wines were then racked and bottled, adding in each variant during the bottling process either 0 or 20 mg/l catechinic tannin (T0 and T20) and using or not carbon dioxide for protection against oxygen. The main sensory parameters of wines were analyzed by using a protocol developed in our laboratory and also ranked by using the OIV-UIO score sheet for wine contests. Sensory profiles for each variant were obtained and discussed. The parameters which affected the sensory profiles of the wines were statistically analyzed for groups of samples containing the same type of treatment, to determine the treatments with significant influences on the sensory parameters.

It was observed that in normal cellar conditions, where protection against oxygen is difficult to maintain in all winemaking stages, the GSH did not improve the aroma intensity and the floral scent perceived in the wines treated, the control wines scoring better for these parameters. The dose of 40 mg/l GSH improved both parameters as compared to the dose of 20 mg/l, but they were still under the values of the control samples. Also, the GSH used without AA increased the perception of bitterness, while in the presence of AA the bitterness induced by GSH was diminished. The tannin did not change the bitterness or the intensity of aroma, but influenced the floral scent, which was better perceived in the samples treated with it. Carbon dioxide treatments during bottling did not have a significant influence on any of the tested parameters.

Key words*: glutathione, ascorbic acid, catechinic tannin, sensory analysis.*

INTRODUCTION

Glutathione (GSH), ascorbic acid (AA), catechinic tannin (T) and carbon dioxide (CO_2) are usually used in white wine technologies in order to preserve for a longer time their primary and fermentation aroma. They are all naturally occurring in wines and are usually supplemented when necessary to act as complementary antioxidants to sulphur dioxide and to ensure the protection of sensitive compounds against the detrimental effect of oxygen. These antioxidants have various mechanisms of reaction in wine, thus the final composition and sensory properties related to them differ, too. On one hand, AA reacts with soluble oxygen, but forms hydrogen peroxide (Bradshaw et al., 2003; Scollary, 2002; Bradshaw et al., 2011), which can be even more detrimental then O_2 and sulphur dioxide is necessary to limit this effect. However, the combination of AA with SO_2, although highly important to prevent many detrimental actions of AA in wines, does not prevent the formation of certain phenolic pigment precursors (Barril et al., 2012) and possibly the oxidation of several aroma compounds too.

On the other hand GSH itself, although is an effective antioxidant, during its consumption generates degradation products which can

induce oxidative coloration in model wines (Sonni et al., 2011a), which is a sign that the aromatic profile may be affected too. Recent studies also showed that the combination of ascorbic acid and glutathione under some specific conditions may act as pro-oxidants in the wine matrix (Wegmann-Herr et al., 2015; Cojocaru and Antoce, 2016).

These added antioxidants also have an impact on the speed with which the dissolved oxygen is consumed. For example, some authors showed that the most active compound which reduces the dissolved oxygen is the SO_2, followed by AA, and GSH (Comuzzo et al., 2015). Other studies (Grant-Preece et al., 2013) also showed that the consumption of dissolved oxygen in wines preserved with SO_2 and other compounds is slower in the case of high concentrations of GSH added (174 mg/l) and faster in the case of 100 mg/l AA added. GSH however, occurs naturally in musts and wines in small concentrations, the maximum identified being 35 mg/l (du Toit et al., 2007) and its supplementation in musts and wine is only permitted by the International Organisation of Vine and Wine in doses of maximum 20 mg/l (OIV, 2016). Since it was under consideration and later approved as a valuable oenological practice, the studies of the effects of addition of glutathione in wine increased, extending also to sparkling wines (Webber et al., 2014, 2017).

As in the case of AA, the combination of GSH with sulphur dioxide is required. According to Panero et al. (2015), unlike AA and oenological tannins, the presence of GSH does not increase the consumption of SO_2, showing that the combination can ensure a longer protection.

Moreover, in the mechanisms of these antioxidants the catalytic role of metals cannot be disregarded either (Danilewicz, 2003, 2012). Thus, it is known already that some of these compounds used for wine protection have, in certain conditions, not only antioxidant behaviours, but also pro-oxidant actions.

Tannins are natural antioxidants too and their usage is more common in wine. Exogenous addition is used mainly in red wines, but it is also used in white wines or in sparkling wines (Fracassetti et al., 2016.). Several types of tannins are known, but in this study we used tannins extracted from the tea plant, which are very close to the tannins from grape seeds and are based on (+)-catechin, epicatechin and epicatechin gallate units (Prieur et al., 1994) and are known to contribute to wine bitterness and/or astringency (Cheynier et al., 2006). The addition of tannins proved beneficial in maintaining the ester concentrations over certain levels in wine stored for 1 year (Sonni et al., 2011b), likely due to their oxygen- and radical-scavenging ability (Danilewicz et al., 2008), as also suggested in other papers, where the tannins were added before fermentation (Sonni et al., 2009).

Interactions of various types of tannin with aroma compounds can also affect their volatility in different ways correlated with the concentration and type of tannin, thus influencing the overall aroma intensity and quality perceived (Mitropoulou et al., 2011).

Taking into account all these complex mechanisms and the difficulty to predict the evolution of wines under certain treatments, this research attempted to narrow down for further studies the effects of those antioxidants and their combinations and the sensory perceivable beneficial effects in white wines.

MATERIALS AND METHODS

The wine samples were prepared in September 2015 at the Department of Viticulture and Enology of the University of Agronomic Sciences and Veterinary Medicine of Bucharest from the Feteasca regala grapes cultivated in the experimental field.

The must was obtained by classical technology (using open destemmer-crusher and hydraulic bladder wine press equipment from Enoveneta, Italy) and left for 24 h to decant and clarify at 10°C. The clarified free-run must was then introduced in 5 stainless steel 50 l tanks, treated with glutathione (Carl Roth GmbH, L-glutathione reduced, purity min. 98% for biochemistry) and ascorbic acid (Carl Roth GmbH, L(+)-ascorbic acid ≥99 %, p.a. for biochemistry) in various concentrations. Physico-chemical parameters of the clarified free-run must were: 21.6% Brix; 82.65 meq/l total titratable acidity; pH 3.33 and 135 mg/l for YAN. The fermentation was performed with *Saccharomyces cerevisiae* Premium blanc 12 V yeast (Enologica Vason) with 20 g/hl activator (V Starter TF, Enologica Vason) added to

achieve a YAN in the optimum range (Cojocaru and Antoce, 2014).

After 3 weeks of alcoholic fermentation, the resulted wines were racked and treated with 70 mg/l sulfur dioxide and then held for another nine weeks at low temperature just above 0°C to achieve a better clarification and stabilisation. Then the wines were racked and bottled, each variant being additionally treated with a dose of 25 mg/l SO_2, with or without 20 mg/l catechinic tannin (Ti Premium, Enologica Vason) and/or filled with carbon dioxide (purity 99.99%) at the time of bottling. The treatments are described in Table 1.

The wines were evaluated in March 2016, 3 months after bottling.

The physico-chemical parameters of the wine variants are included in Table 2. The parameters determined were: the alcoholic strength (% vol. alc., OIV-MA-AS312-01A), total acidity (TA, meq/l, OIV-MA-AS313-01), pH, volatile acidity (VA, meq/l, OIV-MA-AS313-02), free SO_2 (ppm, OIV-MA-AS323-04A), total SO_2 (ppm, total OIV-MA-AS323-04A), dry extract (g/l, OIV-MA-AS2-03B), reducing sugars (g/l, OIV-MA-AS311-01A and non-reducing extract (g/l, OIV-MA-AS2-03B). The concentration of glutathione was not evaluated.

Table 1. Sample codes and antioxidant treatments

Sample No.	Tank/Sample cod	Gluta-thione (mg/l)	Ascorbic acid (mg/l)	Catechinic tannin (mg/l)	Carbon dioxide*
	Additive coding	**G**	**A**	**T**	**CO_2**
	Time of addition	**Winemaking**		**Bottling**	
1	G0_A0_T0	0	0	0	no
2	G20_A50_T0	20	50	0	no
3	G40_A50_T0	40	50	0	no
4	G20_A0_T0	20	0	0	no
5	G40_A0_T0	40	0	0	no
6	G0_A0_T20	0	0	20	no
7	G20_A50_T20	20	50	20	no
8	G40_A50_T20	40	50	20	no
9	G20_A0_T20	20	0	20	no
10	G40_A0_T20	40	0	20	no
11	G0_A0_T0_CO2	0	0	0	yes
12	G20_A50_T0_CO2	20	50	0	yes
13	G40_A50_T0_CO2	40	50	0	yes
14	G20_A0_T0_CO2	20	0	0	yes
15	G40_A0_T0_CO2	40	0	0	yes
16	G0_A0_T20_CO2	0	0	20	yes
17	G20_A50_T20_CO2	20	50	20	yes
18	G40_A50_T20_CO2	40	50	20	yes
19	G20_A0_T20_CO2	20	0	20	yes
20	G40_A0_T20_CO2	40	0	20	yes

*(bottle head-space filling)

Table 2. Physico-chemical parameters of Feteasca regala wines, 3 months after bottling

Sample cod	% vol. alc.	TA, meq/l	pH	VA, meq/l	Free SO_2, ppm	Total SO_2, ppm	Total dry extract, g/l	Reducing sugars, g/l	Non-reducing extract, g/l
G0_A0_T0	13.70	52.97	3.48	5.64	18.05	122.50	21.0	1.38	19.62
G20_A50_T0	13.60	53.56	3.50	5.37	22.52	130.83	20.8	1.24	19.56
G40_A50_T0	13.70	52.87	3.53	5.55	24.68	129.60	21.0	1.17	19.83
G20_A0_T0	13.70	54.05	3.50	5.38	18.67	124.20	21.2	1.43	19.77
G40_A0_T0	13.80	54.15	3.51	6.80	17.59	125.74	22.8	2.83	19.97
G0_A0_T20	13.80	53.36	3.53	6.89	19.59	120.80	21.1	1.43	19.67
G20_A50_T20	13.80	52.87	3.52	7.45	28.54	135.15	21.0	1.26	19.74
G40_A50_T20	13.65	52.67	3.52	8.05	31.94	140.39	21.1	1.32	19.78
G20_A0_T20	13.85	54.55	3.51	7.11	22.99	132.53	22.5	2.53	19.97
G40_A0_T20	13.60	53.86	3.52	6.93	18.67	122.65	20.8	1.17	19.63
G0_A0_T0_CO2	13.70	52.77	3.53	5.49	28.54	132.06	22.4	2.46	19.94
G20_A50_T0_CO2	13.65	53.66	3.53	4.96	29.00	138.39	20.9	1.17	19.73
G40_A50_T0_CO2	13.80	52.27	3.50	7.20	38.11	145.79	21.1	1.28	19.82
G20_A0_T0_CO2	13.70	53.95	3.50	7.73	28.39	139.16	21.0	1.33	19.67
G40_A0_T0_CO2	13.80	55.83	3.52	7.42	31.63	139.01	21.7	1.83	19.87
G0_A0_T20_CO2	13.80	54.15	3.52	6.08	26.38	126.82	20.8	1.17	19.63
G20_A50_T20_CO2	13.80	53.36	3.52	6.00	31.32	135.00	22.5	2.63	19.87
G40_A50_T20_CO2	13.55	52.97	3.52	7.45	32.71	143.79	21.2	1.68	19.52
G20_A0_T20_CO2	13.80	54.35	3.54	7.54	28.39	130.06	22.3	2.51	19.79
G40_A0_T20_CO2	13.70	54.25	3.52	7.31	25.92	133.45	20.8	1.13	19.67

For the sensory analyses 3 bottles of each variant were used. The sensory analyses were performed with a panel of tasters of 5 persons in accordance with the methodology developed in the project Sensofood and a registered patent (Antoce 2005; Antoce and Namolosanu, 2007). From the Sensofood evaluation sheet the following parameters were selected as being representative for this type of samples:

perception of acidity, sweetness, astringency, bitterness, mouth feel, as well as aroma intensity and the overall floral scent.
The parameters are all evaluated on continuous intensity scales with maximum value of 10, anchored with appropriate descriptors. Only the perception of floral aroma was evaluated on discontinuous scales of 5 points, with the values of 2, 4, 6, 8 and 10.
The significance of the values obtained for various sensory parameters was tested with the Origin 9.0 one-way ANOVA and the Tukey post-hoc analysis for the multiple comparison of the mean differences. The averages that are significantly different from others are indicated with different letters, while a similar letter means there was not a significant difference between those groups at $p > 0.05$. All the bottle samples were analyzed in triplicate.

RESULTS AND DISCUSSIONS

In order for wine tasters to be able to compare samples in a meaningful way, the variants were grouped in accordance to a main antioxidant component. Thus, the following groups are formed (Table 3).

Table 3. Groups of wine variants assembled in accordance with the type of antioxidant treatment

Sample No.	Control sample	Group name	Tank/Sample code
1	Control absolute	**Control**	G0_A0_T0
11	Control CO_2		G0_A0_T0_CO2
6	Control tannin		G0_A0_T20
16	Control tannin + CO_2		G0_A0_T20_CO2
6	Control tannin	**T20**	G0_A0_T20
7			G20_A50_T20
8			G40_A50_T20
9			G20_A0_T20
10			G40_A0_T20
4	Control G20	**G20**	G20_A0_T0
14			G20_A0_T0_CO2
9			G20_A0_T20
19			G20_A0_T20_CO2
5	Control G40	**G40**	G40_A0_T0
15			G40_A0_T0_CO2
10			G40_A0_T20
20			G40_A0_T20_CO2
2	Control G20A50	**G20A50**	G20_A50_T0
12			G20_A50_T0_CO2
7			G20_A50_T20
17			G20_A50_T20_CO2
3	Control G40A50	**G40A50**	G40_A50_T0
13			G40_A50_T0_CO2
8			G40_A50_T20
18			G40_A50_T20_CO2

Sensory profiles

In accordance to the groups described in Table 3, general group sensory profiles were determined, based on each parameter average for the respective group. The general profiles of groups are shown all in Figure 1.

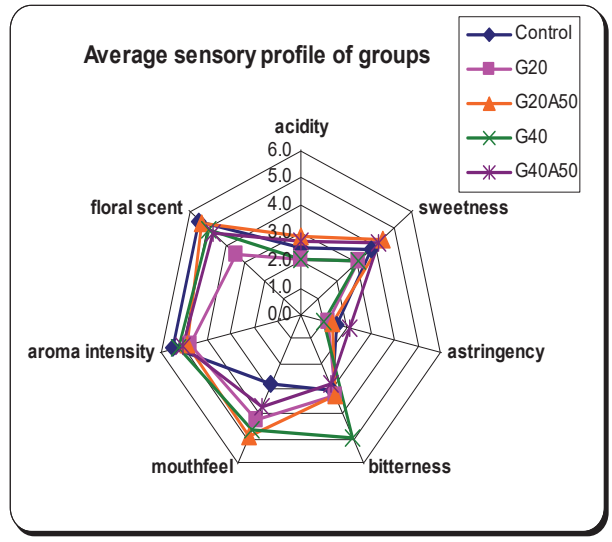

Figure 1. Average sensory profile of the wines grouped in accordance to the antioxidant treatments: glutathione 20 mg/l and 40 mg/l (groups G20 and G40), glutathione 20 mg/l and 40 mg/l plus ascorbic acid 50 mg/l (groups G20A50 and G40A50) and Control group

It can be seen that the samples included in the groups G20 (treated at fermentation with 20 mg/l glutathione) were considered by the tasters less floral and less intensely aromatic than the control samples, while the samples of G40 (treated at fermentation with 40 mg/l glutathione) were superior from this viewpoint.
Generally, the sensory profiles of the groups containing samples also treated with ascorbic acid (G20A50 and G40A50) are very similar, suggesting that the presence of ascorbic acid is dominant in the sensory profile, the dose of glutathione being less important when the wines are also treated with ascorbic acid.
As each group was composed of variants containing additional treatments, some with tannin and/or carbon dioxide used at the bottling time, profiles of the samples included in each group were also generated and discussed.
Figures 2 contain the profiles for each group of wine samples and their samples treated or not with tannin and carbon dioxide.

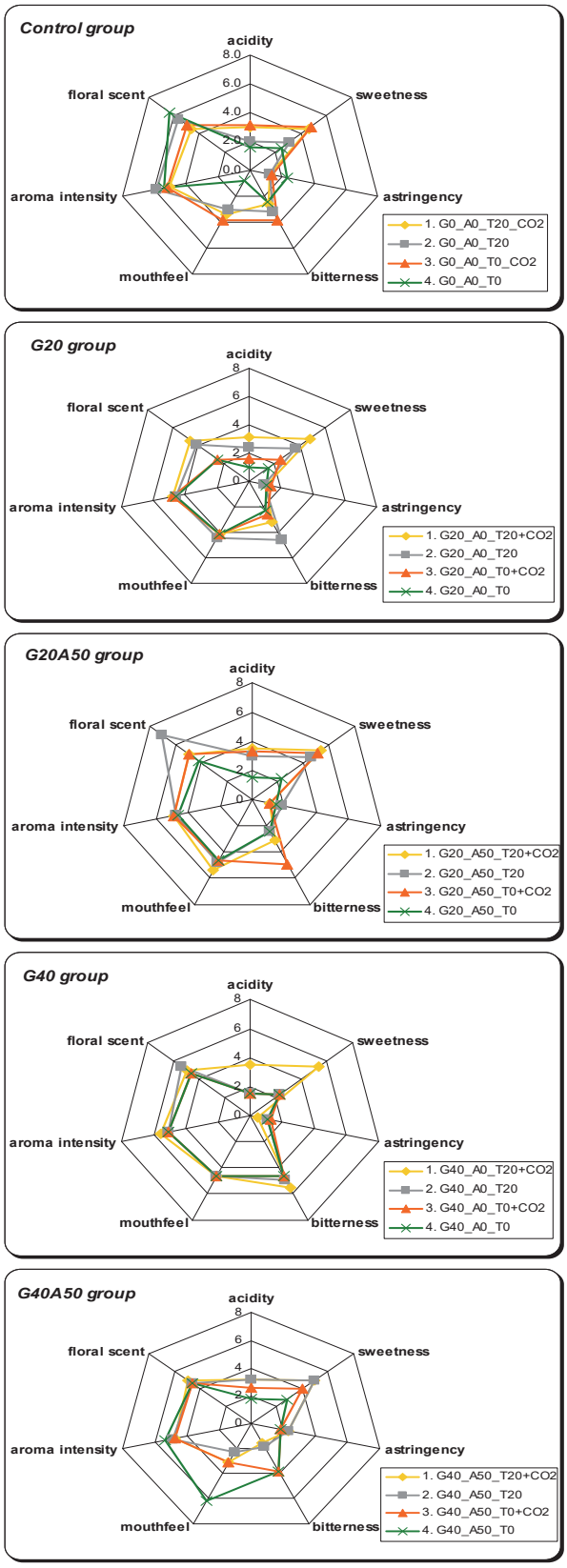

Figure 2. Sensory profile of the wines included in the groups of Control, G20, G20A50, G40 and G40A50, in accordance to the additional antioxidant treatments: 0 or 20 mg/l catechinic tannin (T0, T20) and presence or absence of carbon dioxide (CO2) at bottling time. The numbers allocated to samples represent the rank after an additional sensory evaluation based on the 100 points OIV score sheet

The wines of each group were also analyzed by the tasters using the OIV-UIO score sheet of 100 points (OIV, 2009 a and b) and based on their results (not shown) the samples were ranked from 1 to 4, 1 being the wine with the highest score in the group and 4 the lowest score in the group. To be more suggestive, the highest score sample was colored in gold, the second in silver, the third in bronze and the last one in green, which does not necessarily imply they obtained the scores usually required to be awarded such medals in OIV wine contests. The scores of wines ranged from 78 to 85.

As it can be seen the sensory parameters that had positive impact on the appreciation of wines were the sweetness, aroma intensity and the floral scent, while the bitterness had a negative impact.

In order to determine how these sensory parameters are influenced by the antioxidant treatments, statistical analysis was used to determine the significant differences induced by several types of treatments and combinations of treatments.

Thus, each main sensory parameter was analyzed by using the one way analysis of variance (ANOVA) and Tukey test.

The perception of acidity

When we compared the groups of samples containing treatments with GSH and AA, irrespective of the content of tannin or CO_2 the perception of acidity is not significantly different (Table 4).

Table 4. Acidity sensory values for samples in groups with 0, 20 and 40 mg/l GSH with or without 50 mg/l AA, irrespective of other treatments

Group	Control	G20	G40	G20A50	G40A50
Average*	**2.4[a]**	**2.0[a]**	**2.0[a]**	**2.8[a]**	**2.7[a]**
Standard Deviation	0.7	0.9	1.0	0.9	0.7
Standard Error	0.4	0.5	0.5	0.5	0.3

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 0.78, while the probability that the null hypothesis for the full model is true (the perception of acidity in al groups is similar) is Prob>F = 0.55

As the presence of ascorbic acid in the samples seems to increase the acidity, we tested the values obtained for all the samples with and without AA and found that the difference is still not significant, in spite of better values obtained for F (Table 5).

Table 5. Acidity sensory values for samples treated with GSH, irrespective of the dosage (G), without (group G) or with AA (group GA50), irrespective of other treatments

Group	G	GA50
Average*	**2.8**[a]	**2.0**[a]
Standard Deviation	0.7	0.9
Standard Error	0.3	0.3

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 3.4, while Prob>F is 0.09.

The perception of sweetness

The perception of sweetness is mostly dependent on the perception of acidity, but also on the completion of fermentation in all samples. The results were tested with ANOVA (Table 6) and no significant difference was found among the groups (F value is 0.78, Prob>F is 0.55).

Table 6. Sweetness sensory values for samples in groups with 0, 20 and 40 mg/l GSH with or without 50 mg/l AA

Group	Control	G20	G40	G20A50	G40A50
Average*	**3.7**[a]	**3.1**[a]	**3.1**[a]	**4.3**[a]	**4.2**[a]
Standard Deviation	1.1	1.4	1.5	1.4	1.0
Standard Error	0.6	0.7	0.8	0.7	0.5

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 0.78, while Prob>F is 0.55.

The perception of astringency was rather low in all the samples, varying from 0.9 to 2.3 on a scale out of 10, therefore the significance of its variation was not tested.

The perception of bitterness

Bitterness is one of the important parameters, especially in the white wines, where, unlike in the case of some red wines, it is mainly undesirable for the overall harmony of the wine.

The bitterness was perceived as clearly different only for the wine in the group containing GSH in the concentration of 40 mg/l.

The Tukey test revealed that the samples treated with 40 mg/l GSH are significantly different from the samples treated with the same concentration of GSH, but also containing AA (Table 7).

Table 7. Bitterness sensory values for samples in groups with 0, 20 and 40 mg/l GSH with or without 50 mg/l AA, irrespective of other treatments

Group	Control	G20	G20A50	*G40*	*G40A50*
Average*	**3**[a,b]	**3.2**[a,b]	**3.2**[a,b]	***4.9***[b,d]	***2.8***[a,c]
Standard Deviation	0.6	1.0	1.2	0.4	1.2
Standard Error	0.3	0.5	0.6	0.2	0.6

*At the level of 0.05 the population means of the groups are significantly different. The F value is 3.2, while Prob>F is 0.04.

As the bitterness is expected to be influenced by the presence of tannin, the results were also tested taking into account the presence/absence of tannin. Thus, the groups of samples with and without tannin were analyzed and we found out that the presence of tannin increases the bitterness in the samples treated with GSH, but not in the ones containing also AA. The effect is mostly evident in the case of groups GT and GA50T, where the values of bitterness are significantly different, 4.6 ± 1.0 and 2.2 ± 0.7, respectively (Table 8).

Table 8. Bitterness sensory values for samples with and without tannin (T), grouped in accordance with the presence of GSH (G), Ascorbic acid (A50)

Group	G	GA50	*GT*	*GA50T*
Average*	**3.5**[a,b]	**3.8**[a,b]	***4.6***[b,d]	***2.2***[a,c]
Standard Deviation	1.2	1.0	1.0	0.7
Standard Error	0.6	0.5	0.5	0.3

*At the level of 0.05 the population means of the groups are significantly different. The F value is 3.8, while Prob>F is 0.04.

However, when the treatment with AA is disregarded and we compared the samples with tannin and without tannin, there was no significant difference between the group without tannin (bitterness 3.7 ± 1.0) and the group with tannin (bitterness value 3.4 ± 1.5), the F value being 0.15 and Prob>F being 0.7.

The perception of aroma intensity

The aroma intensity is the parameter that was considered the most important in this study, as the fermentation of wines in the presence of GSH was meant to enhance the aromatic profile of the wines produced in this way.

Also, the treatment with tannin and the addition of CO_2 during bottling were also factors meant to preserve the primary and fermentation aroma of the wines.

The comparison of the wine sample groups, irrespective of the tannin and CO_2 added is presented in Table 9.

Table 9. Aroma intensity sensory values for samples in groups with 0, 20 and 40 mg/l GSH with or without 50 mg/l AA, irrespective of other treatments

Group	*Control*	*G20*	G40	*G20A50*	G40A50
Average*	***5.4***[c,d]	***4.7***[a,b]	**5.2**[a,c]	***4.8***[a,b]	**5.0**[a,d]
Standard Deviation	0.3	0.1	0.2	0.1	0.3
Standard Error	0.2	0.1	0.1	0.1	0.1

*At the level of 0.05 the population means of the groups are significantly different. The F value is 5.7, while Prob>F is 0.005.

In spite of the small differences among the average values, the perception of aroma intensity of the control group is clearly

different from the rest of the groups. Moreover, the values show that the control samples have the most intense aroma, but they are confirmed to differ significantly only from the samples treated with the 20 mg/l GSH (irrespective of the presence of ascorbic acid). Thus, it appears that the treatment with GSH did not lead to an increase of the aroma intensity, but, on the contrary, the dose of 20 mg/l induced a decrease of aroma intensity (from 5.4 ± 0.3 in control to 4.7 ± 0.1 in G20 and 4.8 ± 0.1 in G20A50). In the same time, the sample with 40 mg/l GSH was not significantly different from the control samples (5.2 ± 0.1 in G40 and 5.0 ± 0.3 in G40A50 group).

If we also disregard the effect of ascorbic acid, by including the samples treated with AA in the groups with the same amount of GSH added at fermentation, the same effect of GSH treatment is observed (Table 10).

Table 10. Aroma intensity sensory values for samples treated with 20 and 40 mg/l GSH, irrespective of the presence of ascorbic acid, tannin or CO_2

Group	*Control*	*G20a*	G40a
Average*	***5.4***^{a}	***4.8***^{b}	**5.1**^{a,b}
Standard Deviation	0.3	0.1	0.3
Standard Error	0.2	0.1	0.1

*At the level of 0.05 the population means of the groups are significantly different. The F value is 10.0, while Prob>F is 0.001.

However, from Table 10 it seems that the samples with 40 mg/l GSH tend to have slightly increased aroma intensity as compared to the case of samples with 20 mg/l GSH. To test this hypothesis, we compared the groups with samples containing 20 mg/l and 40 mg/l GSH, irrespective of the other treatments (Table 11).

This time it was confirmed that an increased dosage of GSH significantly increases the aroma intensity.

Table 11. Aroma intensity sensory values for samples with 20 and 40 mg/l GSH (G20 and G40) irrespective of any other treatment

Group	G20	G40
Average*	**4.8**^{a}	**5.1**^{b}
Standard Deviation	0.1	0.3
Standard Error	0.1	0.1

*At the level of 0.05 the population means of the groups are significantly different. The F value is 9.4, while Prob>F is 0.008.

In order to assess the tannin influence on the aroma intensity, we compared on one hand the groups of wine samples with and without tannin (T), with GSH treatments of 20 and 40 mg/l, but irrespective of the presence of AA (Table 12- groups G20, G20T, G40, G40T) and on the other hand the groups of wine samples with and without tannin (T) with treatments of GSH irrespective of the dosage, in the presence or absence of AA (Table 13 – groups G, GT, GA50, GA50T).

Table 12. Aroma intensity sensory values for samples with and without tannin (T), grouped in accordance with the dosage of GSH (G20 and G40) irrespective of the dosage of ascorbic acid

Group	G20	G20T	G40	G40T
Average*	**4.7**^{a}	**4.8**^{a}	**5.1**^{a}	**5.1**^{a}
Standard Deviation	0.1	0.1	0.3	0.3
Standard Error	0.1	0.1	0.1	0.2

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 2.72, while Prob>F is 0.09.

Table 13. Aroma intensity sensory values for samples with and without tannin (T), grouped in accordance with the presence of AA (A50) and GSH (G) irrespective of its dosage

Group	G	GT	GA50	GA50T
Average*	**4.9**^{a}	**5.0**^{a}	**4.9**^{a}	**4.8**^{a}
Standard Deviation	0.2	0.4	0.3	0.1
Standard Error	0.1	0.2	0.2	0.1

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 0.21, while Prob>F is 0.89.

As we can see from both Tables 12 and 13, the tannin treatment at bottling does not significantly influence the intensity of the wine sample aroma, irrespective of the other treatments.

In order to assess the influence of carbon dioxide on the preservation of the aroma intensity, we compared the groups of wine samples bottled with and without carbon dioxide (CO_2). In Table 14 we compared groups with and without carbon dioxide also treated with 20 and 40 mg/l GSH, irrespective of the presence of AA (groups G20, G20CO2, G40, G40CO2), while in Table 15 we compared groups with and without carbon dioxide also treated with GSH irrespective of dosage, but in the presence or absence of AA (groups G, GCO2, GA50, GA50CO2).

Table 14. Aroma intensity sensory values for samples with and without carbon dioxide (CO_2), grouped in accordance with the dosage of GSH (G20 and G40) irrespective of the dosage of ascorbic acid

Group	*G20*	G20CO2	*G40*	G40CO2
Average*	***4.7***^{a}	**4.8**^{a,b}	***5.1***^{b}	**5.0**^{a,b}
Standard Deviation	0.1	0.1	0.2	0.4
Standard Error	0.1	0.1	0.1	0.2

*At the level of 0.05 the population means of the groups are significantly different. The F value is 3.731, while Prob>F is 0.04.

Table 15. Aroma intensity sensory values for samples with and without carbon dioxide (CO_2), grouped in accordance with the presence of AA (A50) and GSH (G) irrespective of its dosage

Group	G	GCO2	GA50	GA50CO2
Average*	**4.8^{a}**	**5.0^{a}**	**4.9^{a}**	**4.8^{a}**
Standard Deviation	0.3	0.4	0.3	0.1
Standard Error	0.1	0.2	0.2	0

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 0.41 while Prob>F is 0.75.

As we can see, from Tables 14 and 15, the presence of carbon dioxide induced no significant difference in the perception of the aroma intensity of the samples. Although the average values of the groups in Table 14 are statistically determined to be different (G40 group having a significantly higher average value than the group G20), this is not due to the effect of protection conferred by the carbon dioxide, but to the treatment with GSH, the samples treated with 40 mg/l GSH being more intense (5.1 ± 0.2) than those treated with only 20 mg/l GSH (4.7 ± 0.1). Aside of this the Tukey test did not show any other significant difference for this sensory parameter. Thus, we can safely say that the carbon dioxide has no major effect on aroma intensity under the conditions employed.

The perception of floral scent

Due to the reported effect of GSH in white wines produced under reductive conditions, the fermentation in the presence of additional glutathione concentrations is expected to be beneficial for the primary and secondary wine aroma preservation.

Thus, the evaluation of the floral scent perceived by the tasters in the samples produced with GSH (with or without AA) is important for the confirmation of this hypothesis. Thus, in Table 16 the results for floral scent evaluation are presented for the groups of wines produced with 20 and 40 mg/l GSH, with or without 50 mg/l AA.

Table 16. Sensory values for floral scent perception for samples in groups with 0, 20 and 40 mg/l GSH with or without 50 mg/l AA, irrespective of other treatments

Group	*Control*	*G20*	G40	G20A50	G40A50
Average*	***5.4^{a}***	***3.4^{b}***	**4.9^{a,b}**	**5.3^{a,b}**	**4.7^{a,b}**
Standard Deviation	0.8	1.1	0.4	1.3	0.2
Standard Error	0.4	0.6	0.2	0.6	0.1

*At the level of 0.05 the population means of the groups are significantly different. The F value is 3.41, while Prob>F is 0.004.

As it can be seen, the floral aroma differs among the groups, with the control wines ranking among the most floral ones, in spite of the reasonable expectations for this semi-aromatic variety that the treated wines would have an enhanced floral aroma. We observed, and the Tukey test confirmed, that as in the case of aroma intensity sensory parameter, the treatment with only 20 mg/l GSH actually decreased the floral scent in our wines as compared to the control wine group. As far as this parameter was concerned the effect of AA was not conclusive.

When the effect of ascorbic acid is also disregarded, by including the samples treated with AA in the groups with the same amount of GSH added at fermentation, the control group seems again to have the most floral aroma (Table 17). However, a larger standard deviation in the group containing treatments with 20 mg/l GSH the statistical tests could not confirm any significant difference among the three groups.

Table 17. Sensory values for floral scent perception for samples treated with 20 and 40 mg/l GSH, irrespective of the presence of ascorbic acid, tannin or CO_2

Group	Control	G20a	G40a
Average*	**5.4^{a}**	**4.4^{a}**	**4.8^{a}**
Standard Deviation	0.8	1.5	0.3
Standard Error	0.4	0.5	0.1

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 1.4, while Prob>F is 0.27.

For this parameter too, in order to assess the influence of the added tannin on the floral scent of the bottled wines, in Table 18 there were compared the groups of wine samples with and without tannin (T), with treatments of GSH of 20 and 40 mg/l, irrespective of the presence of AA, while in Table 19 there were compared the samples with treatments of GSH irrespective of the dosage, in the presence or absence of AA. In both cases no significant differences occurred regarding the perception of a floral aroma, irrespective of the other treatments.

Table 18. Sensory values for floral scent perception for samples with and without tannin (T), grouped in accordance with the dosage of GSH (G20 and G40) irrespective of the dosage of ascorbic acid

Group	G20	G20T	G40	G40T
Average	**3.5^{a}**	**5.2^{a}**	**4.6^{a}**	**5.0^{a}**
Standard Deviation	1.2	1.3	0.1	0.3
Standard Error	0.6	0.6	0.1	0.2

At the level of 0.05 the population means of the groups are not significantly different. The F value is 2.7, while Prob>F is 0.09.

Table 19. Sensory values for floral scent perception for samples with and without tannin (T), grouped in accordance with the presence of AA (A50) and GSH (G) irrespective of its dosage

Group	G	GT	GA50	GA50T
Average*	**3.6**[a]	**4.8**[a]	**4.6**[a]	**5.4**[a]
Standard Deviation	1.2	0.5	0.3	1.2
Standard Error	0.6	0.3	0.1	0.6

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 2.9, while Prob>F is 0.08.

However, from Table 18 it seems that the samples with tannin tend to have slightly increased aroma intensity. To test this hypothesis, we compared the groups with sample groups containing GSH irrespective of dosage (G), with the sample group with GSH irrespective of dosage and 20 mg/l tannin (GT) and the results are included in Table 20.

Table 20. Sensory values for floral scent perception for samples with GSH (G) or with GSH and 20 mg/l tannin (GT), irrespective of GSH dosage or other treatments

Group	G	GT
Average*	**4.1**[a]	**5.1**[b]
Standard Deviation	1.0	0.9
Standard Error	0.4	0.3

*At the level of 0.05 the population means of the groups are significantly different. The F value is 4.7, while Prob>F is 0.0.05.

As clearly shown by the statistical parameters, the treatment with tannin before bottling of the samples produced with GSH added at fermentation confers an advantage for the preservation of the floral aroma of these wines.
To also test if the GSH dosage tends to have a perceivable influence on the floral aroma we compared the groups containing samples treated with 20 mg/l GSH (G20) and 40 mg/l GSH (G40), irrespective of the other treatments (Table 21).

Table 21. Sensory values for floral scent perception for samples with 20 and 40 mg/l GSH (G20 and G40) irrespective of any other treatment

Group	G20	G40
Average*	**4.4**[a]	**4.8**[a]
Standard Deviation	1.5	0.3
Standard Error	0.5	0.1

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 0.6, while Prob>F is 0.0.45.

Although the dosage of 40 mg/l seems to be more helpful than the 20 mg/l, due to a higher variability in the group G20, statistically no significant difference was found among the groups as regards the floral scent parameter.
In order to assess the carbon dioxide influence on the floral scent perception, the groups of wine samples with and without carbon dioxide (CO_2) were compared, on one hand taking into account the GSH dosage of 20 and 40 mg/l (Table 22) and on the other hand taking into account the presence or absence of AA in the presence of GSH irrespective of the dosage (Table 23).
In both cases the bottling with CO_2 did not cause any significant perceivable difference in the floral scent of wines.

Table 22. Sensory values for floral scent perception for samples with and without carbon dioxide (CO_2), grouped in accordance with the dosage of GSH (G20 and G40) irrespective of the dosage of ascorbic acid

Group	G20	G20CO2	G40	G40CO2
Average*	**4.5**[a]	**4.2**[a]	**4.8**[a]	**4.8**[a]
Standard Deviation	1.9	1.2	0.4	0.2
Standard Error	1.0	0.6	0.2	0.1

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 0.22, while Prob>F is 0.88.

Table 23. Sensory values for floral scent perception for samples with and without carbon dioxide (CO_2), grouped in accordance with the presence of AA (A50) irrespective of the dosage of GSH (G)

Group	G	GCO2	GA50	GA50CO2
Average*	**4.2**[a]	**4.2**[a]	**5.1**[a]	**4.8**[a]
Standard Deviation	1.2	1.1	1.3	0.2
Standard Error	0.6	0.6	0.7	0.1

*At the level of 0.05 the population means of the groups are not significantly different. The F value is 0.83 while Prob>F is 0.5.

CONCLUSIONS

A combination of antioxidant substances such as SO_2, GSH, AA, tannin and CO_2 added during fermentation or at bottling time could improve the sensory perception of some wine characteristics, but the results depends on the combination and winemaking conditions.
In reductive winemaking conditions GSH was demonstrated by several authors to have a positive effect on white wine aroma preservation. Other antioxidants, such as AA, tannin or CO_2, are also reported to be efficient when used for the same purpose in various phases of winemaking. Many of the tests, however, were conducted in wine model solutions or in highly controlled laboratories or cellars, but not in normally equipped wine cellars, where the wines are difficult to protect from oxygen in all the production steps. Thus, the present study investigated the sensory perceivable effects of these antioxidants on the final wines when a total protection from oxygen is not ensured.

It was shown that even in such conditions some sensory characteristic differences are perceivable and should be taken into account when deciding a specific treatment.
The ranking of the samples inside each group of treatment (based on GSH of 20 and 40 mg/l, with or without 50 mg/l AA) by using the OIV wine contest score sheet showed that the sensory parameters that had positive impact on the appreciation of wines were the sweetness, aroma intensity and the floral scent, while the bitterness had a negative impact.

The influence of GSH
Under the conditions of our study and for the Feteasca regala wine, we observed that the GSH did not improve the aroma intensity and the floral scent perceived in the wines treated, the control wines scoring better for these parameters.
The intensity of the aroma was not enhanced by any of the treatments. The control samples are the most intense (5.4 ± 0.3), but they are confirmed to differ significantly only from the samples treated with the 20 mg/l GSH group, irrespective of the presence of AA, which shows the lowest value for the parameter (4.7 ± 0.1). However, the samples with 40 mg/l GSH tend to have slightly increased aroma intensity as compared to the case of samples with 20 mg/l GSH and this is statistically confirmed when comparing only the wines grouped only according to GSH dose criterion.
The perception of floral scent is as well highest in the control group, the treatment with only 20 mg/l GSH actually decreasing the floral scent in wines as compared to the control wine group.
Thus, we can say that the dose of 40 mg/l GSH improved both parameters (aroma intensity and floral scent) as compared to the dose of 20 mg/l, but they were still under the values of the control samples.
The perception of acidity and sweetness is not significantly influenced by the presence of GSH, but we observed that the bitterness was increased at higher doses of GSH.

The influence of AA
The AA did not significantly influence the perception of the acidity, as well as that of the sweetness.
Although the GSH used without AA increased the perception of bitterness, the presence of AA diminished the bitterness induced by GSH. Thus, the samples treated with 40 mg/l GSH (with or without tannin) are significantly bitter (4.9 ± 0.4) than the samples similarly treated, but also containing AA (2.8 ± 1.2). We can say that in this experiment the increase in bitterness was mostly due to the treatment with a higher dose of GSH and the decrease was due to the treatment with the AA.
Generally, the sensory profiles of the groups containing samples treated both with GSA and AA (G20A50 and G40A50) appeared very similar, the effect of ascorbic acid being dominant in the sensory profile, covering the effect of GSH, irrespective of the dosage.
Regarding the influence of AA on the floral scent of wines the results were not conclusive.

The tannin influence
The combination of GSH, AA and tannin affected the bitterness in specific ways. Basically, the tannin did not change the perceived bitterness. We observed that the presence of tannin seemed to increase the bitterness in the samples treated with GSH, but not in the ones containing also AA. The effect of AA in lowering the bitterness was mostly evident in the case of groups GT and GA50T, where the values of bitterness were significantly different, 4.6 ± 1.0 and 2.2 ± 0.7, respectively. But when the treatment with AA was disregarded there was no significant difference in bitterness perception between the group without tannin (3.7 ± 1.0) and the group with tannin (value 3.4 ± 1.5).
The tannin and the carbon dioxide treatments at bottling did not significantly influence the intensity of the wine aroma, irrespective of the other treatments applied.
For the floral scent perception in wine, the treatment with tannin before bottling of the samples produced with GSH added at fermentation confers an advantage proven by statistical analysis.
The dosage of GSH used with the tannin seems also important, but due to the high variability of the samples, this effect of GSH dose could not be proven.

The influence of carbon dioxide

Carbon dioxide treatments at bottling did not have a significant influence on the perceivable floral scent of wines, nor on the intensity of the wine aroma, irrespective of the other treatments applied. Basically, carbon dioxide treatments at bottling did not have any significant influence on any of the tested parameters.

The overall conclusion of this study, derived from the sensorial perception of the final wine quality, is that under normal cellar conditions the usage of GSH appears to be technically and economically unjustified.

Conflicts of interest

The authors declare no financial or commercial conflicts of interest.

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