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ORIGINAL RESEARCH ARTICLE

Effect of SO_2, glutathione and gallotannins on the shelf-life of a Cortese white wine bottled with different oxygen intakes

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ABSTRACT

The work aimed to study the effect of adding reduced glutathione (GSH) and/or gallotannins at bottling on the shelf-life of a Cortese white wine to limit the use of SO_2.

The experimental trial was carried out following a full factorial plan consisting of 8 experimental trials that differed for the content of free SO_2 (2 levels: 20 and 40 mg/L), the absence/presence of gallotannins (0 and 40 mg/L) and GSH (0 and 20 mg/L). The wines of the eight trials were oxygenated respectively at 5.5 ppm and 4.0 ppm of O_2. The wines oxygenated at 5.5 ppm were bottled in 135 mL bottles and monitored during 12 months of storage (colour, polyphenolic composition, GSH, free and total SO_2); the wines oxygenated at 4.0 ppm of O_2 were bottled in 750 mL bottles, then analysed and tasted after 15 months. The oxygen consumption rate (OCR) was measured with a luminescence-based technology. The OCR followed a first-order kinetic in all the wines, and a significant OCR acceleration was observed when increasing the concentration of free SO_2. A significant increase in OCR was also observed in the samples with GSH, albeit to a lesser extent than with SO_2, while the addition of gallotannins caused a decrease in the OCR. It is possible that similar mechanisms are at the basis of the acceleration of OCR observed with GSH and SO_2. However, unlike SO_2, GSH showed poor antioxidant efficacy in the protection of both the colour and the aromatic component, probably also due to its lower molar concentration.

The presence of GSH limited the oxidative losses of SO_2, mostly in wines with higher SO_2 levels. However, the effect of GSH decreased over time, since after 8 months, GSH was only present in traces. The resistance to oxidative browning of wines during bottle storage depends on the concentration of free SO_2 present in the medium (> 10 mg/L), therefore, higher GSH intakes, such as to ensure sufficient preservation of the free SO_2 content during storage, will have to be tested to evaluate the possibility of prolonging the shelf-life of wines.

KEYWORDS: sulphur dioxide, glutathione, gallotannins, white wines, oxygen consumption rate

INTRODUCTION

Oxidation of white wines with consequent browning and loss of typical aroma is a long-standing problem in white winemaking. Phenolics, being the major substrate for oxygen, are compounds of must and wine responsible for browning (Singleton, 1987). Oxidation can be enzymatic or non-enzymatic (chemical).

The enzymatic oxidation occurs in grape musts after pressing, and it is very fast; the enzymes involved are the polyphenol oxidases (PPO): natural grapes tyrosinase and, in grapes infected by *Botrytis cinerea*, laccase, which oxidise must phenols into the respective o-quinones (Singleton, 1987). The enzymatic browning of a grape must is highly related to the content of hydroxycinnamates such as caffeoyltartaric acid (caftaric acid) and para-coumaroyltartaric acid (coutaric acid) (Cheynier *et al.*, 1990), and it is promoted by flavan-3-ols (Cheynier *et al.,* 1995).

Non-enzymatic (chemical) oxidations occur during wine ageing. The cascade of the oxidative process starts with the oxidation of polyphenols containing an ortho-dihydroxybenzene (catechol) ring, such as (+)-catechin, (−)–epicatechin, caffeic acid and its esters, or a 1,2,3-trihydroxybenzene (galloyl) group, such as (–)–epigallocatechin, gallic acid and its esters, that are the most readily oxidisable compounds in wine (Singleton, 1987; Singleton, 2000; Kilmartin *et al.*, 2001; Danilewicz, 2003; Li *et al*., 2008). These substrates are oxidised to semiquinones and quinones, while oxygen is reduced to hydrogen peroxide. Since oxygen cannot react directly with phenolic compounds, for the limited reactivity of triplet oxygen, the reaction is catalysed by transition metal ions, in particular by the redox cycle of Fe^{3+}/Fe^{2+} together with Cu^{2+}/Cu^{+} (Danilewicz, 2003; Danilewicz *et al*., 2008; Waterhouse and Laurie, 2006). Compounds with more isolated phenolic groups, such as malvidin, para-coumaric acid and resveratrol, are oxidised at higher potentials (Kilmartin *et al*., 2001).

Quinones formed from the oxidation of polyphenols are unstable and electrophilic, so they may react with nucleophilic compounds, such as phenols and thiols: in the first case form dimers or polymers, which have lower reduction potential than the initial phenols and, therefore, are much more readily oxidised (Li *et al*., 2008; Singleton, 1987).

Hydrogen peroxide (H_2O_2), in association with ferrous ions, generates hydroxyl radical ($HO^{\cdot}$) (Fenton reaction). $HO^{\cdot}$ is a strong oxidant, capable of non-specifically oxidizing virtually all organic constituents in proportion to their abundance: first of all, ethanol and tartaric acid, but also glycerol, sugars and other organic acids (Danilewicz 2003; Danilewicz 2007; Waterhouse and Laurie 2006; Li *et al*., 2008).

The oxidation products in wine are acetaldehyde from ethanol and keto-acids from organic acids: for instance, tartaric acid forms glyoxylic acid (Danilewicz, 2003; Li *et al.*, 2008; Singleton, 2000), furfural and 5-hydroxymethyl furfural, which are both sugar degradation products (through caramelization or Maillard reaction). These aldehydes can react with flavanols and form yellow–orange xanthylium compounds (Es-Safi *et al.*, 2000).

Furthermore, wine oxidation leads to the loss of aromas, especially fruity and floral notes, and to the appearance of a range of off-flavours that very often occur before the colour change becomes apparent (Escudero *et al.*, 2002). If at low concentrations, these aromas might improve wine complexity; at certain levels, they are dangerous for aroma quality (Singleton *et al*., 1979).

Many studies were carried out to reproduce in the laboratory the taste of aroma degradation related to oxidative spoilage (Simpson, 1982; Escudero *et al.*, 2002; Bueno *et al.,* 2010; Ferreira *et al.*, 1998; Silva Ferreira *et al.*, 2003). The most important descriptors related to the typical aroma of "oxidative spoiled white wines" were reported as being "honey-like, farm-feed, hay, woody-like, nutty, spicy (Silva Ferreira *et al*., 2002), vegetal aroma resembling asparagus or straw, acetaldehyde (Noble *et al.*, 1987); the appearance of bitterness was also reported (Rankine, 1995).

The attested antioxidant, antioxidasic and antimicrobial properties of SO_2 make sulphur dioxide the most common additive for the preservation of wines. However, it has been widely proven that a prolonged absorption of SO_2 can cause health problems and an allergenic effect in sensitive subjects (Ribereau-Gayon *et al*., 2004). In the future, a decrease in the limits for SO_2 concentration in wine is expected, and, in some cases, the prospective is a completely SO_2-free wine.

Other molecules are now being studied for their antioxidant and antiradical properties, such as reduced glutathione (GSH) and oenological tannins (condensed, ellagic and gallic tannins).

Several works have been published (Elias *et al*., 2010; Danilewicz *et al*., 2008; Danilewicz *et al*., 2010; Danilewicz, 2011) that deeply address the mechanisms that regulate the oxidation reactions, with the aim of defining the theoretical basis for the reduction in the use of SO_2 in wines. However, these experiments were mostly performed on a laboratory scale with model solutions and only in a few cases with real wines. Therefore, to reduce/replace sulphites in wine, it is mandatory to investigate the effectiveness of other molecules added to different wine typologies in preserving wine from ageing oxidation.

This work, performed under oenological conditions, was aimed at studying the possibility of adding GSH and/or gallic tannins at bottling to limit the use of SO_2 in white wines.

To date, the studies on wine have been mainly focused on the effect of GSH on aroma evolution during bottle ageing of wines with volatile thiols, in particular, Sauvignon Blanc. On the other hand, little information is available on the influence of GSH on the oxidative evolution of wine colour.

Furthermore, since the level of dissolved oxygen is a critical parameter to consider during the winemaking process (Guaita *et al*., 2013), two different levels of oxygen were considered to study the effect of different bottling conditions

on the oxidative evolution of white wine with different additives.

MATERIALS AND METHODS

1. Experimental trial

This experiment was carried out at CREA-VE's Experimental Cellar with a Cortese white wine provided by Cantina Sociale di Nizza (Nizza Monferrato, AT, Italy). The wine used for the experiment had the following general composition: ethanol 11.7 % v/v, titratable acidity 5.25 g /L as tartaric acid, pH 2.96, volatile acidity 0.24 g/L as acetic acid, malic acid 1.38 g/L, total polyphenols 62 mg/L, catechins 6.2 mg/L as flavans reactive to para-dimethylcinnamaldehyde, acetaldehyde 17.6 mg/L, copper 0.07 mg/L, iron 0.63 mg/L, GSH 3.6 mg/L, free SO_2 20.3 mg/L, total SO_2 60.3 mg/L; the wine was stable against tartaric and protein precipitations.

According to the experimental protocol reported in Table 1, the wine was divided into two aliquots and oxygenated, respectively, up to 4.0 ppm and 5.5 ppm, then SO_2, GSH and gallic tannins were added to both the aliquots. Eight trials have been set up for each oxygen level, following a full factorial design, where each factor studied (SO_2, GSH and gallotannins) varied on two levels: 20 and 40 mg/L of free SO_2, 0 and 40 mg/L of gallotannins, 0 and 20 mg/L of GSH.

Samples with 4.0 ppm of dissolved oxygen were bottled in 750 mL bottles, flushed with nitrogen before and after filling, closed with a synthetic closure, and stored neck upwards at 20 °C.

The dissolved oxygen and the headspace oxygen were measured at bottling, and their evolution was followed over time (12 months). The measurement was performed two days a week during the first month after bottling, 1 day a week during the second and the third month of storage, and once a month during the remaining months.

Chemical controls (free and total SO_2, volatile acidity, acetaldehyde, absorbance at 420 nm, CIELab, total polyphenols) and sensory analysis were performed after 15 months of bottle ageing.

Samples with 5.5 ppm of dissolved oxygen were bottled in 135 ml bottles (oxygenation test), sealed with a crown cap and stored at 20 °C. Two bottles for each sample equipped with a sensor (sensor spot) were also filled to measure the oxygen content during the experiment until its complete consumption (< 0.1 mg/L).

The analytical controls performed during the storage period (1, 3, 8 and 12 months after bottling) regarded some parameters related to the oxidative evolution of wines: free and total SO_2, GSH, HCTA, absorbance at 420 nm, CIELab, catechins, total polyphenols.

Furthermore, the accelerated browning test (Simpson, 1982), a test to predict the tendency of wine to brown, was performed after 1 and 8 months of bottle ageing.

2. Analytical methods

2.1. Conventional analyses

Ethanol concentration, total extract, pH, total acidity, volatile acidity, free and total SO_2 were determined according to EEC methods (EEC Regulation 2676/90). Organic acids were determined by high-performance liquid chromatography (HPLC) (Cane, 1990). Briefly, 1 mL of wine was acidified with an equal volume of H_3PO_4 1N, then passed through a Sep-Pak C18 cartridge (Waters Corp., Milford, MA, USA); the hydrophilic phase was collected and made up to 10 mL with H_3PO_4 5×10^{-3} M. The sample was filtered at 0.2 µm cut-off for chromatographic analysis. A Phenomenex Synergy Hydro-RP 80 A column (250 × 4.60 mm, 4 µm) was used for organic acids quantification. The analysis was performed at 25 °C with isocratic elution with H_3PO_4 5×10^{-3} M, 0.6 mL/min flow rate and the detector was set at 210 nm. Identification was based on retention time, and quantification was made with a calibration curve.

2.2. Spectrophotometric analyses

Wine colour parameters, including the CIELab indices (cylindrical coordinates: L* lightness, C* chroma, h* hue) and the absorbance at 420 nm were determined according to Piracci (1994) (10 mm o.p., sample filtration with a 0.45 µm polypropylene filter). The phenolic composition (total polyphenols and catechins) was determined according

TABLE 1. Experimental plan for studying the effect of the addition of reduced glutathione (GSH) and/or gallotannins at bottling on the shelf-life of a Cortese white wine containing two different levels of SO_2.

	Oxygen (ppm)		SO_2 (mg/L)	GSH (mg/L)	Gallotannins (mg/L)
(1)	4	5.5	20	0	0
s	4	5.5	40	0	0
t	4	5.5	20	0	40
st	4	5.5	40	0	40
g	4	5.5	20	20	0
sg	4	5.5	40	20	0
gt	4	5.5	20	20	40
sgt	4	5.5	40	20	40

to Di Stefano *et al*. (1989). Acetaldehyde was measured with a colorimetric method (Di Stefano and Ciolfi, 1982). The accelerated browning test consisted in measuring the absorbance at 420 nm of the wine before and after 6 days at 50 °C in a thermostat, in a 50 mL flask filled for 2/3 and closed with cotton to allow oxygen to diffuse into wine. The difference between the two measures gives an index of the tendency to browning (Simpson, 1982).

2.3. Atomic absorption spectroscopy analysis of metals

The content of iron (Fe) and copper (Cu) was determined by atomic absorption spectroscopy according to EU methods (EEC Regulation 2676/90).

2.4. HPLC analysis of hydroxycinnamyl tartaric acids and 2-S-glutathionilcaftaric acid (GRP)

The hydroxycinnamyl tartaric acids (HCTA) and 2-S-glutathionilcaftaric acid (GRP) were determined by HPLC (Di Stefano and Cravero, 1991) after filtration with a 0.45 µm polypropylene filter (VWR International, Milan, Italy); the injection volume was 20 µL, and the signal was monitored and recorded at 320 nm. Hydroxycinnamyl tartaric acids (trans-caftaric acid, cis- and trans-coutaric acids, cis- and trans-fertaric acids) and 2-S-glutathionyl-caffeoyl-tartaric acid (GRP) were separated on an ODS Hypersil RP-C18 reversed-phase HPLC column (200 mm × 2.1 mm i.d., 5 µm packing, Thermo Scientific, Waltham, MA) at 25 °C. The peaks were identified according to both the retention time and the UV absorption spectrum, compared with references reported in the scientific literature (Baranowski and Nagel, 1981). HCTAs were quantified using an external standard curve: the calibration, due to the lack of commercial standards for caftaric, coutaric and fertaric acids, was performed using caffeic, coumaric and ferulic acids, respectively. Each standard was injected in triplicate to assess both the linearity and the repeatability of the method.

2.5. HPLC analysis of reduced glutathione (GSH)

The reduced glutathione (GSH) was quantified with the method proposed by Park *et al*. (2000) and modified to use twice the amount of the derivatizing agents as the authors.

Pre-column derivatization of glutathione with o-phthalaldehyde (OPA) and 2-aminoethanol was performed. The resulting isoindole derivatives were separated on a Synergy Hydro RP-C18 reversed-phase HPLC column (150 mm × 4.6 mm I.D., 4 µm packing, Phenomenex, Torrance, CA, USA) and detected by a fluorescence detector with excitation and emission wavelengths of 340 and 450 nm, respectively. The identification of the GSH peak was made by comparison with the retention time of a pure standard that was injected under the same analytical conditions. The GSH concentration was determined with a calibration curve obtained by adding increasing quantities (six levels) of pure reference compound to a model solution. Each point of the curve was injected in triplicate to assess both the linearity and reproducibility of the method. The quantification of GSH was performed using the Chemstation software (Agilent Technologies, Palo Alto, CA, USA).

2.6. Chemicals and equipment

The methanol for the HPLC mobile phase (HPLC grade), p-coumaric acid, GSH, N-acetyl-L-cysteine, EDTA, OPA, and 2-aminoethanol were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). The caffeic and ferulic acid standards were purchased from Extrasynthese (Genay, France). Ultrapure water from a Milli-Q gradient A10 instrument system (Millipore Corporation, Billerica, MA, USA) was used throughout this experiment.

The equipment used were the following: UV-Vis JASCO V-630 spectrophotometer (JASCO, USA); Perkin Elmer 5100 PC AA atomic absorption spectrophotometer (Perkin Elmer, Norwalk, CT, USA); Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, DAD UV-Vis detector, fluorescence detector (FLD).

2.7. Dissolved and head-space oxygen measurement

The concentration of dissolved and headspace oxygen was measured with a luminescence-based technology (NomaSense™ O2 Trace, PreSens GmbH, Regensburg, Germany). It is a trace-oxygen meter with a fibre-optic oxygen minisensor based on a 2 mm polymer optical fibre. The NomaSense™ O_2 Trace system detects oxygen (oxygen partial pressure) in solutions (dissolved oxygen) and in the gaseous space (headspace) using separate sensors, which are glued into bottles before bottling.

When 135 mL bottles were used, the measure regarded only oxygen in solution (dissolved oxygen), while in trials with 750 mL bottles, both dissolved and headspace oxygen were measured with separate sensors.

2.8. Sensory analysis

After 15 months of bottle ageing, the wines were submitted to descriptive sensory analysis, following a method previously described (Cravero *et al.*, 2012; Guaita *et al.*, 2013).

Two preliminary sensory sessions were carried out to choose the olfactory attributes. During the first preliminary session, the assessors were asked to indicate the wine olfactory descriptors using as reference a predefined odour list (Guinard and Noble, 1986). After the first session, 15 descriptors with a frequency of citation at least equal to 28 (8 wines*14 assessor/4), which represents 25 % were chosen.

During the second preliminary sensory session, the panel verified the suitability of the 15 chosen descriptors for the wines, helping themselves with reference standards prepared as reported in Table 2. Ten odour attributes were finally confirmed based on their identification frequency: acacia flowers, lemon, pineapple, yellow golden delicious apple (abbreviated "golden apple"), and six related to oxidative ageing: cut apple-oxidised apple/acetaldehyde (abbreviated "acetaldehyde"), honey, liquorice, walnut, green beans, hay/straw) (Silva Ferreira *et al*., 2002; Noble *et al*., 1987).

The attributes of hay and straw were considered together.

TABLE 2. Odour attributes and reference standards composition were presented during the second preliminary sensory session carried out to choose the odour attributes.

Odour attributes	Standards
Acacia flowers	Acacia flowers essence
Lemon	1 lemon cut into pieces*
Yellow golden delicious apple	1 yellow golden delicious apple cut into pieces*
Green apple	1 granny smith apple cut into pieces*
Oxidised apple/Cut apple/ Acetaldehyde	1 yellow golden apple cut into pieces and exposed to air
Pineapple	pure pineapple juice*
Honey	honey (20 mL)
Liquorice	liquorice root
Almond	some almonds cut into pieces*
Walnut	some walnuts cut into pieces*
Hazelnut	some hazelnuts cut into pieces*
Cut grass	cis-3-hexen-1-ol (2 mg/L)*
Green beans	vegetation water of cooked green beans (100 mL)*
Hay	a few strands of hay
Straw	some straw

*in white wine (about 300 mL) for about 24 hours.

Moreover, the panel defined 1 visual attribute (straw yellow) and 4 taste attributes (acidity, bitterness, softness, and structure).

Finally, the quantitative evaluation of the 15 attributes (1 visual, 10 olfactory and 4 gustatory) was carried out in duplicate using unstructured scales.

The wine samples, identified with a three-digit code, were presented randomly and evaluated within 1 hour after pouring.

The trained panel was made up of 14 trained assessors from CREA-VE Asti. The wines were served at the temperature of 16 ± 1 °C in ISO (3591-1977) approved glasses in an ISO (8589- 2007) tasting room.

2.9. Statistical analysis

Chemical data were processed with a complete three-factor ANOVA (SO_2, gallic tannins and GSH) to study the main effect of the three factors considered in each trial and their interactions.

The results of the sensory analysis were processed as well with a complete three-factor ANOVA (factors: wines, assessors, and sensory sessions).

For this purpose, SPSS for Windows version 15.0 (SPSS Inc., Chicago, Il USA, 2004) was used.

RESULTS AND DISCUSSION

1. Oxygen consumption rate (OCR)

The trend of the consumption of dissolved O_2 (oxygen consumption rate: OCR) in the wines of the different trials bottled in 135 mL bottles was monitored from bottling to its disappearance (concentration values around 0.1 mg/L) observed between 73 and 200 days from bottling.

The kinetics of oxygen consumption of the different trials was modelled based on the content of dissolved oxygen ($Ln[O_2]$) over time (from bottling up to the 73rd day, when the oxygen concentration in the fastest oxygen consumer trial was lower than 0.1 mg/L) to find the order of reactions (Figure 1).

The results show that the trends were always fitted by a first-order kinetic model with $0.91 < R^2 < 0.99$. Table 3 shows the first-order equations that describe the relationships between oxygen concentration and time according to the model: $Ln[O_2]_t = -Kt + ln[O_2]_0$, where $[O_2]_0$ was the concentration of dissolved oxygen at bottling (constant value of the equation), $[O_2]_t$ the concentration of dissolved oxygen at time t and K the constant rate of oxygen consumption (representing the slope of the kinetic curve).

Our results agree with the observations of Jeremic *et al*. (2020) operating on model wine solutions and on Chianti red wines treated with tannins of different origins.

Boulton (2020), working with model catechin solutions, model wine with free SO_2 and white wines, proved that the trend of OCR, variable among wines, follows either first or pseudo-first order kinetics only if the concentration of the ferric ion is constant, that could happen if part of the ferrous ions is quickly oxidised back to the oxidised form.

Anyway, since the OCR is influenced by different parameters, the kinetics of oxygen consumption in wines can be more complex than in model solution and not always characterised as first or pseudo-first-order kinetic, as observed by some authors for red wines (del Alamo-Sanza *et al*., 2014; Ferreira *et al*., 2015).

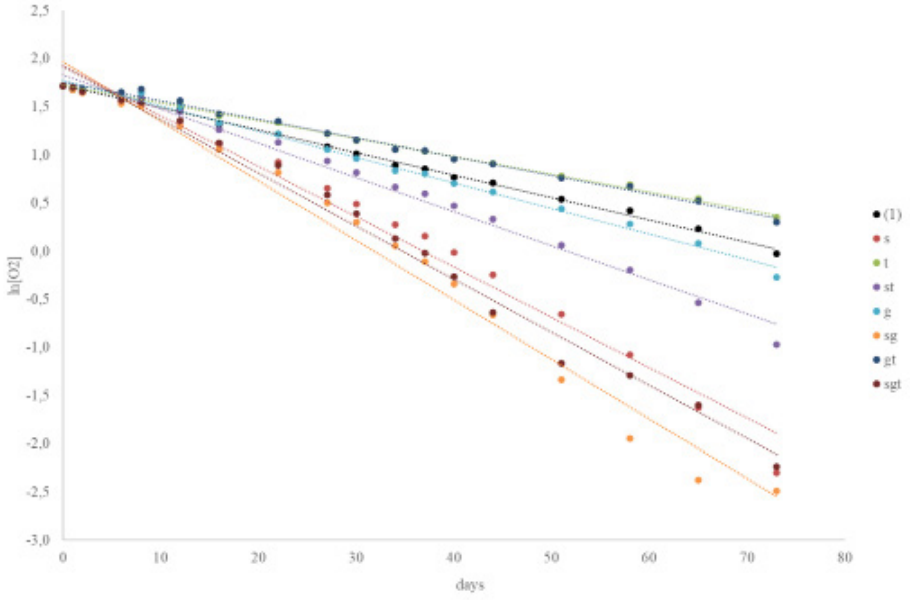

FIGURE 1. Regression lines of oxygen consumption kinetic in a Cortese white wine oxygenated at 5.5 ppm (135 mL bottles) and treated with different concentrations of SO_2, GSH and gallotannins at bottling to obtain eight different trials for each dose of oxygen.

(1) = Control = 20 mg/L of free SO_2; s = 40 mg/L of free SO_2; t = 20 mg/L of free SO_2 and 40 mg/L of gallic tannins; g = 20 mg/L of free SO_2 and 20 mg/L of GSH; st = 40 mg/L of free SO_2 and 40 mg/L of gallic tannins; sg = 40 mg/L of free SO_2 and 40 mg/L of gallic tannins, gt = 20 mg/L of GSH and 40 mg/L of gallic tannins, sgt = 40 mg/L of free SO_2, 20 mg/L of GSH and 40 mg/L of gallic tannins. Each sample has been prepared in duplicate.

The K values (constant rate of oxygen consumption), which express the average rate of oxygen consumption of the different trials, were processed with complete three-factor ANOVA to study the main effect of the three factors (SO_2, gallic tannins and GSH) on the OCR in wines, and their interactions. All three studied factors showed a significant effect on the OCR.

The most pronounced effect concerned SO_2: in the wines with 40 mg/L of SO_2, the K parameter increased by 2.4 times: from 0.022 (lower SO_2 concentration) to 0.053 (higher SO_2 concentration) unities ($p \leq 0.001$). The same trend was observed in our previous work with white wines (Panero *et al*., 2015).

This result agrees with those obtained by Danilewicz *et al*. (2008) and by Danilewicz and Wallbridge (2010) with model solutions: samples with a higher level of SO_2 were significantly faster oxygen consumers than samples with lower SO_2.

Indeed, according to Danilewicz *et al*. (2008), the increase in OCR observed for higher SO_2 levels depends on the fact that when polyphenols are oxidised in the presence of SO_2, a part of the quinones formed reacts with bisulphite to produce sulfonic acid adducts, and most of the remainder is reduced back to the corresponding phenolic forms. Danilewicz (2012) observed that in model solutions without SO_2, the oxidation reaction of diphenols to quinones associated with the reduction of O_2 to hydrogen peroxide is very slow as the value of the redox potential of the two half-reactions is similar (ΔE values equal to 0.012 and ΔG close to zero). The regeneration of *orto*-diphenols from quinones caused by SO_2, therefore, determines the rightward shift of the reaction with the consequent OCR acceleration (Danilewicz *et al*., 2008).

The addition of GSH also caused an acceleration of OCR, with a much lower efficacy (average increases in the K parameter equal to approximately 1.2 times from 0.034 to 0.049 unities, $p \leq 0.05$). An increase in OCR was also observed by Danilewicz *et al*. (2008) when they added cysteine in model wine: the increase observed by Danilewicz *et al*. was much more evident than the one observed in our study by adding GSH to white wine. Fracassetti *et al*. (2013) observed a slightly faster consumption rate only when they added GSH (67.5 mg/L) to a clarified white wine and to a model wine with high SO_2 content (50 mg/L of total SO_2) but not in the model wine when the SO_2 content was low (17 mg/L of total SO_2). This could prove a slight synergistic effect of GSH when coupled with SO_2, which, however, was not observed in our study (no significant interactions between SO_2 and GSH). Our results are consistent with Nikolantonaki *et al*. (2014), who observed, working with model solution, a similar reactivity of SO_2 alone or coupled with GSH as regards the reduction of 4-methyl-1,2-benzoquinone to *ortho*-diphenol. This result could further confirm the hypothesis of Danilewicz *et al*. (2008) that the increase in OCR with increasing doses of SO_2, either in model solution or in wine, is due to the capacity of SO_2 to reduce quinones back to *otho*-diphenols.

Conversely, the addition of gallotannins (40 mg/L) caused a significant but modest reduction (–20 %) of the K parameter from 0.041 to 0.034 unities ($p \leq 0.05$).

TABLE 3. First-order kinetic equations for the consumption of oxygen in Cortese white wines oxygenated, respectively, at 5.5 ppm (135 mL bottles) and 4.0 ppm (750 mL bottles) and treated with different concentrations of SO_2, GSH and gallotannins at bottling to obtain eight different trials for each dose of oxygen.

	135 mL bottles		750 mL bottles	
	Equations of the regression lines	K value ± std error	Equations of the regression lines	K value ± std error
-1	y = –0.020X + 1.704 (R2 = 0.996)	0.02 ± 0.000	y = –0.049X + 1.461 (R2 = 0.984)	0.049 ± 0.002
-1	y = –0.026X + 1.740 (R2 = 0.997)	0.026 ± 0.000	y = –0.048X + 1.468 (R2 = 0.986)	0.048 ± 0.002
s	y = –0.051X + 1.900 (R2 = 0.988)	0.051 ± 0.001	y = –0.087X + 1.574 (R2 = 0.976)	0.087 ± 0.004
s	y = –0.053X + 1.958 (R2 = 0.974)	0.053 ± 0.002	y = –0.075X + 1.469 (R2 = 0.971)	0.075 ± 0.004
t	y = –0.017X + 1.725 (R2 = 0.993)	0.017 ± 0.000	y = –0.038X + 1.461 (R2 = 0.975)	0.038 ± 0.002
t	y = –0.020X + 1.729 (R2 = 0.996)	0.020 ± 0.000	y = –0.037X + 1.440 (R2 = 0.979)	0.037 ± 0.002
st	y = –0.043X + 1.864 (R2 = 0.984)	0.043 ± 0.001	y = –0.072X + 1.618 (R2 = 0.967)	0.072 ± 0.004
st	y = –0.042X + 1.874 (R2 = 0.976)	0.042 ± 0.002	y = –0.071X + 1.634 (R2 = 0.971)	0.071 ± 0.004
g	y = –0.032X + 1.802 (R2 = 0.993)	0.032 ± 0.001	y = –0.050X + 1.536 (R2 = 0.976)	0.050 ± 0.002
g	y = –0.021X + 1.744 (R2 = 0.997)	0.021 ± 0.000	y = –0.054X + 1.445 (R2 = 0.989)	0.054 ± 0.002
sg	y = –0.058X + 1.976 (R2 = 0.979)	0.058 ± 0.002	y = –0.095X + 1.482 (R2 = 0.935)	0.095 ± 0.008
sg	y = –0.065X + 1.957 (R2 = 0.976)	0.065 ± 0.003	y = –0.092X + 1.112 (R2 = 0.914)	0.092 ± 0.009
gt	y = –0.019X + 1.758 (R2 = 0.994)	0.019 ± 0.000	y = –0.042X + 1.441 (R2 = 0.983)	0.042 ± 0.002
gt	y = –0.019X + 1.746 (R2 = 0.994)	0.019 ± 0.000	y = –0.038X + 1.440 (R2 = 0.976)	0.038 ± 0.002
sgt	y = –0.046X + 1.987 (R2 = 0.930)	0.046 ± 0.003	y = –0.071X + 1.766 (R2 = 0.926)	0.071 ± 0.006
sgt	y = –0.064X + 1.848 (R2 = 0.958)	0.064 ± 0.003	y = –0.089X + 1.622 (R2 = 0.970)	0.089 ± 0.005

(1) = Control = 20 mg/L of free SO_2; s = 40 mg/L of free SO_2; t = 20 mg/L of free SO_2 and 40 mg/L of gallic tannins; g = 20 mg/L of free SO_2 and 20 mg/L of GSH; st = 40 mg/L of free SO_2 and 40 mg/L of gallic tannins; sg = 40 mg/L of free SO_2 and 40 mg/L of gallic tannins, gt = 20 mg/L of GSH and 40 mg/L of gallic tannins, sgt = 40 mg/L of free SO_2, 20 mg/L of GSH and 40 mg/L of gallic tannins. Each sample has been prepared in duplicate. K = angular coefficient of the equation = constant rate of oxygen consumption.

The same result was observed by Jeremic *et al*. (2020), who added 100 mg/L of different tannins (gallotannins, ellagitannins, condensed tannins from seeds and skins) to Chianti wine and measured the different OCRs after saturation: after the first saturation, only the thesis with gallotannins showed a reduction in OCR compared to the control.

Conversely, when working with model solutions (hydroalcoholic solutions at pH 3.5 containing transition metals), the addition of tannins always leads to an increase in OCR (Pascual *et al*., 2017; Vignault *et al*., 2018; Motta *et al*., 2020), probably because the added tannins provide an easily oxidizable substrate, previously absent. However, these works showed different OCRs related to the tannin typology: gallotannins resulted in the slowest oxygen consumers compared to the other classes of tannins.

During the oxidation process, the different typologies of tannins can also evolve into new compounds with different redox properties. Furthermore, the tannins could bind to the metals to create compounds less oxidizing than the metals themselves, reducing or eliminating the catalytic effect of metals and/or reducing the formation of free radicals and forming complexes more difficult to oxidise (Jeremic *et al*., 2020).

Finally, no significant interactions were observed between the factors SO_2, GSH and gallic tannin as regards the OCR of wines.

The OCR was also measured in the 750 mL bottles during storage, and the data were processed from bottling (DO = 4 mg/L) to the total consumption of oxygen in the fastest oxygen consumer sample (DO < 0.1 mg/L). In addition, in this case, the trend of oxygen consumption in the different samples was well-fitted by a first-order kinetic (Table 3). The K parameter values were processed with a complete three-factor ANOVA, as described above. The results were the same as those obtained in the previous experiment. The addition of SO_2 caused a highly significant ($p < 0.001$) increase in the K parameter from 0.045 to 0.082 unities, whereas the addition of GSH and gallotannins caused, respectively, a significant increase (from 0.060 to 0.066 unities) and a highly significant decrease (from 0.069 to 0.057 unities). Finally, no interactions between factors were observed.

2. Evolution of the physicochemical parameters of bottled wines

2.1. Effect of SO_2

The protective effect of SO_2 against browning was confirmed. Significantly lower values of A420 were observed in the $+SO_2$ trials from the first month after bottling (first sampling), and the differences between $–SO_2$ and $+SO_2$ trials increased during the storage period (Table 4).

The CIELab parameters were also influenced by the content of SO_2: higher SO_2 contents correspond to higher L* and lower h* and C* parameters. The differences were almost always significant and increased over time.

These results are related to the role of SO_2 in reducing the *ortho*-quinones of polyphenols back to the original *ortho*-diphenols and in interacting with them to produce sulfur derivatives, thus, slowing down the formation of brown pigments produced by polymerization between quinones and polyphenols (Makhotkina and Kilmartin, 2009). These Authors assessed by cyclic voltammetry how the reactivity of SO_2 with *ortho*-quinones varied with the type of polyphenol: it was higher for the quinone of caffeic acid, lower for that of quercetin (flavonol), and intermediate between the two for the quinone of catechin (flavanol). Furthermore, the same authors verified how the reactivity between free SO_2 and quinones increased as the SO_2 content increased from 0 to 32 mg/L. This result could explain the colour differences we observed between the wines with different SO_2 content.

The concentration of total polyphenols (GAE index) and its trend over time was stable and not influenced by the SO_2 level. In addition, the content of catechins reactive with p-DACA remained unchanged and similar between the two trials.

The HCTA content was monitored from the first month after bottling till the 8th month of storage. The Cortese wine used in this experiment had a lower HCTA content compared to the values reported by Panero *et al*. (2015). Statistically significant differences between the experimental trials were observed when comparing the values at each sampling. Anyway, these differences were modest, and without any practical interest, they probably resulted significant in the excellent repeatability of the analysis (low error variances and high F values). Indeed, the HCTA content remained nearly constant during bottle ageing in all samples: the HCTA did not participate in the browning reactions. These results agree with Du Toit *et al*. (2006), who observed low correlations between HCTA concentration and white wine susceptibility to browning.

During a previous bottle storage experiment carried out with a Montepulciano wine, we observed (Guaita *et al*., 2013) a decrease in HCTA content over time, but also, in that case, it resulted independent from the consumed oxygen, and probably associated with the hydrolysis of HCTA, especially of caftaric acid to the respective caffeic acid.

According to literature, the white wine phenolic molecules mainly involved in the browning process seem to be flavan-3-ols, especially (+)-catechin, (–)–epicatechin and dimeric procyanidins B1-B4 (Du Toit *et al*. 2006; Nikolantonaki *et al.*, 2012).

TABLE 4. Average values of the main physicochemical parameters measured during storage of a Cortese white wine oxygenated at 5.5 ppm and treated with different concentrations of SO_2, GSH and gallotannins at bottling.

Part 1/2

	bottle aging											
	1 month			3 months			8 months			12 months		
	$-SO_2$	$+SO_2$	sign	$-SO_2$	$+SO_2$	sign	$-SO_2$	$+SO_2$	sign	$-SO_2$	$+SO_2$	sign
Free SO_2 (mg/L)	11.1	24.63	***	4.1	20	***	3.68	18.84	***	1.72	15.28	***
Total SO_2 (mg/L)	42.49	71.11	***	30.1	62.2	***	30.24	56.02	***	27.7	63.72	***
Catechins (mg/L)	5.7	5.69	ns	5.79	5.85	ns	5.13	5.27	ns	5.27	5.89	**
Total polyphenols (mg/L)	66.86	67.1	ns	73.5	69.1	*	57.4	57.06	ns	59.49	60.23	ns
A420	0.041	0.036	***	0.047	0.041	***	0.058	0.046	***	0.066	0.046	***
L*	99.51	99.67	*	99.47	99.64	ns	99.12	99.42	**	99.06	99.58	**
h*	-1.35	-1.3	ns	-1.36	-1.31	*	-1.37	-1.33	*	-1.37	-1.32	***
c*	2.93	2.65	*	3.19	2.78	*	4.18	3.38	***	4.78	3.44	***
GSH	8.8	10.8	ns	8.8	10.8	ns	0.5	0.71	ns	nd	nd	_
t-Caftaric acid (mg/L)	10.57	10.54	ns	11.56	10.99	ns	10.74	10.58	**	nd	nd	_
c-Coutaric acid (mg/L)	0.64	0.62	ns	0.62	0.56	*	0.56	0.65	***	nd	nd	_
t-Coutaric acid (mg/L)	0.34	0.36	ns	0.38	0.38	ns	0.64	0.45	***	nd	nd	_
GRP (mg/L)	6.22	6.11	*	6.05	5.86	**	5.71	5.77	ns	nd	nd	_
c+t Fertaric acid (mg/L)	1.56	1.58	ns	1.68	1.58	ns	1.54	1.54	ns	nd	nd	_
Browning test (A420)	0.03	0.03	ns	nd	nd	_	0.05	0.036	*	nd	nd	_

	bottle aging											
	1 month			3 months			8 months			12 months		
	-GSH	+GSH	sign	-GSH	+GSH	sign	-GSH	+GSH	sign	-GSH	+GSH	sign
Free SO_2 (mg/L)	20.1	15.64	**	10.95	13.12	*	9.24	13.28	**	7.44	9.56	ns
Total SO_2 (mg/L)	55.13	58.47	ns	44.4	47.8	ns	39.96	46.3	*	43.84	47.08	ns
Catechins (mg/L)	5.71	5.68	ns	5.84	5.81	ns	5.28	5.13	*	5.63	5.54	ns
Total polyphenols (mg/L)	67.69	66.24	ns	74	68.5	**	57.71	56.75	ns	58.75	60.97	*
A420	0.038	0.039	ns	0.44	0.44	ns	0.055	0.049	***	0.057	0.054	ns
L*	99.62	99.56	ns	99.64	99.47	ns	99.19	99.35	ns	99.31	99.33	ns
h*	-1.31	-1.34	ns	-1.33	-1.33	ns	-1.36	-1.34	ns	-1.35	-1.34	ns
c*	2.84	2.74	ns	2.89	3.09	ns	3.86	3.7	ns	4.17	4.06	ns
GSH	2	16.88	***	0.71	5.33	***	0	1.21	***	nd	nd	_
t-Caftaric acid (mg/L)	10.63	10.48	ns	10.85	11.69	ns	10.67	10.65	ns	nd	nd	_
c-Coutaric acid (mg/L)	0.65	0.61	ns	0.6	0.58	ns	0.6	0.61	ns	nd	nd	_
t-Coutaric acid (mg/L)	0.34	0.36	ns	0.4	0.36	ns	0.57	0.53	*	nd	nd	_
GRP (mg/L)	6.19	6.14	ns	5.99	5.9	ns	5.8	5.7	*	nd	nd	_
c+t Fertaric acid (mg/L)	1.62	1.52	ns	1.7	1.56	ns	1.6	1.59	ns	nd	nd	_
Browning test (A420)	0.026	0.033	**	nd	nd	_	0.036	0.05	*		nd	
	-Tan	+Tan	sign	-Tan	+Tan	sign	-Tan	+Tan	sign	-Tan	+Tan	sign
Free SO_2 (mg/L)	19.71	16.02	**	11.6	12.48	ns	10.88	11.64	ns	8.4	8.6	ns
Total SO_2 (mg/L)	54.76	58.84	ns	47.36	44.88	ns	42.94	43.32	ns	43	44.92	ns
Catechins (mg/L)	5.7	5.7	ns	5.73	5.92	*	5.18	5.22	ns	5.54	5.63	ns
Total polyphenols (mg/L)	59.46	74.46	***	64.9	77.62	***	55.96	58.5	ns	54.84	64.88	***
A420	0.038	0.039	ns	0.04	0.04	ns	0.053	0.051	*	0.057	0.055	ns
L*	99.62	99.56	ns	99.56	99.56	ns	99.2	99.34	ns	99.3	99.33	ns
h*	-1.32	-1.33	ns	-1.33	-1.33	ns	-1.36	-1.34	ns	-1.35	-1.34	ns
c*	2.78	2.8	ns	2.97	3	ns	3.86	3.7	ns	4.25	3.98	**
GSH	8.9	9.99	ns	3.12	2.9	ns	0.46	0.75	ns	nd	nd	_
t-Caftaric acid (mg/L)	10.58	10.53	ns	11.18	11.37	ns	10.69	10.63	ns	nd	nd	_
c-Coutaric acid (mg/L)	0.64	0.62	ns	0.61	0.57	ns	0.6	0.61	ns	nd	nd	_
t-Coutaric acid (mg/L)	0.35	0.35	ns	0.42	0.34	*	0.55	0.54	ns	nd	nd	_
GRP (mg/L)	6.19	6.14	ns	6.05	5.86	**	5.7	5.77	ns	nd	nd	_
c+t Fertaric acid (mg/L)	1.62	1.52	ns	1.63	1.63	ns	1.59	1.61	ns	nd	nd	_
Browning test (A420)	0.028	0.032	ns	nd	nd	_	0.04	0.04	ns	nd	nd	_

*,** and *** and ns indicate differences at P ≥ 95 %; 99 %; 99.9 % and not significant, respectively.

+SO_2 = 40 mg/L of free SO_2, –SO_2 = 20 mg/L of free SO_2; +GSH = +20 mg/L of reduced glutathione; –GSH = no GSH added; +Tan = + 40 mg/L of gallotannins, –Tan = no tannins added.

CIELab: L* = lightness; h* = hue; C* = chroma; GRP = Grape Reaction Product.

No significant effect of SO_2 concentration in limiting the GSH losses was observed. The same results were observed in previous work (Panero *et al*., 2015).

The accelerated browning test was performed 1 and 8 months after bottling to verify the resistance to browning. According to Singleton and Kramlinga (1976), this test is linearly correlated with wine oxidation at room temperature. The test results showed a protective effect of the highest dose of SO_2 after eight months of bottle ageing: +SO_2 samples presented lower values of A420 (Table 4).

On the other hand, no differences were observed between the wines with different SO_2 content when the concentration of free SO_2 in the –SO_2 samples exceeded 10 mg/L (test carried out one month after bottling). This could confirm what was reported by Godden *et al*. (2001) in a previous work, where 10 mg/L of free SO_2 was indicated as the concentration threshold below which white wines are no longer protected from the oxidative evolution of colour and aroma.

2.2. Effect of GSH

A significant increase in the consumption of free SO_2 was observed in +GSH samples only one month after bottling.

However, the trend was reversed during storage: the +GSH samples always had higher free SO_2 content (statistically significant differences were observed 3 and 8 months after bottling). As regards the total SO_2 content, it was averagely higher in +GSH samples (protective effect of GSH), but the differences were statistically significant only after 8 months of bottle ageing (Table 4). The protective effect of GSH towards SO_2 (limiting SO_2 consumption) could be due to the aptitude of GSH to participate in nucleophilic addition reactions with o-quinones like SO_2 does, reconverting them back to *orto*-diphenols and auto-oxidizing itself to disulphite (GSSG), as suggested by Makkhonika and Kilmartin (2009).

More recently, other authors (Gambuti *et al*., 2015) observed an effect of GSH on the inhibition of acetaldehyde production during the micro-oxygenation process, intervening, as occurs for SO_2, on hydrogen peroxide (Fenton reaction). The same authors also hypothesised that GSH could combine acetaldehyde, albeit to a lesser extent than SO_2.

Anyway, the addition of GSH did not show any evident effect on wine colour browning during bottle ageing, differently from SO_2. This result has already been observed by other authors (Panero *et al.* 2015; El Hosry *et al.* 2009). The trend of the A420 parameter during ageing was the same in +GSH and –GSH samples, except after 8 months of bottle ageing when the wines treated with GSH had lower A420 values than the –GSH samples (Table 4).

Although the literature indicates GSH and SO_2 as two excellent protective molecules against the oxidation of polyphenols (Makhotkina and Kilmartin, 2009), the modest result obtained with GSH may be due to the dose used (Motta, 2014). Indeed, 20 mg/L, which is the maximum dose admitted by OIV (Resolution OIV-OENO 446-2015), corresponds to 65 µM, respectively, 5 and 10 times lower than the concentration of SO_2 in $-SO_2$ and $+SO_2$ samples at the beginning of the trial, probably a too low molar concentration to show a lasting protective effect.

The accelerated browning test was performed 1 and 8 months after bottling for all samples to test the tendency to browning at high temperatures and in the presence of high levels of dissolved oxygen. The results showed higher A420 values (higher browning capacity) in +GSH samples and highlighted how the addition of GSH caused an acceleration of the oxidation process, thus, favouring, unlike SO_2, the formation of brown pigments.

During bottle ageing, the GSH content decreased in all trials; in this experiment, the losses were slower compared to a previous experience carried out by Panero *et al*. (2015). After 1 month of bottle ageing, the average GSH content in +GSH samples was almost completely preserved (mean value = 16.9 mg/L), but after 3 months, only 25 % of the original content was detected (mean value = 5.3 mg/L).

As already observed in previous work, SO_2 did not have a significant effect in limiting GSH losses (Panero *et al*., 2015); anyway, during the first three months of storage, the mean GSH content was always averagely higher in $+SO_2$ samples.

The GSH consumption rate could be related to different parameters: SO_2/GSH ratio, free and total SO_2 content, and wine composition, especially the polyphenolic content. These aspects are worth being deepened with specific studies.

The observed results were consistent with a possible increase in colour due to the Maillard reaction: the GSH offers two amino groups, and, in addition, SO_2 combines the carbonyl groups, thus, limiting GSH's ability to start the Maillard reaction. Anyway, even if the results suggested a possible similar behaviour, there was no direct analytical evidence to confirm it: this hypothesis needs to be fully evaluated.

TABLE 5. Average values of the main physicochemical parameters measured after 15 months of bottle ageing of a Cortese white wine oxygenated at 4 ppm and treated with different concentrations of SO_2, GSH and gallotannins at bottling.

	$-SO_2$	$+SO_2$	sign	-GSH	+GSH	sign	–Tan	+Tan	sign
Free SO_2 (mg/L)	1.72	8.8	***	4.72	5.8	ns	4.68	5.84	ns
Total SO_2 (mg/L)	22.36	55.16	***	36.24	41.28	ns	35.24	42.28	ns
Total polyphenols (mg/L)	60.94	59.74	ns	60.99	59.68	ns	56.83	63.85	***
A420	0.072	0.052	***	0.06	0.06	ns	0.06	0.06	ns
L*	99.08	99.55	***	99.25	99.38	**	99.27	99.36	*
h*	–1.38	-1.32	***	–1.32	–1.36	**	–1.35	–1.35	ns
C*	5.39	3.97	***	3.97	4.61	ns	4.67	4.69	ns
Volatile acidity (g/L)	0.15	0.145	ns	0.145	0.16	ns	0.16	0.14	ns
Acetaldehyde (mg/L)	17.5	18	ns	17.94	17.53	ns	18.2	17.3	ns

$+SO_2$ = 40 mg/L of free SO_2, $-SO_2$ = 20 mg/L of free SO_2; +GSH = +20 mg/L of reduced glutathione; –GSH = no GSH added; +Tan = + 40 mg/L of gallotannins, –Tan = no tannins added.

CIELab: L* = lightness; h* = hue; C* = chroma.

*,** and *** and ns indicate differences at p ≥ 95 %; 99 %; 99.9 % and not significant, respectively.

2.3. Effect of gallotannins

No noteworthy effect of gallotannins was observed on the studied physicochemical parameters, except for an increase in polyphenols content in +Tan samples due to the addition itself.

An increase in the rate of SO_2 consumption was observed only 1 month after bottling in +Tan samples, and then the differences disappeared. The reduction of free SO_2 content could be a cause of the initial slowdown in oxygen consumption observed in +Tan samples.

No change in colour was observed after the addition of gallotannins. Furthermore, the colour parameters (A420 and CIELab indices) remained, on average, similar between –Tan and +Tan during storage. Only after 8 and 12 months, +Tan differed significantly from –Tan due to the less intense yellow colour (C* and A420); the differences, however, were modest (Table 4).

2.4. Physicochemical composition 15 months after bottling

Table 5 shows the physicochemical composition of wines stored in 750 mL bottles for 15 months at 20 °C. The concentration of SO_2 at bottling influenced the evolution of wine colour: $+SO_2$ samples were distinguished from $–SO_2$ samples for the significantly lower values of A420, h*, C* and for the higher L* values. No significant effects were observed as regards the other parameters.

The addition of GSH at bottling caused a significant increase in L* and C* and a significant decrease in h*. The slight protective effect of GSH towards SO_2 was confirmed: 15 months after bottling; the +GSH samples had a higher average content of both free and total SO_2.

As regards gallotannins, the only significant difference, except the higher value of total polyphenols, was an increase in L* in the presence of tannins.

However, the effect on the colour of the addition of GSH and gallotannins was negligible, as confirmed by the sensory analysis of wines (next paragraph).

2.5. Sensory analysis

After 15 months of bottle ageing, the wines were subjected to sensory analysis. The data relating to the 15 sensory descriptors (1 visual, 10 olfactory and 4 gustatory) were subjected to ANOVA: the main effects and the first-order interactions between the factors "wine", "assessor" and "sensory session" were calculated. The results are reported in Table 6.

Significant differences between assessors were observed for all descriptors and, between sessions, for 7 out of 15 descriptors.

These differences depend on the different use of the scale by the assessors and on the fact that the positioning on the measurement scale can vary between different sessions. The variability due to the main effect of these two factors (assessor and sensory session) is eliminated in the ANOVA calculation, and the comparisons between the different experimental trials are not affected by these differences.

Conversely, the presence of significant interactions between factors indicates the poor robustness of the considered descriptors. In particular, the presence of significant

TABLE 6. ANOVA results for the sensory data. Significativity of F index for main effects and first-order interactions of the factors "wine", "assessor" and "sensory session".

	Assessor	Wine	Session	Assessor*Session	Assessor*Wine	Wine*Session
Straw yellow	***	***	*	***	*	ns
Acacia flowers	***	ns	*	ns	ns	ns
Lemon	***	***	*	ns	ns	ns
Pineapple	***	ns	***	*	*	ns
Golden apple	***	ns	ns	ns	ns	ns
Acetaldehyde	***	***	ns	*	*	ns
Honey	***	ns	ns	*	ns	ns
Liquorice	***	**	ns	*	ns	*
Walnut	***	**	ns	ns	ns	ns
Green beans	***	**	**	*	ns	ns
Hay/Straw	***	ns	*	**	ns	**
Acidity	***	ns	ns	ns	ns	ns
Bitterness	***	ns	*	ns	ns	ns
Softness	***	ns	ns	ns	ns	ns
Structure	***	ns	ns	*	ns	ns

(1) *,**,*** and ns indicate differences at p ≥ 95 %; 99 %; 99.9 % and not significant, respectively.

interactions between assessors and sessions highlights the existence of inconsistent assessments between the sensory sessions (this interaction was detected for the descriptors straw yellow, pineapple, acetaldehyde, honey, liquorice, green beans, hay/straw and structure).

The presence of significant interactions between wine and sensory session indicates a lack of consistency in the evaluation of wines during the two sensory sessions (this is the case of the descriptors hay/straw and liquorice). The presence of interactions between assessors and wine indicates the discrepancies between assessors in the evaluation of wines (these interactions were detected for the colour and the aroma of pineapple and acetaldehyde). Overall, the most robust sensory descriptors, for which no significant interactions were detected, were acacia flowers, lemon, golden apple, walnut, acidity, bitterness and softness.

The sensory analysis confirmed the positive role of SO_2 in protecting wine from oxidation during ageing, according to the results of physicochemical analyses. The average sensory profile for the $+SO_2$ and $–SO_2$ samples is reported in Figure 2A.

The samples with a higher level of SO_2 at bottling were described as significantly less coloured and with significantly more intense notes of lemon and acacia flowers (freshness descriptors) compared to the $–SO_2$ samples. Furthermore, the $+SO_2$ samples resulted statistically different from the $–SO_2$ samples for less intense notes related to oxidative evolution: acetaldehyde, liquorice, walnut and green beans. No differences were observed between the two samples for the taste descriptors.

The +GSH samples were similar to –GSH ones; they differed significantly from one another only for the more intense aroma of liquorice and the less intense aroma of pineapple (Figure 2B). The addition of gallotannins had no sensory effect (Figure 2C).

CONCLUSION

The results confirmed the key role of SO_2 in the shelf life of bottled wines and in preserving their organoleptic characteristics during ageing. The addition of SO_2 led to an acceleration of OCR, which in all wines followed a first-order kinetic. The increase in OCR was the consequence of the acceleration of the first step of the oxidation process, that is, the oxidation of phenols, which is due to the reduction of quinones back to phenols or to the formation of additional compounds with quinones or the consumption of hydrogen peroxide.

The mechanism of action for GSH is supposed to be the same as for free SO_2. Anyway, the results showed a negligible effect of GSH compared to SO_2: no effect of GSH on colour intensity was observed during bottle ageing, and an increase in colour browning was observed in the +GSH samples after the browning test.

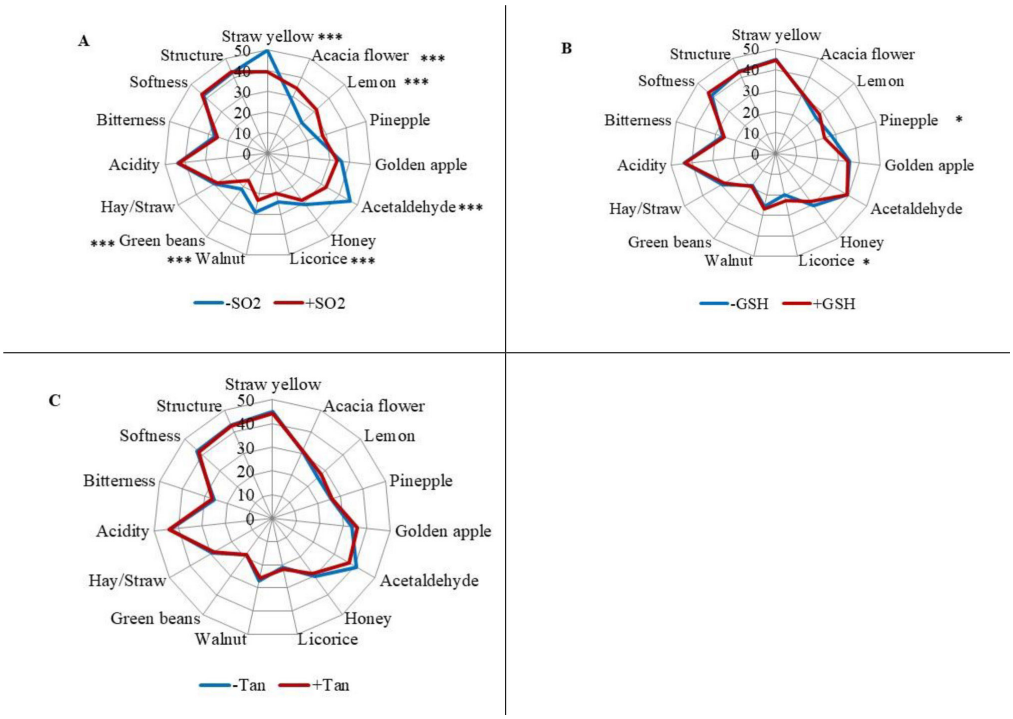

FIGURE 2. Sensory profiles of Cortese wines 15 months after bottling. Effect of SO_2 (Figure A), GSH (figure B) and tannins (figure C).

$+SO_2$ (all the trials added with SO_2), $–SO_2$ (all the trials not added with SO_2), +GSH (all trials added with GSH) and –GSH trials (all trials not added with GSH); +Tannins = all trials treated with tannins, –Tannins = all trials not treated with tannins.

* and *** indicate differences at $p \geq 95$ % and 99.9 %, respectively.

The initial free SO_2 concentration of 40 mg/L allowed one to preserve the organoleptic quality of Cortese wine, subjected to an important oxygen intake at bottling (4 mg/L) during 15 months of bottle ageing. On the contrary, medium-low quantities (20 mg/L) of free SO_2 were not sufficient to ensure adequate protection of the wines during the same storage period.

As it is well known, to choose the right amount of SO_2 to be added to the wine at bottling, it is mandatory to take into account the losses due to the reaction with oxygen dissolved into the wine during bottling and storage (type of closure used), and even the expected permanence in the bottle before the consumption. Regarding the other additives, neither gallotannins (40 mg/L) nor GSH (20 mg/L) improved the shelf life of wines. At the doses used, GSH caused a slight acceleration of OCR and a mild protective effect on SO_2 consumption.

Conversely, a slight slowdown in OCR was observed in +Tan, probably due to the increased initial consumption of SO_2. The effectiveness of GSH and/or gallotannins as partial substitutes for SO_2 should be evaluated for shorter storage times and for controlled oxygen intakes, possibly using higher doses, particularly in the case of GSH. From a practical point of view, to date, the best way to extend the shelf life of wines without adding too high concentrations of SO_2 at bottling is to control the oxygen uptake in bottled wine by improving the bottling systems to limit the amount of oxygen that enters in the bottle and is dissolved into the wine.

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