Application of colloidal silver versus sulfur dioxide during vinification and storage of Tempranillo red wines


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Abstract

**Background and Aims:** The antiseptic effect of the addition of colloidal silver (CAgC) alone or in combination with a small quantity of sulfur dioxide (SO₂) was examined in both vinification and storage of red wines.

**Methods and Results:** Four treatments of grapes and wines with SO₂ and CAgC alone or in combination with a small quantity of SO₂ were established. All treatments of grapes provided similar control of yeast and lactic acid bacteria, however, the three treatments with CAgC were more effective in the control of acetic acid bacteria. The addition of CAgC did not affect fermentation, and the wines had physicochemical, aromatic and sensory characteristics similar to that of the control, but a lower alcohol content. None of the treatments maintained control of bacteria during storage. After 1 month of stabilisation and 4 months of storage, the wines produced with CAgC had a higher colour intensity and a lower concentration of both anthocyanins and total polyphenols. Moreover, the final wines showed little difference in volatile and biogenic amine composition, which affected neither their quality nor their sensory characteristics.

**Conclusions:** Under the conditions studied, addition of SO₂ could be reduced or replaced by CAgC. Overall, wines elaborated with CAgC showed no microbiological problems, and moreover, they had a higher colour intensity and similar aromatic composition and biogenic amines concentration to that of the control; their sensory characteristics were well rated.

**Significance of the Study:** Colloidal silver could be a promising antiseptic in the elaboration and storage of young wines.

**Keywords:** biogenic amine, colloidal silver, sulfur dioxide, volatile compound, wine composition

Introduction

Sulfur dioxide (SO₂) is probably the most important additive in winemaking because of its antiseptic and antioxidant properties – it inhibits the development of a wide range of microorganisms, such as yeasts, lactic acid bacteria (LAB) and, to a lesser extent, acetic acid bacteria (AAB) (Santos et al. 2012). It shows a selective action for yeast; for instance, apiculate yeasts are almost totally inhibited by a SO₂ concentration lower than 50 mg/L, while elliptical yeasts, in particular Saccharomyces cerevisiae, are more resistant (Lustrato et al. 2003). As an antioxidant, SO₂ protects wine phenols from oxidation, reduces the effect of dissolved oxygen and inhibits the action of oxide enzymes, such as polyphenoloxidase, peroxidase, tyrosinase and protease (Ribèreau-Gayon et al. 2006). Despite the advantages of SO₂, sulfite resulting from the addition of SO₂ has been reported to have a toxic effect on human health, such as headache, nausea, abdominal pain and allergic reactions in sensitive consumers (Vally et al. 2009).

Because of these potential health problems that have been associated with SO₂, the legislation concerning the presence of this compound in food is becoming more restrictive. The World Health Organization (2009) has established recommendations that limit or even disallow the use of SO₂ in the treatment of food products. Consequently, the maximum concentration of SO₂ legally allowed in wines has been gradually reduced. The Office Internationale de la Vigne et du Vin (OIV) and the European Economic Community (EEC) have established limits for SO₂ of 150 mg/L for dry red wines and 200 mg/L for dry white wines (European Commission 1990, Office International de la Vigne et du Vin 2012). In the Appellation of Origin (AO) Rioja (Spain), these limits are more restrictive: 140 mg/L for dry red wines and 180 mg/L for dry white wines (Reglamento A.O. Rioja 2004).

Currently, there is a great interest in other substances without health impact or in innovative technologies that can replace or complement the action of SO₂, thereby reducing its use in wines. In recent years, the addition of compounds, such as dimethyl dicarbonate (Costa et al. 2008), bacteriocins (Bartowsky 2009), lysozyme (López et al. 2009a), phenolic substances (Somni et al. 2011), other natural alternatives (Salaha et al. 2008) and the application of physical methods, such as pulsed electric field (PEF), ultrasound, ultraviolet radiation, low electric current and high pressure (Santos et al. 2012), have been assessed.

Silver has been known to mankind since ancient times for its broad spectrum of antimicrobial activity, specifically against Gram-positive and Gram-negative bacteria and fungi (Rathnayake et al. 2012). Currently, it is used to reduce infection in patients with burns, to prevent bacterial colonisation on medical devices, for water treatment and also in the textile
All treatments were in combinations of CAgC and SO2 at the must stage and during storage of red wine made from Tempranillo grapes from the AO Rioja, Spain. Furthermore, the possibility of reducing or eliminating the addition of SO2 to wines without deterioration in wine quality was evaluated.

**Materials and methods**

**Colloidal silver particles**

The study was undertaken with a patented CAgC product containing 99% kaolin (inorganic support/matrix) and 1% metallic silver particles (active material), which is manufactured by Laboratorios Argenol (Zaragoza, Spain; http://www.laboratorios-argenol.com). Colloidal silver is a fine grey powder, insoluble in water and alcohol, with a particle size less than 10 μm.

**Vinification**

The flow chart of the winemaking process is shown in Figure 1. Tempranillo grapes (800 kg) were destemmed and divided into eight homogeneous batches just after crushing. Two batches were treated with 50 mg/kg of SO2 (denoted SO2); two were treated with 1 g/kg of CAgC (denoted CAgC); two were treated with 1 g/kg of CAgC + 25 mg/kg of SO2 (denoted CAg-1); and the other two were treated with 0.5 g/kg of CAgC + 25 mg/kg of SO2 (called as CAg-2). After 24 h, they were inoculated with a commercial yeast strain (Zymaflore RJA44, Laffort, Bordeaux, France). Alcoholic fermentation (AF) was monitored daily by measurement of the density. When the AF was finished (sugar concentration lower than 5 g/L), the wines were racked and placed in 25 L stainless steel vats. The wines underwent controlled malolactic fermentation (MLF) at 20°C, after inoculation with a malolactic starter (Uvaferm α, Lallemand, Toulouse, France). This fermentation was controlled by periodically measuring malic acid content until residual malic acid was less than 0.2 g/L.

Samples were taken from the fermentation vats at four times for analysis: must before and 24 h after addition of SO2 and CAgC, end of AF and end of MLF.

**Storage**

The wines treated with 50 mg/kg of SO2 and 1 g/kg of CAgC were used to examine the effect of CAgC on storage (Figure 1). After the completion of MLF, because of their high pH, 2 g/L of tartaric acid was added to the wines. Each wine was divided into eight homogeneous batches after which they were subjected to treatments that consisted of the addition of: (i) 30 mg/L of SO2 (denoted SO2); (ii) 1 g/L of CAgC (denoted CAgC); (iii) 1 g/L of CAgC + 15 mg/L of SO2 (denoted CAg-1); and (iv) 0.5 g/L of CAgC + 15 mg/L of SO2 (denoted CAg-2). Two replications were established for each treatment. The tanks were stabilised at 5°C for 1 month after which the wines were bottled and stored for 4 months at 16°C.

Wine samples were taken before addition of the SO2 and CAgC and at the end of storage for LAB and AAB counts, species identification of LAB, physicochemical analysis and analysis of volatile compounds and biogenic amines.

**Microbiological analysis**

Yeasts numbers were determined before and after the treatments by serial dilution of samples. The samples were spread on Petri plates containing two media: chloramphenicol glucose agar for yeasts and lysine agar (Scharlau Chemie S.A., Barcelona, Spain) for non-Saccharomyces yeasts. Diphenyl crystals were added to the media in order to inhibit the development of mould. The plates were incubated at 25°C for 48 h, and viable counts were reported as CFU/mL.

In order to isolate and count LAB, samples were diluted in a sterile saline solution and placed on a Man Rogosa and Sharpe (MRS) agar (Scharlau Chemie S.A.) plates with 10% v/v tomato juice, 6 g/L fructose, 0.5 g/L cysteine-HCl, 5 g/L D,L-malic acid and 100 mg/L of pimaricin (Acofarma, S. Coop., Terrassa, Spain). Samples were incubated at 30°C under strict anaerobic conditions (Gas Pak System, Oxoid Ltd, Basingstoke, England) for at least 10 days, after which viable counts were reported as CFU/mL. Samples were taken at four times: must before and 24 h after addition of SO2 and CAgC, wine after MLF and 5 months later. From each plate, 20 colonies in must and 15 in the other samples were randomly selected for species identification. Isolates were stored in glycerol (30%) and modified MRS (1:1 v/v) at −20°C.

Species were identified by previously recommended methods, which included bacterial morphology, Gram staining

![Flow chart of vinification and storage of wines that were treated with colloidal silver (CAgC), sulfur dioxide (SO2) and combinations of CAgC and SO2 at the must stage and during 5-month storage of the wines. During storage the treatments were: (i) 30 mg/L of SO2 (denoted SO2); (ii) 1 g/L of CAgC (denoted CAgC); (iii) 1 g/L of CAgC + 15 mg/L of SO2; and (iv) 0.5 g/L of CAgC + 15 mg/L of SO2. All treatments were in duplicate.](Image 1)
and catalase (Holt et al. 1994); and sequencing of polymerase chain reaction products of partial 16S rRNA genes using WLAB1 and WLAB2 as previously described by López et al. (2003). Polymerase chain reaction products were sequenced by Macrogen Inc. (Seoul, South Korea), and sequences were used for comparison to the data in GenBank using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990).

Acetic acid bacteria were counted in samples of must before and after addition of SO₂ and CasC and of wines which were spread on 2.5% mannitol agar plates with 0.5% yeast extract, 0.3% peptone w/v and 100 mg/L pimaricin (Acofarma). Plates were incubated at 26°C for 2 days under aerobic conditions.

Must and wine analysis
The composition of must and wines was determined according to the OIV (Office International de la Vigne et du Vin 2012) and EEC official methods (European Commission 1990). Anthocyanins were determined through decoloration with SO₂, (Ribéreau-Gayon and Stronestreet 1965); total polyphenols index by measuring the absorbance at 280 nm after conventional dilution of samples (Somers and Evans 1974); and ionised anthocyanins and the polymerisation index were determined according to Glories (1978) and Ruiz (1999), respectively. Silver was determined with a Zeeman graphite furnace atomic absorption spectrometer Varian model AA240ZGTA 120 (Varian Inc., Walnut Creek, CA, USA), after ashing the sample and dissolving in nitric acid, following the OIV method (Office International de la Vigne et du Vin 2012).

Analysis of volatile compounds by gas chromatography
The volatile compounds of the wines after vinification and after storage were analysed by the method of Ortega et al. (2001) with modifications (López et al. 2011). First, the sample was extracted by mixing 3 mL of sample, 9.5 mL of (NH₄)₂SO₄ saturated solution, 15 μL of an internal standard solution (40 μg/100 mL of ethanol each of 2-butanol, 4-methyl-2-pentanol, 4-hydroxy-4-methyl-2-pentanone, 2-octanol and heptanoic acid) and 200 μL of dichloromethane in a 15-mL screw-cap centrifuge tube. The tube was shaken for 1 h at 400 rpm and then centrifuged at 1258 g for 10 min. Once the phases separated, the dichloromethane phase was recovered with a 0.5 mL syringe and transferred to a 0.3 mL vial. An aliquot of the extract (2 μL) was injected onto a Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) 6890 series II gas chromatograph equipped with an automatic injector (Agilent 6890 Series Injector) and a Hewlett-Packard FID detector. The volatile compounds were separated on a DB-Wax capillary column (60 m × 0.32 mm ID, 0.5 μm film thickness; J&W Scientific, Folsom, CA, USA). The injection was in split/splitless mode without division for 30 s. The temperature program was as follows: 40°C for 5 min, then raised up to 220°C at a rate of 3°C/min. The carrier gas was N₂ at a flow rate of 3 mL/min. Split flow was 30 mL/min, injector temperature was 220°C and detector temperature was 280°C. Compounds were identified by comparison of their retention time with that of pure reference standards using a Hewlett-Packard GCD Series II gas chromatograph electron ionisation detector under the same chromatographic conditions. The volatile compounds were quantified using an internal standard method which was based on calibration curves of the respective standards in a 12% ethanol (v/v) solution at pH 3.6. These standard solutions underwent the same process of extraction as the samples.

Analysis of biogenic amines by high-performance liquid chromatography (HPLC)
The biogenic amines in the wines after vinification and after storage were analysed by reverse-phase HPLC by the method of López et al. (2012) with some modifications with a Hewlett Packard Series 1100 liquid chromatograph equipped with an ALS automatic liquid sampler (Hewlett Packard 1100 Series), an Agilent 1100 fluorometric detector and a Hewlett Packard UV-DAD 1100 Series detector. Samples were submitted to an automatic precolumn derivatisation with s-phthalaldehyde (OPA Reagent, Agilent Technologies). An aliquot (20 μL) of the derivatised sample was injected onto a Hypersil ODS (250 × 4.0 mm, ID 5 μm) column (Agilent Technologies) which was maintained at a constant temperature of 40°C. Solvents and gradient conditions for analysis of the biogenic amines were as follows. Two eluents were used as mobile phases: eluent A – 75 mM sodium acetate, 0.018% triethylamine (pH 6.9) + 0.3% tetrahydrofuran; and eluent B – water, methanol and acetonitrile (10:45:45 v/v/v). All reagents, samples and standards were first filtered with 0.45 μm Millipore filters (Merck Millipore, Darmstadt, Germany). The gradient profile was: 0–15 min, 0–47.5% B, 1.630 mL/min; 15–15.01 min, 47.5% B, 0.800 mL/min; 15.01–25 min, 47.5–60% B, 0.800 mL/min; 25–25.01 min, 60% B, 1.630 mL/min; 25.01–26.01 min, 60–100% B, 1.630 mL/min; 26.01–26.51 min, 100% B, 2.500 mL/min; 26.51–34.01 min, 100% B, 1.630 mL/min; 34.01–36.01 min, 100–0% B, 1.630 mL/min. Separated compounds were detected by a fluorometric detector, λ excitation = 340 nm, λ emission = 450 nm and a UV-DAD detector (λ = 338 nm). Compounds were identified by comparison of their retention time with that of pure reference standards and quantified with an internal standard (heptalamine).

Sensory analysis
The wines after vinification and after the 5-month storage period were subjected to sensory analysis by ten experienced wine sensory panellists. The analysis included six descriptors grouped by visual phase, olfactory phase (flavour intensity and quality), taste phase (taste intensity and quality) and harmony. Each attribute was rated on a reverse scale where a higher score implies lower quality. Also, some aroma and taste descriptors were rated on a scale of 0–10 (where 0: absence of a descriptor; and 10: maximum intensity).

Statistical analysis
Statistical analysis of the data was performed using SPSS Version 15.0 statistical package for Windows (SPSS, Chicago, IL, USA). The coefficient of variation for all compounds was within an acceptable range. Data were analysed using analysis of variance; differences between means were compared using the Tukey test at 0.05 probability level.

Results and discussion

Vinification

Microbiological populations. There was no difference in the population of the non-Saccharomyces yeasts, when must was treated with CasC, SO₂ and combinations of CasC and SO₂ for 24 h (Figure 2a). The population of Saccharomyces yeasts, however, was slightly lower in must treated with 1 g/kg of CasC, independently of the addition of SO₂. Furthermore, no treatment was able to completely control the LAB population, given that after 24 h, the population of

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In contrast, the application of other compounds or technologies to reduce SO₂ has shown that lysozyme was linked to wines with a colour intensity and total polyphenols content lower than that of wines made only with SO₂. This could be due to the higher proportion of ionised anthocyanins found in the former wines. Izquierdo-Cañañas et al. (2012) did not find a difference in colour intensity between wines made with CAgC (with or without SO₂) showed greater colour intensity than that of wines made only with SO₂. This could be due to the higher proportion of ionised anthocyanins found in the former wines. Izquierdo-Cañañas et al. (2012) did not find a difference in colour intensity between wines made with CAgC and SO₂, but they observed a significant reduction in the content of anthocyanins and total polyphenols in wines where silver had been added.

In contrast, the application of other compounds or technologies to reduce SO₂ has shown that lysozyme was linked to wines with a colour intensity and total polyphenols content lower than that of the control wines (López et al. 2009), and that the application of a PEF treatment led to wines with higher colour intensity, total polyphenols and tannins (López et al. 2009b).

The concentration of silver in the finished wines was between 0.01 and 0.03 mg/L, which was below the limit of 0.1 mg/L recommended by the OIV (Office International de la Vigne et du Vin 2012).

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Colloidal silver and sulfur dioxide in winemaking

Table 1. Number of isolates and proportion of LAB species in initial must and 24 h after treatment with colloidal silver (CAgC) and sulfur dioxide (SO₂) and combinations of CAgC and SO₂.

<table>
<thead>
<tr>
<th>LAB species</th>
<th>Proportion in initial must (%)</th>
<th>Proportion after 24 h of treatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SO₂† CAgC‡ CAg-1§ CAg-2¶</td>
</tr>
<tr>
<td>Lactobacillus plantarum</td>
<td>31</td>
<td>72 43 0 79</td>
</tr>
<tr>
<td>Lactobacillus keunkei</td>
<td>11</td>
<td>7 7 0 0</td>
</tr>
<tr>
<td>Lactobacillus malii</td>
<td>0</td>
<td>7 0 0 0</td>
</tr>
<tr>
<td>Lactobacillus pentosus</td>
<td>0</td>
<td>0 0 0 7</td>
</tr>
<tr>
<td>Pediococcus sp.</td>
<td>58</td>
<td>14 7 0 7</td>
</tr>
<tr>
<td>Pediococcus pentosaceus</td>
<td>0</td>
<td>0 43 100 7</td>
</tr>
<tr>
<td>Number of isolates</td>
<td>20</td>
<td>15 15 15 15</td>
</tr>
</tbody>
</table>

Treatments consisted of the addition of: †50 mg/kg of SO₂; ‡1 g/kg of CAgC; §1 g/kg of CAgC + 25 mg/kg of SO₂; ¶500 mg/kg of CAgC + 25 mg/kg of SO₂.

Table 2. Wine composition after vinification with added colloidal silver (CAgC) and sulfur dioxide (SO₂) and combinations of CAgC and SO₂.

<table>
<thead>
<tr>
<th></th>
<th>SO₂†</th>
<th>CAgC‡</th>
<th>CAg-1§</th>
<th>CAg-2¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol content (% v/v)</td>
<td>13.6b</td>
<td>13.3a</td>
<td>13.2a</td>
<td>13.2a</td>
</tr>
<tr>
<td>pH</td>
<td>4.22</td>
<td>4.17</td>
<td>4.16</td>
<td>4.18</td>
</tr>
<tr>
<td>Total acidity (g/L of tartaric acid)</td>
<td>4.09</td>
<td>4.31</td>
<td>4.46</td>
<td>4.24</td>
</tr>
<tr>
<td>Volatile acidity (g/L of acetic acid)</td>
<td>0.35</td>
<td>0.37</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Potassium (mg/L)</td>
<td>2155</td>
<td>2059</td>
<td>2004</td>
<td>1931</td>
</tr>
<tr>
<td>Tartaric acid (g/L)</td>
<td>2.71</td>
<td>2.66</td>
<td>2.66</td>
<td>2.62</td>
</tr>
<tr>
<td>Malic acid (g/L)</td>
<td>0.06</td>
<td>0.04</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Lactic acid (g/L)</td>
<td>2.50</td>
<td>2.40</td>
<td>2.68</td>
<td>2.49</td>
</tr>
<tr>
<td>Reducing sugars (g/L)</td>
<td>2.72</td>
<td>2.75</td>
<td>2.80</td>
<td>2.68</td>
</tr>
<tr>
<td>Anthocyanins (mg/L)</td>
<td>889</td>
<td>829</td>
<td>833</td>
<td>816</td>
</tr>
<tr>
<td>Total polyphenols index</td>
<td>59.2</td>
<td>57.3</td>
<td>55.5</td>
<td>57.6</td>
</tr>
<tr>
<td>Colour intensity</td>
<td>7.79</td>
<td>9.02</td>
<td>8.61</td>
<td>8.69</td>
</tr>
<tr>
<td>Polymersisation index</td>
<td>1.39</td>
<td>1.49</td>
<td>1.34</td>
<td>1.42</td>
</tr>
<tr>
<td>Ionised anthocyanins (%)</td>
<td>16.2a</td>
<td>21.8 c</td>
<td>19.3b</td>
<td>18.5b</td>
</tr>
</tbody>
</table>

In each row, different letters indicate significant differences (P ≤ 0.05) between samples. Treatments consisted of the addition of: †50 mg/kg of SO₂; ‡1 g/kg of CAgC; §1 g/kg of CAgC + 25 mg/kg of SO₂; ¶500 mg/kg of CAgC + 25 mg/kg of SO₂. All treatments were in duplicate.

Volatile compounds. Table S1 shows the concentration of volatile compounds in the wines after vinification. With the exception of ethyl lactate, the formation of volatile compounds was not affected by the treatment, which is similar to the findings of Garde-Cerdán et al. (2008) and Sonni et al. (2011). These authors observed that the synthesis of most of the volatile compounds was not affected by the presence or absence of SO₂. Colloidal silver increased the synthesis of ethyl lactate, in particular when the dose was 1 g/kg, i.e. treatments CAgC and CAg-1; however, Izquierdo-Cañas et al. (2012) found a higher concentration of ethyl lactate in wines fermented in presence of SO₂.

Biogenic amines. Table 3 shows the concentration of biogenic amines in the wines after vinification. A significant difference between treatments was observed only for putrescine, with a lower value in the treatments where 1 g/kg of CAgC was added. This amine is derived from arginine and ornithine (Ferrer et al. 2007), so the presence of CAgC could have affected the metabolism of these amino acids and/or the synthesis of putrescine from them during the AF and/or MLF. Neither histamine nor isoamylamine were found in the wines (Table 3). Putrescine was the most abundant amine, which agrees with results of other authors (Bover-Cid et al. 2006, López et al. 2012). Secondary amines, such as putrescine and cadaverine, can also react with nitrite forming carcinogenic nitrosamines (ten Brink et al. 1990). In alcoholic beverages, doses considered negative for health range between 8 and 20 mg/L for histamine and 25 and 40 mg/L for tyramine, while as little as 3 mg/L for phenylethylamine can cause negative physiological effects (Soufleros et al. 1998). Moreover, the amine concentration can affect the commercialisation of wines as some countries have set a maximum limit for biogenic amines in wine (Beneduce et al. 2010). In our study, none of the treatments resulted in a concentration of biogenic amines that may adversely affect human health or wine quality. No significant difference in the concentration of total biogenic amines between treatments was observed, probably because there were no significant difference in LAB population (Figure 2b), which is mainly responsible for the formation of biogenic amines (Landete et al. 2007).

Sensory analysis. The sensory evaluation of the wines confirmed the results of the wine analysis, i.e. the difference between treatments was negligible (Figure 3a). All the wines were scored as ‘good’ (between 24 and 44 points); however, some features were evident: the wine elaborated with 1 g/kg of CAgC was slightly better liked, receiving the lowest score (higher quality) in the aroma phase; the wines treated with 1 g/kg of CAgC (CAgC and CAg-1) were the highest in the flavour attributes astringency (Figure 3b); and wines treated with 1 g/kg of CAgC (CAgC and CAg-1) were the highest in the flavour attributes structure (Figure 3c).

Storage

Microbial population. At the end of MLF, the microbial population was similar between the two initial wines, being 2 × 10⁶ CFU/mL for LAB (Figure 4), AAB were not isolated. After 5-month storage, the LAB population remained at a high level independent of the treatment applied (Figure 4); this means that none of the treatments applied after MLF were sufficient to control successfully LAB during storage. Other authors have also reported a high LAB population in wines after

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storage with SO₂ and/or lysozyme (López et al. 2009a, 2012, Polo et al. 2011). At the end of storage, differences were found between treatments, with the LAB population lowest in the wines treated with 30 mg/L of SO₂. Identification of species present in all wines showed that all isolates were *Oenococcus oeni*.

**Wine composition.** A significant difference was observed only in the colour parameters 5 months after the end of MLF (Table 4). The wines with higher colour intensity were those produced and stored without SO₂ and those with lower colour intensity were produced and stored without CAgC. Colour intensity values were correlated with the concentration of ionised anthocyanins in wines. Therefore, wines produced and stored with SO₂ (with or without CAgC) had a lower colour intensity and higher anthocyanins and total polyphenols concentration than those made and stored with CAgC (with or without SO₂). This could be due to the fact that SO₂ has antioxidant properties and also favours the extraction of anthocyanins and polyphenols during skin maceration (Ribéreau-Gayon et al. 2006). Thus, the wines made and stored in the presence of SO₂ may have a high concentration of these compounds (Table 4). Sulfur dioxide, however, reacts with several compounds, including anthocyanins, forming colorless compounds, so the colour intensity of the wine could decrease in the presence of SO₂.

The silver concentration of the wines after 5-month storage ranged between 0.01 and 0.05 mg/L, which, as for the wines after vinification, was below the limit recommended by the Office International de la Vigne et du Vin (2012).

**Volatile compounds.** Table S2 shows the concentration of volatile compounds in the wines after storage for 5 months. During this time, some changes were observed in the concentration of methionol; of the esters, isoamyl acetate, diethyl succinate and ethyl lactate; of the acids, isobutyric, butyric + γ-butyrolactone and isovaleric; and of acetoin and diacetyl, whereas the concentration of other volatile compounds stayed practically constant (Tables S1, S2) (statistical results not shown). The concentration of methionol, diethyl succinate, ethyl lactate, acids, acetoin and diacetyl increased while that of...
isoamyl acetate decreased. These variations in the concentration of volatile compounds can be explained by the hydrolysis and esterification reactions that occur during wine storage (Ramey and Ough 1980, Makhotkina et al. 2012).

The concentration of $n$-butanol, isobutanol, isoamyl alcohols, $n$-hexanol and cis-3-hexen-1-ol was not affected by the treatments applied, but the concentration of the remaining four alcohols showed significant differences as a function of the treatment (Table S2). For a given storage treatment, when significant differences were observed, the concentration of $n$-propanol, 2-phenylethanol, benzyl alcohol and methionol in the wines where the initial treatment was with CAgC was higher than that in wines where the initial treatment was with SO$_2$. These subtle differences after the storage period could be explained by the greater presence of oxygen in samples produced without SO$_2$, as this favours the formation of higher alcohols in wines (Valero et al. 2002) (Table S2). For the wines with an initial SO$_2$ or CAgC treatment, no significant difference in the concentration of $n$-propanol, 2-phenylethanol, benzyl alcohol or methionol was observed, with the exception of the wine made with SO$_2$ and stored with CAgC, in which the concentration of methionol was significantly higher than that of the wine made with SO$_2$ and stored using CAg-2.

The treatments significantly affected the concentration of four of the 11 studied esters (Table S2). In contrast to the alcohols, when a significant difference occurred, the concentration of isoamyl acetate and ethyl octanoate was higher in the wines coming from the initial treatment with SO$_2$ than in those treated initially with CAgC, irrespective of the storage treatment. Garde-Cerdán and Ancín-Azpilicueta (2007) and Sonni et al. (2011) also observed that when there was a significant difference in the concentration of esters, it was higher in wines stored with SO$_2$ than without SO$_2$. Ethyl lactate showed, however, the same trend described for the alcohols. Moreover, as with the alcohols, no significant difference was observed in the concentration of isoamyl acetate, 2-phenylethyl acetate, ethyl octanoate and ethyl lactate as a function of the storage treatment applied to wines with an initial treatment of either SO$_2$ or CAgC.

A significant difference in the concentration of the four acids, isobutyric, butyric + $\gamma$-butyrolactone, isovaleric and hexanoic, was found, while the concentration of other acids was not affected by the treatment (Table S2). In contrast to the alcohols and esters, no significant difference was observed after storage for wines having a different initial treatment. The concentrations of the acids were unaffected by the treatments applied. However, the treatment with CAgC + SO$_2$ resulted in a decrease in the concentration of the acids compared to the treatment with SO$_2$ alone.

**Figure 4.** Total lactic acid bacteria population in wines produced by adding either (a) sulfur dioxide (SO$_2$) to the must or (b) colloidal silver (CAgC) which were then stored for 5 months following the addition of: 30 mg/L of SO$_2$ (SO$_2$); 1 g/L of CAgC (CAgC); 1 g/L of CAgC + 15 mg/L of SO$_2$ (CAg-1); and 500 mg/L of CAgC + 15 mg/L of SO$_2$ (CAg-2). In each column, different letters indicate a significant difference ($P \leq 0.05$) between samples.

**Table 4.** Composition of wines vinified with either sulfur dioxide (SO$_2$) or colloidal silver (CAgC) and then stored for 5 months following treatment with SO$_2$, CAgC and combinations of CAgC and SO$_2$.

<table>
<thead>
<tr>
<th></th>
<th>Elaboration with SO$_2$</th>
<th>Elaboration with CAgC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SO$_2$†</td>
<td>CAgC‡</td>
</tr>
<tr>
<td>pH</td>
<td>3.94</td>
<td>3.95</td>
</tr>
<tr>
<td>Total acidity (g/L of tartaric acid)</td>
<td>4.53</td>
<td>4.70</td>
</tr>
<tr>
<td>Volatile acidity (g/L of acetic acid)</td>
<td>0.39</td>
<td>0.37</td>
</tr>
<tr>
<td>Tartaric acid (g/L)</td>
<td>1.41</td>
<td>1.65</td>
</tr>
<tr>
<td>Citric acid (mg/L)</td>
<td>4.83</td>
<td>5.06</td>
</tr>
<tr>
<td>Glycerol (g/L)</td>
<td>8.09</td>
<td>8.08</td>
</tr>
<tr>
<td>Anthocyanins (mg/L)</td>
<td>720 d</td>
<td>634 b</td>
</tr>
<tr>
<td>Total polyphenols index</td>
<td>55.1 d</td>
<td>54.3 bcd</td>
</tr>
<tr>
<td>Colour intensity</td>
<td>8.00 a</td>
<td>9.17 cd</td>
</tr>
<tr>
<td>Polymerisation index</td>
<td>1.54 a</td>
<td>1.62 abc</td>
</tr>
<tr>
<td>Ionised anthocyanins (%)</td>
<td>14.5 a</td>
<td>21.0 cd</td>
</tr>
</tbody>
</table>

In each row, different letters indicate a significant difference ($P \leq 0.05$) between samples. Treatments were: †30 mg/L of SO$_2$; ‡1 g/L of CAgC; §1 g/L of CAgC + 15 mg/L of SO$_2$; ¶500 mg/L of CAgC + 15 mg/L of SO$_2$. All treatments were in duplicate.

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exception was isovaleric acid, the concentration of which was lower in wines produced and stored with SO2 than in those produced with SO2 and stored with CAgC or either of the CAg-1 and CAg-2 combinations.

Finally, the concentration of acetic acid in wines was highly dependent on the treatment applied during storage, while that of acetaldehyde and diacetyl was independent on the treatment (Table S2). The concentration of acetic acid in all treatments applied during storage was higher in the wines where the initial treatment was SO2 than in those treated initially with CAgC. Moreover, the concentration of acetic acid in the wines made and stored with SO2 was higher than that found in the wines produced with SO2 and stored with CAgC, or either of the CAg-1 or CAg-2 combinations; also, the concentration of acetic acid in the wines made with CAgC and stored with SO2 was higher than that in wines produced and stored with CAgC. Acetic acid is formed by the reduction of diacetyl (Ribéreau-Gayon et al. 2006); as a result, formation of acetic acid was probably favoured in the presence of SO2, which provides better protection from oxygen. However, although other authors have found that SO2 can result in an increased acetaldehyde concentration (Romano and Suzzi 1993, Sonni et al. 2011), in our wines, no significant difference between wines was observed, as the concentration of this compound was low in all wines (Tables S1,S2).

Regardless of the treatment, the final wines contained volatile compounds within the ranges reported in the literature (Margalit 1997, Ugliano and Henschke 2009, López et al. 2011).

**Biogenic amines.** Five months after vinification, the concentration of biogenic amines in the wines was lower or remained nearly constant, except for ethylamine which increased during this period (Tables 3 and 5) (statistical results not shown). Other authors have found no clear pattern of the formation of biogenic amines during wine storage (Marques et al. 2008, Polo et al. 2011). In our study, these amines were not formed during wine storage, probably because of a low concentration of amino acid precursors and/or the lack of decarboxylase activity of the LAB strains present. *Oenococcus oeni* strains were the major LAB isolated after wine storage, and it is known that the formation of biogenic amines is strain dependent within the same species (López et al. 2012). In our study, the formation of biogenic amines during wine storage was similar, independent of the presence or absence of SO2 and/or CAgC, and only the final concentration of putrescine and cadaverine was significantly affected by the treatment (Table 5). For the wines initially treated with SO2, the concentration of putrescine at the end of storage was lower than that for the 15 mg/L of SO2 + 500 mg/L of CAgC treatment; however, for the wines initially treated with CAgC, it was higher. The storage treatment did not affect the concentration of cadaverine when the wines had been initially treated with CAgC, but for wines initially treated with SO2, the cadaverine concentration was lower for the CAg-1 treatment (Table 5). The concentration of biogenic amines was low in all wines and thus had no impact on wine quality.

**Sensory analysis.** The sensory analysis did not show a large difference between the two storage treatments (Figures 5,6). All the wines were qualified as ‘good’ (between 24 and 44 points): those with better qualification (lower score) were stored with 1 g/L of CAgC and a small amount of SO2 (15 mg/L) in both experiments (Figures 5a,6a). Both wines (CAg-1) showed strong varietal character and fruitiness, and a higher score for the attributes of astringency and structure (Figures 5b,c,6b,c). The wines stored using only SO2 were also more astringent too (Figures 5c,6c), and the wines produced with CAgC and stored only with SO2 were more bitter (Figure 6c). The overall aroma and flavour differences between the wines were insignificant.

**Conclusions** The addition of CAgC controlled yeast and LAB growth similar to that of SO2 addition, and it was more effective in the control of AAB; this latter observation could be interesting for botrytised grapes. At the dosage employed, CAgC had no influence over the AF or MLF. The wines produced using CAgC had an alcohol content lower than that of the wines with added SO2, although both had similar composition and sensory characteristics. This suggests that it may be possible to replace SO2 with CAgC in red wine production.

During storage of wine, CAgC addition, with or without SO2, reduced the LAB population slightly when compared with that

### Table 5. Biogenic amines concentration of wines vinified with either sulfur dioxide (SO2) and colloidal silver (CAgC) and then stored for 5 months following treatment with SO2, CAgC and combinations of CAgC and SO2.

Table 5. Biogenic amines concentration of wines vinified with either sulfur dioxide (SO2) and colloidal silver (CAgC) and then stored for 5 months following treatment with SO2, CAgC and combinations of CAgC and SO2.

<table>
<thead>
<tr>
<th></th>
<th>Elaboration with SO2</th>
<th>Elaboration with CAgC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SO2†</td>
<td>CAgC‡</td>
</tr>
<tr>
<td>Methylamine</td>
<td>0.309</td>
<td>0.305</td>
</tr>
<tr>
<td>Ethylamine</td>
<td>0.230</td>
<td>0.303</td>
</tr>
<tr>
<td>Tyramine</td>
<td>0.131</td>
<td>0.145</td>
</tr>
<tr>
<td>Putrescine</td>
<td>1.76 c</td>
<td>1.60 bc</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>0.914 ab</td>
<td>0.917 ab</td>
</tr>
<tr>
<td>Phenylethylamine</td>
<td>0.240</td>
<td>0.233</td>
</tr>
<tr>
<td>Total biogenic amines</td>
<td>3.65 c</td>
<td>3.50 bc</td>
</tr>
</tbody>
</table>

In each row, different letters indicate a significant difference (P ≤ 0.05) between samples. Treatments were: †30 mg/L of SO2; ‡1 g/L of CAgC; §1 g/L of CAgC + 15 mg/L of SO2; ¶500 mg/L of CAgC + 15 mg/L of SO2. All treatments were in duplicate. n.d., not detected.

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where only \( \text{SO}_2 \) was added. In addition, wines stored using CAgC had a higher colour intensity, although they had a lower concentration of anthocyanins and polyphenols. Wines treated with \( \text{SO}_2 \) and CAgC showed minor differences in aroma composition, in the concentration of biogenic amines, and in sensory characteristics and perceived quality. These results show that CAgC could be used for the storage of young wines, but further study is required in ageing wines.

Figure 5. (a) Sensory analysis, (b) aroma (c) and taste descriptors of wines elaborated with an initial addition of \( \text{SO}_2 \), which were then stored for 5 months following the addition of: \( \text{SO}_2 \) (30 mg/L of \( \text{SO}_2 \)) (●); CAgC (1 g/L of CAgC) (◇); CAg-1 (1 g/L of CAgC + 15 mg/L of \( \text{SO}_2 \)) (▲); CAg-2 (500 mg/L of CAgC + 15 mg/L of \( \text{SO}_2 \)) (X).

Figure 6. (a) Sensory analysis, (b) aromatic and (c) taste descriptors of wines elaborated with initial addition of colloidal silver (CAgC) and later stored for 5 months. The storage treatments were the addition of: \( \text{SO}_2 \) (30 mg/L of \( \text{SO}_2 \)) (●); CAgC (1 g/L of CAgC) (◇); CAg-1 (1 g/L of CAgC + 15 mg/L of \( \text{SO}_2 \)) (▲); CAg-2 (500 mg/L of CAgC + 15 mg/L of \( \text{SO}_2 \)) (X).
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Supporting information

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**Table S1.** Concentration of volatile compounds (mg/L) after vinification \((n=2)\) with added colloidal silver (CAgC) and sulfur dioxide (SO2) and combinations of CAgC and SO2.

In each row, different letters indicate significant differences \((P \leq 0.05)\) between samples.

Treatments consisted of the addition of: †50 mg/kg of SO2; ‡1 g/kg of CAgC; §1 g/kg of CAgC + 25 mg/kg of SO2; ¶500 mg/kg of CAgC + 25 mg/kg of SO2.

**Table S2.** Concentration of volatile compounds (mg/L) of wines \((n=2)\) vinified with either sulfur dioxide (SO2) and colloidal silver (CAgC) and then stored for 5 months following treatment with SO2, CAgC and combinations of CAgC and SO2.

In each row, different letters indicate significant differences \((P \leq 0.05)\) between samples.

Treatments were: †30 mg/L of SO2; ‡1 g/L of CAgC; §1 g/L of CAgC + 15 mg/L of SO2; ¶500 mg/L of CAgC + 15 mg/L of SO2.