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

T. GARDE-CERDÁN, R. LÓPEZ, P. GARIJO, L. GONZÁLEZ-ARENZANA, A.R. GUTIÉRREZ, I. LÓPEZ-ALFARO and P. SANTAMARÍA

Instituto de Ciencias de la Vid y del Vino (Gobierno de La Rioja, CSIC, Universidad de La Rioja), Ctra. Mendavia-Logroño NA 134, Km 90. 26071 Logroño, La Rioja, Spain

Corresponding author: Dr Pilar Santamaría, email microbiologia.cida@larioja.org

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 (SO_2) was examined in both vinification and storage of red wines.

Methods and Results: Four treatments of grapes and wines with SO_2 and CAgC alone or in combination with a small quantity of SO_2 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 SO_2 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 (SO_2) 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 SO_2 concentration lower than 50 mg/L, while elliptical yeasts, in particular *Saccharomyces cerevisiae*, are more resistant (Lustrato et al. 2003). As an antioxidant, SO_2 protects wine phenols from oxidation, reduces the effect of dissolved oxygen and inhibits the action of oxidase enzymes, such as polyphenoloxidase, peroxidase, tyrosinase and protease (Ribéreau-Gayon et al. 2006). Despite the advantages of SO_2, sulfite resulting from the addition of SO_2 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 SO_2, 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 SO_2 in the treatment of food products. Consequently, the maximum concentration of SO_2 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 SO_2 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 SO_2, 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 (Sonni 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

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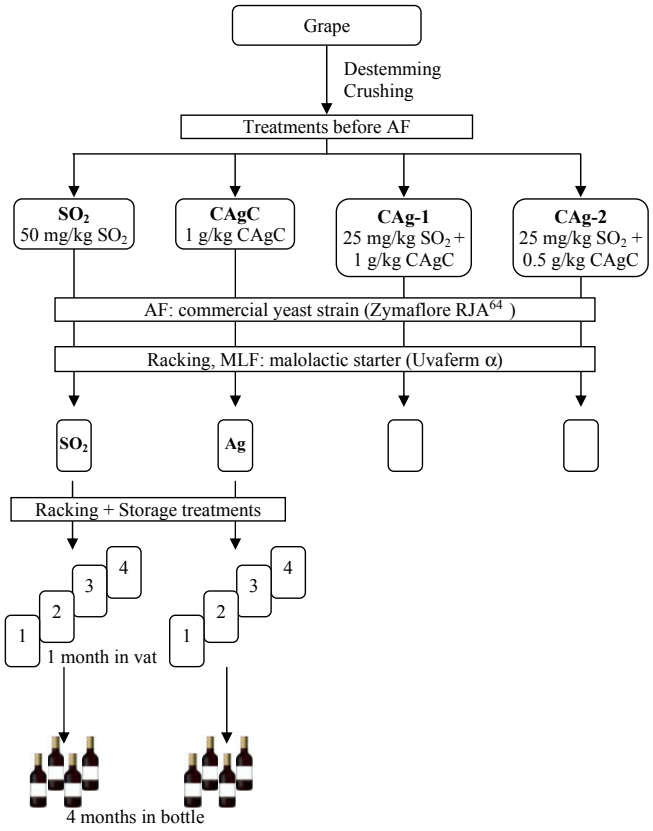

Figure 1. Flow chart of vinification and storage of wines that were treated with colloidal silver (CAgC), sulfur dioxide (SO_2) and combinations of CAgC and SO_2 at the must stage and during 5-month storage of the wines. During storage the treatments were: (1) 30 mg/L SO_2; (2) 1 g/L CAgC; (3) 1 g/L CAgC + 15 mg/L SO_2; and (4) 0.5 g/L CAgC + 15 mg/L SO_2. All treatments were in duplicate.

industry (Silver 2003). Clothing, respirators, antibacterial sprays, detergent, dietary supplements, cutting boards, shoes, cell phones, laptop keyboards, household water filters, face creams, supermarket products for washing vegetables and children's toys are among the products that exploit the antimicrobial properties of silver materials (Marambio-Jones and Hoek 2010).

The primary goal of this study was to assess the application of colloidal silver (CAgC) particles either alone or in combination with a small quantity of SO_2, both in winemaking 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 SO_2 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 SO_2 (denoted SO_2); two were treated with 1 g/kg of CAgC (denoted CAgC); two were treated with 1 g/kg of CAgC + 25 mg/kg of SO_2 (denoted CAg-1); and the other two were treated with 0.5 g/kg of CAgC + 25 mg/kg of SO_2 (called as CAg-2). After 24 h, they were inoculated with a commercial yeast strain (Zymaflore RJA^{64}, 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 SO_2 and CAgC, end of AF and end of MLF.

Storage

The wines treated with 50 mg/kg of SO_2 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 SO_2 (denoted SO_2); (ii) 1 g/L of CAgC (denoted CAgC); (iii) 1 g/L of CAgC + 15 mg/L of SO_2 (denoted CAg-1); and (iv) 0.5 g/L of CAgC + 15 mg/L of SO_2 (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 SO_2 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 SO_2 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

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 SO_2 and CAgC 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 SO_2 (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 $(NH_4)_2SO_4$ saturated solution, 15 µL of an internal standard solution (40 mg/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 N_2 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 a pure reference standard 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 *o*-phthaldialdehyde (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 (heptylamine).

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 CAgC, SO_2 and combinations of CAgC and SO_2 for 24 h (Figure 2a). The population of *Saccharomyces* yeasts, however, was slightly lower in must treated with 1 g/kg of CAgC, independently of the addition of SO_2.

Furthermore, no treatment was able to completely control the LAB population, given that after 24 h, the population of

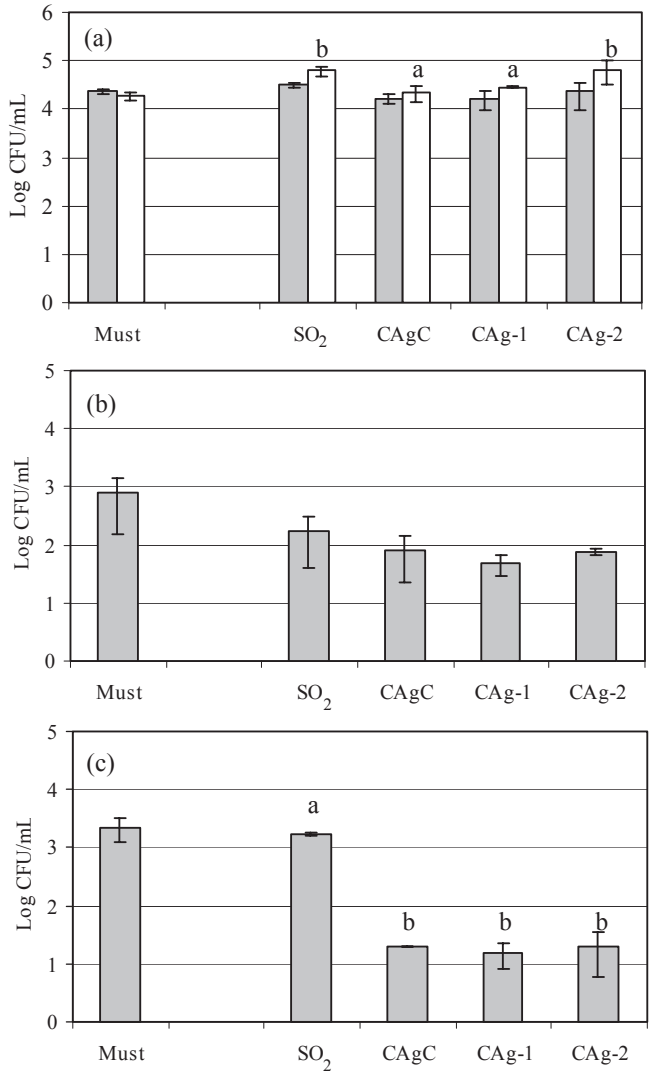

Figure 2. Effect of colloidal silver (CAgC), sulfur dioxide (SO_2) and combinations of CAgC and SO_2 on (a) the population of *Saccharomyces* (□) and non-*Saccharomyces* (■) yeasts, (b) total lactic acid bacteria population and (c) total acetic acid bacteria population of must before and after 24 h of treatment with SO_2 (50 mg/kg of SO_2); CAgC (1 g/kg of CAgC); CAg-1 (1 g/kg of CAgC + 25 mg/kg of SO_2); CAg-2 (500 mg/kg of CAgC + 25 mg/kg of SO_2). In each column, different letters indicate significant differences ($P \leq 0.05$) between samples.

LAB was present at nearly 2 log CFU/mL (Figure 2b). The residual LAB population was not surprising as previous studies had shown that 30–100 mg/L of SO_2 was insufficient to eliminate LAB (Polo et al. 2011). Neither the addition of 200–500 mg/L lysozyme produced a significant effect on the LAB population (López et al. 2009a, Polo et al. 2011). Among the LAB identified in the initial must, 42% of species belonged to the *Lactobacillus* genus and 58% to the *Pediococcus* genus (Table 1). The incidence of LAB genera was dependent on the treatment. Thus, 24 h after treatment, a high proportion of *Lactobacillus* was detected in both SO_2 and CAg-2-treated musts compared with 50% *Lactobacillus* and 50% *Pediococcus* in CAgC-treated must; only *Pediococcus* was identified in CAg-1-treated must. Therefore, the impact of CAgC and SO_2 on the distribution of LAB species was inconsistent.

The viable AAB count in must 24 h after treatment with SO_2 and CAgC varied considerably (Figure 2c). The addition of SO_2 did not affect the AAB population whereas CAgC reduced it by 2 log CFU/mL. Additionally, the combination of CAgC and SO_2 did not improve the antiseptic effect compared with that of CAgC. In contrast, Izquierdo-Cañas et al. (2012) reported that red and white wines prepared from must treated with either CAgC (1 g/kg) or SO_2 (50 mg/kg) had a similar AAB population.

The results obtained in this study indicated that CAgC had antiseptic properties better than that of SO_2. It was able to control yeasts and LAB in the must similar to SO_2, and its control over AAB was greater than that of SO_2.

Oenological parameters and rate of fermentation. The initial must had the following composition: potential alcoholic strength 13.4% v/v; pH 3.54; total acidity 6.18 g/L; tartaric acid 6.94 g/L and malic acid 3.29 g/L. Alcoholic fermentation and MLF were completed in 8 and 11 days, respectively, regardless of the treatment applied. Viable yeasts count during AF and viable LAB count during MLF were also similar for all treatments (data not shown).

Table 2 shows the composition of the wines after vinification. All wines treated with CAgC had a lower alcohol content and a higher content of ionised anthocyanins. A previous study also found a low alcohol content in white and red wines produced using silver particles when compared with that for wines with added SO_2 (Izquierdo-Cañas et al. 2012). These authors suggested that the presence of silver modified yeast metabolism leading to a lower ethanol synthesis. Other studies, in which SO_2 was fully or partially substituted by either lysozyme (López et al. 2009a, Sonni et al. 2011), oenological tannins (Sonni et al. 2011), the application of electrochemical treatment (Lustrato et al. 2003) or PEF treatment (Garde-Cerdán et al. 2008), did not find a significant reduction in the alcohol content of the wine. Moreover, among other factors, the proportion of ionised anthocyanins depends on pH and SO_2 (Ribéreau-Gayon et al. 2006). As pH increases, the concentration of ionised anthocyanins decreases. Also, the combination of these compounds with SO_2 leads to the formation of a new colourless compound that is not ionised. In our study, at the end of vinification, the wines showed a similar pH value (Table 2), so the combination with SO_2 probably explained why the proportion of ionised anthocyanins was inversely proportional to SO_2 concentration. For the remaining parameters, no significant difference was found between treatments (Table 2); however, although the differences were not significant, all wines treated with CAgC (with or without SO_2) showed greater colour intensity than that of wines made only with SO_2. This could be due to the higher proportion of ionised anthocyanins found in the former wines. Izquierdo-Cañas et al. (2012) did not find a difference in colour intensity between wines made with CAgC and SO_2, 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 SO_2 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).

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 (SO_2) and combinations of CAgC and SO_2.

LAB species	Proportion in initial must (%)	Proportion after 24 h of treatment (%)			
		SO_2†	CAgC‡	CAg-1§	CAg-2¶
Lactobacillus plantarum	31	72	43	0	79
Lactobacillus kunkeei	11	7	7	0	0
Lactobacillus mali	0	7	0	0	0
Lactobacillus pentosus	0	0	0	0	7
Pediococcus sp.	58	14	7	0	7
Pediococcus pentosaceus	0	0	43	100	7
Number of isolates	20	15	15	15	15

Treatments consisted of the addition of: †50 mg/kg of SO_2; ‡1 g/kg of CAgC; §1 g/kg of CAgC + 25 mg/kg of SO_2; ¶500 mg/kg of CAgC + 25 mg/kg of SO_2.

Table 2. Wine composition after vinification with added colloidal silver (CAgC) and sulfur dioxide (SO_2) and combinations of CAgC and SO_2.

	SO_2†	CAgC‡	CAg-1§	CAg-2¶
Alcohol content (% v/v)	13.6b	13.3a	13.2a	13.2a
pH	4.22	4.17	4.16	4.18
Total acidity (g/L of tartaric acid)	4.09	4.31	4.46	4.24
Volatile acidity (g/L of acetic acid)	0.35	0.37	0.36	0.36
Potassium (mg/L)	2155	2059	2004	1931
Tartaric acid (g/L)	2.71	2.66	2.66	2.62
Malic acid (g/L)	0.06	0.04	0.04	0.05
Lactic acid (g/L)	2.50	2.40	2.68	2.49
Reducing sugars (g/L)	2.72	2.75	2.80	2.68
Anthocyanins (mg/L)	889	829	833	816
Total polyphenols index	59.2	57.3	55.5	57.6
Colour intensity	7.79	9.02	8.61	8.69
Polymerisation index	1.39	1.49	1.34	1.42
Ionised anthocyanins (%)	16.2a	21.8 c	19.3b	18.5b

In each row, different letters indicate significant differences ($P \leq 0.05$) between samples. Treatments consisted of the addition of: †50 mg/kg of SO_2; ‡1 g/kg of CAgC; §1 g/kg of CAgC + 25 mg/kg of SO_2; ¶500 mg/kg of CAgC + 25 mg/kg of SO_2. 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 during AF was unaffected by the presence or absence of SO_2. 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 SO_2.

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 50 mg/kg SO_2 had a higher intensity of the attribute varietal (Figure 3b); and wines treated with 1 g/kg of CAgC (CAgC and CAg-1) were the highest in the flavour attributes astringency and structure (Figure 3c).

Storage

Microbial population. At the end of MLF, the microbial population was similar between the two initial wines, being 2×10^7 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

storage with SO_2 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 SO_2. 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 SO_2 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 SO_2 (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 SO_2). This could be due to the fact that SO_2 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 SO_2 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 SO_2.

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).

Table 3. Biogenic amines concentration after vinification with added colloidal silver (CAgC) and sulfur dioxide (SO_2) and combinations of CAgC and SO_2.

	Concentration (mg/L)			
	SO_2†	CAgC‡	CAg-1§	CAg-2¶
Histamine	n.d.	n.d.	n.d.	n.d.
Methylamine	0.318	0.287	0.286	0.330
Ethylamine	0.115	0.178	0.161	0.178
Tyramine	0.129	0.158	0.135	0.157
Putrescine	1.91b	1.53a	1.56a	1.66ab
Cadaverine	0.959	1.00	0.980	0.886
Phenylethylamine	0.236	0.264	0.253	0.235
Isoamylamine	n.d.	n.d.	n.d.	n.d.
Total biogenic amines	3.67	3.46	3.38	3.45

In each row, different letters indicate significant differences ($P \leq 0.05$) between samples. Treatments consisted of the addition of: †50 mg/kg of SO_2; ‡1 g/kg of CAgC; §1 g/kg of CAgC + 25 mg/kg of SO_2; ¶500 mg/kg of CAgC + 25 mg/kg of SO_2. All treatments were in duplicate.

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

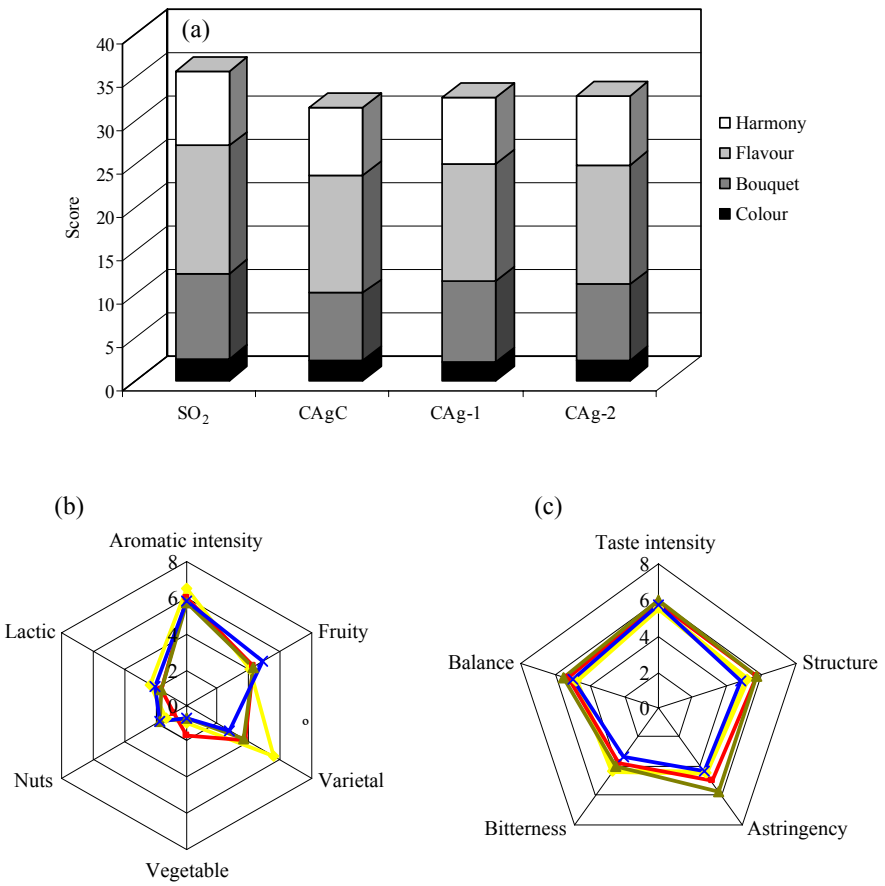

Figure 3. (a) Sensory analysis, (b) aroma (c) and taste descriptors of wines treated with colloidal silver (CAgC) and sulfur dioxide (SO_2) as follows: SO_2 (50 mg/kg of SO_2) (◆); CAgC (1 g/kg of CAgC) (■); CAg-1 (1 g/kg of CAgC + 25 mg/kg of SO_2) (▲); CAg-2 (500 mg/kg of CAgC + 25 mg/kg of SO_2) (✕).

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).

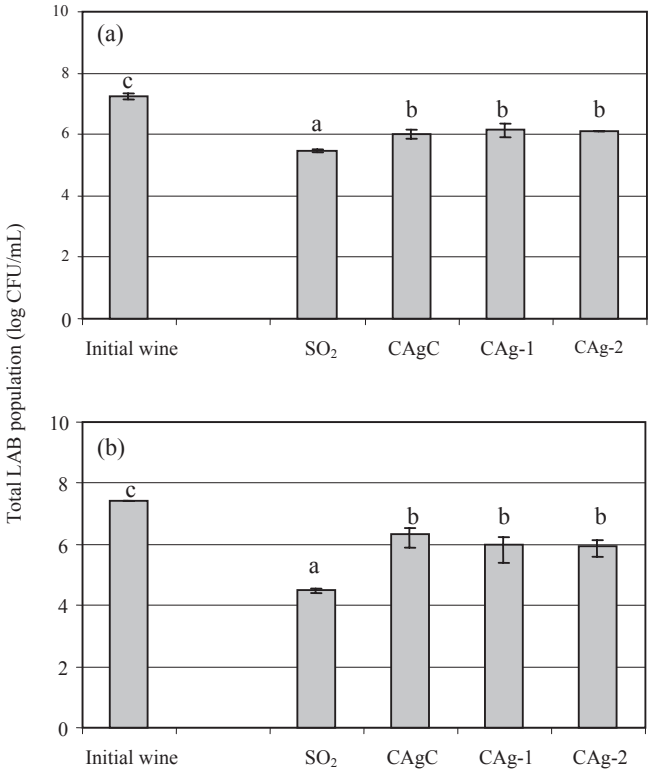

Figure 4. Total lactic acid bacteria population in wines produced by adding either (a) sulfur dioxide (SO_2) to the must or (b) colloidal silver (CAgC) which were then stored for 5 months following the addition of: 30 mg/L of SO_2 (SO_2); 1 g/L of CAgC (CAgC); 1 g/L of CAgC + 15 mg/L of SO_2 (CAg-1); 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.

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 + γ-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

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.

	Elaboration with SO_2				Elaboration with CAgC			
	SO_2†	CAgC‡	CAg-1§	CAg-2¶	SO_2†	CAgC‡	CAg-1§	CAg-2¶
pH	3.94	3.95	3.95	3.94	3.89	3.89	3.89	3.88
Total acidity (g/L of tartaric acid)	4.53	4.70	4.61	4.48	4.52	4.65	4.46	4.48
Volatile acidity (g/L of acetic acid)	0.39	0.37	0.38	0.39	0.42	0.43	0.43	0.43
Tartaric acid (g/L)	1.41	1.65	1.50	1.55	1.46	1.63	1.60	1.63
Citric acid (mg/L)	4.83	5.06	4.14	5.99	3.45	4.37	5.29	2.76
Glycerol (g/L)	8.09	8.08	8.16	8.07	7.92	8.01	8.01	7.93
Anthocyanins (mg/L)	720 d	634 b	691 cd	693 cd	646 bc	567 a	610 ab	606 ab
Total polyphenols index	55.1 d	54.3 bcd	54.3 bcd	54.5 cd	53.0 ab	52.2 a	52.5 a	53.3 abc
Colour intensity	8.00 a	9.17 cd	8.46 b	8.69 bc	8.88 bc	10.2 e	9.68 de	9.71 de
Polymerisation index	1.54 a	1.62 abc	1.58 a	1.60 ab	1.69 bcd	1.74 d	1.69 bcd	1.72 cd
Ionised anthocyanins (%)	14.5 a	21.0 cd	17.0 ab	17.2 ab	17.9 bc	24.7 e	22.5 de	22.3 de

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.

Table 5. Biogenic amines concentration of wines vinified with either sulfur dioxide (SO_2) and colloidal silver (CAgC) and then stored for 5 months following treatment with SO_2, CAgC and combinations of CAgC and SO_2.

	Concentration (mg/L)							
	Elaboration with SO_2				Elaboration with CAgC			
	SO_2†	CAgC‡	CAg-1§	CAg-2¶	SO_2†	CAgC‡	CAg-1§	CAg-2¶
Histamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Methylamine	0.309	0.305	0.298	0.285	0.274	0.291	0.304	0.300
Ethylamine	0.230	0.303	0.286	0.266	0.259	0.263	0.245	0.254
Tyramine	0.131	0.145	0.143	0.159	0.126	0.153	0.162	0.142
Putrescine	1.76 c	1.60 bc	1.66 c	1.31 a	1.40 a	1.33 a	1.45 ab	1.59 bc
Cadaverine	0.914 ab	0.917 ab	0.854 a	1.12 b	0.776 a	0.799 a	0.846 a	0.798 a
Phenylethylamine	0.240	0.233	0.220	0.274	0.260	0.284	0.263	0.265
Isoamylamine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total biogenic amines	3.65 c	3.50 bc	3.47 abc	3.41 abc	3.09 a	3.12 ab	3.27 abc	3.35 abc

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. n.d., not detected.

exception was isovaleric acid, the concentration of which was lower in wines produced and stored with SO_2 than in those produced with SO_2 and stored with CAgC or either of the CAg-1 and CAg-2 combinations.

Finally, the concentration of acetoin 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 acetoin in all treatments applied during storage was higher in the wines where the initial treatment was SO_2 than in those treated initially with CAgC. Moreover, the concentration of acetoin in the wines made and stored with SO_2 was higher than that found in the wines produced with SO_2 and stored with CAgC, or either of the CAg-1 or CAg-2 combinations; also, the concentration of acetoin in the wines made with CAgC and stored with SO_2 was higher than that in wines produced and stored with CAgC. Acetoin is formed by the reduction of diacetyl (Ribéreau-Gayon et al. 2006); as a result, formation of acetoin was probably favoured in the presence of SO_2, which provides better protection from oxygen. However, although other authors have found that SO_2 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 SO_2 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 SO_2, the concentration of putrescine at the end of storage was lower than that for the 15 mg/L of SO_2 + 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 SO_2, 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 SO_2 (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 SO_2 were also more astringent too (Figures 5c,6c), and the wines produced with CAgC and stored only with SO_2 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 SO_2 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 SO_2, although both had similar composition and sensory characteristics. This suggests that it may be possible to replace SO_2 with CAgC in red wine production.

During storage of wine, CAgC addition, with or without SO_2, reduced the LAB population slightly when compared with that

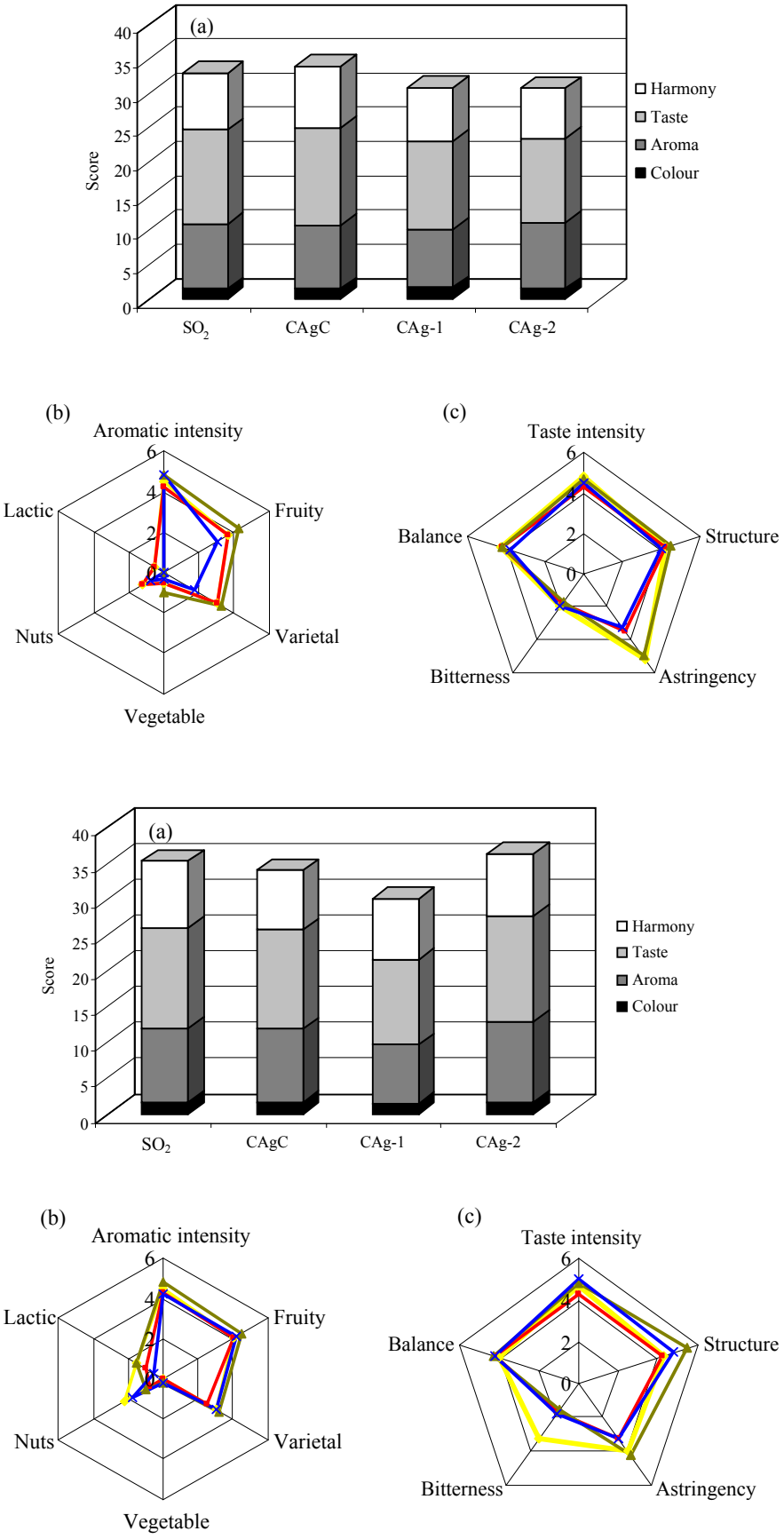

Figure 5. (a) Sensory analysis, (b) aroma (c) and taste descriptors of wines elaborated with an initial addition of SO_2, which were then stored for 5 months following the addition of: SO_2 (30 mg/L of SO_2) (◆); CAgC (1 g/L of CAgC) (■); CAg-1 (1 g/L of CAgC + 15 mg/L of SO_2) (▲); CAg-2 (500 mg/L of CAgC + 15 mg/L of SO_2) (✕).

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: SO_2 (30 mg/L of SO_2) (◆); CAgC (1 g/L of CAgC) (■); CAg-1 (1 g/L of CAgC + 15 mg/L of SO_2) (▲); CAg-2 (500 mg/L of CAgC + 15 mg/L of SO_2) (✕).

where only 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 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.

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Table S1. Concentration of volatile compounds (mg/L) after vinification ($n = 2$) with added colloidal silver (CAgC) and sulfur dioxide (SO_2) and combinations of CAgC and SO_2.

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

Treatments consisted of the addition of: [†]50 mg/kg of SO_2; [‡]1 g/kg of CAgC; [§]1 g/kg of CAgC + 25 mg/kg of SO_2; [¶]500 mg/kg of CAgC + 25 mg/kg of SO_2.

Table S2. Concentration of volatile compounds (mg/L) of wines ($n = 2$) vinified with either sulfur dioxide (SO_2) and colloidal silver (CAgC) and then stored for 5 months following treatment with SO_2, CAgC and combinations of CAgC and SO_2.

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