

Protection of Aroma Volatiles in a Red Wine with Low Sulphur Dioxide by a Mixture of Glutathione, Caffeic Acid and Gallic Acid

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The levels of several esters and other volatiles in Merlot-Cabernet Sauvignon blend red wines with typical sulphur dioxide (35 mg/L), with low sulphur dioxide (25 mg/L), and with low sulphur dioxide (25 mg/L) plus a mixture of antioxidants (glutathione 20 mg/L, caffeic acid 60 mg/L and gallic acid 20 mg/L), were evaluated at bottling, and after 18 and 36 months of ageing. Most volatiles decreased during wine storage. At bottling and after 18 months of storage, all three wines exhibited similar levels of volatiles. After 36 months of storage, wines with low sulphur dioxide exhibited lower levels of many volatiles, such as ethyl acetate, ethyl hexanoate, ethyl octanoate and 2-phenylethanol. On the other hand, wines with low sulphur dioxide plus the mixture of antioxidants exhibited similar levels of most volatiles in comparison with wines with typical sulphur dioxide. The present results indicate that a mixture of glutathione, caffeic acid and gallic acid can protect esters and other volatiles in young red wine with low sulphur dioxide, and can replace part of sulphur dioxide typically used.

INTRODUCTION

Oxidative spoilage of both white and red wines is a well-known problem in winemaking. The first step of oxidation is characterised by the transformation of aroma compounds, leading to a loss of characteristic wine aromas and, subsequently, to the formation of new aromas characteristic of older wines or atypical aromas associated with wine deterioration (Singleton, 1987; Vaimakis & Roussis, 1996; Ferreira *et al.*, 1997; Roussis *et al.*, 2005).

Sulphur dioxide is the most common preservative used in winemaking, exhibiting both antioxidant and antimicrobial properties. However, when free sulphur dioxide levels are between 15 and 40 mg/L, most individuals begin to detect a distinctive burnt match odour. Moreover, the consumption of high concentrations of sulphites may have adverse health effects on humans, such as giving rise to asthma. As a result of these disadvantages of sulphur dioxide addition to wine, the trend is to limit its use.

As far as wine aroma compounds are concerned, it has been reported that sulphur dioxide protects several aroma volatiles, such as esters, alcohols and fatty acids, during wine ageing (Garde-Cerdan & Ancin-Azpilicueta, 2007; Roussis *et al.*, 2007; Roussis & Sergiannitis, 2008).

The antioxidant tripeptide glutathione is a natural constituent, and the main thiol, of wines. Phenolics are major wine antioxidants. Among them, caffeic acid is the main hydroxycinnamic acid and gallic acid the main (hydroxy)

benzoic acid in wines. Regarding wine aroma compounds, it has been found previously that glutathione, caffeic acid, gallic acid, and also wine phenolic extracts slow the decrease of several esters and terpenes during white wine storage (Roussis *et al.*, 2005; Lambropoulos & Roussis, 2007a, 2007b; Papadopoulou & Roussis, 2008), and glutathione slows the decrease of volatile thiols during the storage of Sauvignon wine (Dubourdieu & Lavigne-Cruege, 2004). Moreover, it has been observed that the addition of a mixture of glutathione and caffeic acid to a white wine containing low free sulphur dioxide has a protective effect on volatile esters and terpenes (Roussis *et al.*, 2007).

In the present study, we investigated the ability of a mixture of glutathione, caffeic acid and gallic acid to protect aroma volatiles during the storage of a red wine with low sulphur dioxide.

MATERIALS AND METHODS

Glutathione 98% , caffeic acid 98% and gallic acid 97%, as well as the absolute ethanol used, were purchased from Sigma (St. Louis, MO, USA). A Merlot-Cabernet Sauvignon (60-40) blend red wine produced in the industry was used; the producers recommend that the wine is consumed within three years after production. Cryoextraction was applied during winemaking, and decantations were applied after alcoholic and malolactic fermentation. Micro-oxygenation was applied to the young wines for about three months; the

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delivered quantity of oxygen was 2.5 mg/L of wine per week. After clarification and stabilisation, the wine was bottled in bottles of 750 mL, using natural corks for moderate ageing (Alves Portugal Cork).

In the control wines, sulphur dioxide was added at bottling and the levels of sulphur dioxide were typical, *i.e.* 35 mg/L free sulphur dioxide and 75 mg/L total sulphur dioxide. In the red wine with low sulphur dioxide, no addition of sulphur dioxide was done at bottling. Sulphur dioxide levels were 25 mg/L free sulphur dioxide and 58 mg/L total sulphur dioxide. The third experimental wine was the wine with low sulphur dioxide to which the mixture of glutathione, caffeic acid and gallic acid was added at bottling. For this, 5 mL was removed per 1 000 mL of wine, and 5 mL of 50% ethanol containing glutathione, caffeic acid and gallic acid was added in order for the final concentration of the three antioxidants in the wine to be 20 mg/L, 60 mg/L and 20 mg/L respectively. In the control wine and the wine with low sulphur dioxide, 5 mL of wine were removed per 1 000 mL and 5 mL of 50% ethanol were added.

The gross composition of the red wine at bottling was as follows: alcohol content 13.0 % vol, residual sugars 1.4 g/L, pH 3.46, total acidity 5.6 g/L as tartaric acid, and volatile acidity 0.3 g/L as acetic acid. Alcohol was determined with a hydrometer, reducing sugars were determined by the Lane–Eynon method, pH with a pH meter, total acidity by volumetric analysis, and volatile acidity by steam distillation. Total and free sulphur dioxide was determined by the Ripper method. The bottles were stored in a dark room at 18°C. After 0, 18 and 36 months of storage, bottles were taken and wine samples were analysed for volatiles. All wine samples were analysed by solid phase microextraction (SPME) along with gas chromatography–mass spectrometry (GC–MS) (Vas & Vekey, 2004; Castro *et al.*, 2008). A polydimethylsiloxane 100 µm fibre (Supelco, Bellefonte, PA, USA) was used for the absorption of volatiles.

Two mL of each wine sample and 50 µL of internal standard in 10% ethanol (4-methyl-1-pentanol, 5 mg/L in final solution) were transferred to a 4 mL screw-capped glass vial with a Teflon-rubber septum (red, 12 mm, Sun-Sri, Rockwood, TN, USA). The contents were stirred for 15 min at 40°C, after which a constant length of the fibre was exposed to the headspace for another 20 min at 40°C without stirring. Desorption of volatiles took place at 250°C using a 0.75 mm ID liner (Supelco, Bellefonte, PA, USA) for 5 min. Split mode was used, and the split ratio was 2:1. GC–MS analysis was carried out on an HP 5973 quadrupole mass spectrometer directly coupled to an HP 6890 gas chromatograph.

MS conditions were as follows: source temperature: 230°C, quadrupole temperature: 150°C, transfer line temperature: 270°C. Acquisition was performed in electron impact (EI) mode (70 eV) by 2.02 scans s⁻¹, and the mass range was 29 to 400 m/z. Solvent delay was applied up to 6.0 min to avoid the ethanol peak.

A DB-5 fused-silica capillary column was used (60 m x 0.32 mm internal diameter, 1.0 µm film thickness, J&W Scientific, Folsom, CA, USA). The flow rate of the helium carrier gas was 1.1 mL min⁻¹. The oven temperature was programmed to start at 40°C for 5 min, and then raised to 260°C at a rate of

5°C/min, and it was held at 260°C for 10 min. All peaks were identified by comparing mass spectra to those obtained from the Wiley library (Wiley 275; J. Wiley & Sons Ltd., West Sussex, England).

The identification of many peaks was confirmed with mass spectra and retention times of standard compounds determined under the same analysis conditions. Authentic standards used were ethyl acetate 99%, isoamyl acetate 99%, n-hexyl acetate 99%, 2-phenyl ethyl acetate 99%, ethyl butanoate 99%, ethyl hexanoate 99%, ethyl octanoate 99%, ethyl decanoate 99%, ethyl dodecanoate 99%, 1-hexanol 98% (Merck, Darmstadt, Germany), hexanoic acid 98%, octanoic acid 98%, decanoic acid 98%, and 4-methyl-pentanol 99% (Sigma, St. Louis, MO, USA). Semiquantitative data were expressed in milligrams per litre [(area of compound/area of internal standard) · concentration of internal standard]. The whole experiment was repeated three times and the results reported are the means of the three trials. The one-way analysis of variance (ANOVA), using the Bonferroni test at a level of significance of $P < 0.05$, was used for statistical analysis (SPSS 17.0) (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

The levels of volatiles of a young Merlot-Cabernet Sauvignon blend wine a) with typical sulphur dioxide (35 mg/L), designated as TSD wine, b) with low sulphur dioxide (25 mg/L), designated as LSD wine, and c) with low sulphur dioxide (25 mg/L) plus a mixture of antioxidants (glutathione 20 mg/L, caffeic acid 60 mg/L and gallic acid 20 mg/L), designated as LSDA wine, were evaluated during wine storage (Table 1).

Most volatiles decreased during the storage of TSD wine. These are the esters isoamyl acetate, 5-methyl-3-heptanoate, ethyl hexanoate, n-hexyl acetate, ethyl octanoate, 2-phenyl ethyl acetate, ethyl decanoate and ethyl dodecanoate, the alcohols 1-hexanol and 2-phenylethanol, the polyols 1,3-butanediol and 2,3-butanediol, and the fatty acids hexanoic acid, octanoic acid and decanoic acid. However, some other volatiles, particularly ethyl acetate and also ethyl butanoate and ethyl 9-decanoate, were stable during wine storage. A decrease of most esters, alcohols and fatty acids during the storage of white and red wines has been reported by others (Perez-Prieto *et al.*, 2003; Roussis *et al.*, 2007).

At bottling (0 months) and after 18 months of storage, the LSD and LSDA wines exhibited similar levels of volatiles compared to the TSD wine. These results indicate that red wines with low sulphur dioxide exhibited similar levels of volatiles during storage for a significant period. Moreover, the results indicate that the addition of the mixture of antioxidants did not change the levels of volatiles during wine storage for a significant period.

After 36 months of storage, the LSD wine exhibited lower levels of many volatiles – esters, fatty acids and alcohols – compared to the TSD wine. Among them were ethyl acetate, isoamyl acetate, 5-methyl-3-heptanoate, ethyl hexanoate, ethyl octanoate, 1-hexanol, 2-phenylethanol, 1,3-butanediol and hexanoic acid. These results indicate that the addition of sulphur dioxide at bottling and the control of its concentration at typical levels (35 mg/L) protected these volatiles during long storage of the wine. The protection of

TABLE 1

Relative concentrations (mg/L) of volatiles in experimental Merlot-Cabernet Sauvignon red wines during storage.

Volatiles	TSD wine 0 months	TSD wine 18 months	TSD wine 36 months	LSD wine 36 months	LSDA 36 months
Ethyl acetate	20.3 ^A ± 2.7	21.4 ^A ± 1.5	23.1 ^{Aa} ± 1.9	9.8 ^b ± 1.0	22.5 ^a ± 1.1
Ethyl butanoate	1.1 ^A ± 0.1	1.0 ^A ± 0.1	0.7 ^{Aa} ± 0.1	0.5 ^b ± 0.0	0.8 ^a ± 0.0
Isoamyl acetate	9.6 ^A ± 1.5	4.1 ^B ± 0.3	1.5 ^{Ca} ± 0.2	1.1 ^b ± 0.1	1.4 ^a ± 0.1
5-methyl-3-heptanoate	7.2 ^A ± 0.1	4.7 ^B ± 0.3	3.5 ^{Ba} ± 0.5	2.5 ^b ± 0.09	3.4 ^a ± 0.2
Ethyl hexanoate	28.3 ^A ± 3.0	21.8 ^B ± 1.9	12.6 ^{Ba} ± 0.2	10.8 ^b ± 0.6	14.4 ^a ± 1.0
n-hexyl acetate	1.4 ^A ± 0.2	0.4 ^B ± 0.1	0.0	0.0	0.0
Ethyl octanoate	138.1 ^A ± 18.0	95.6 ^B ± 8.6	48.6 ^{Cb} ± 1.1	40.5 ^c ± 1.5	60.7 ^a ± 1.4
2-phenyl ethyl acetate	1.8 ^A ± 0.3	0.9 ^B ± 0.1	0.3 ^{Aa} ± 0.1	0.2 ^a ± 0.1	0.3 ^a ± 0.1
Ethyl decanoate	57.7 ^A ± 10.4	34.2 ^B ± 1.6	13.2 ^{Cb} ± 1.0	12.9 ^b ± 1.5	17.1 ^a ± 0.5
Ethyl dodecanoate	2.6 ^A ± 0.4	2.2 ^A ± 0.4	0.6 ^{Ba} ± 0.1	0.5 ^a ± 0.2	0.7 ^a ± 0.1
1,3-butanediol	9.2 ^A ± 1.3	7.6 ^A ± 0.5	3.4 ^{Ba} ± 0.4	2.1 ^b ± 0.5	3.9 ^a ± 0.6
2,3-butanediol	1.7 ^A ± 0.2	1.3 ^B ± 0.1	0.5 ^{Cb} ± 0.2	0.4 ^b ± 0.2	0.9 ^a ± 0.1
1-hexanol	6.5 ^A ± 0.4	4.2 ^B ± 0.2	3.1 ^{Ca} ± 0.1	2.2 ^b ± 0.1	3.1 ^a ± 0.2
2-phenylethanol	64.8 ^A ± 14.6	43.9 ^B ± 4.2	26.1 ^{Ca} ± 1.0	19.1 ^b ± 1.4	29.1 ^a ± 1.7
Hexanoic acid	3.6 ^A ± 0.4	2.6 ^A ± 0.2	1.1 ^{Ba} ± 0.2	0.6 ^b ± 0.1	0.6 ^b ± 0.1
Octanoic acid	7.0 ^A ± 2.0	7.4 ^A ± 1.0	3.4 ^{Ba} ± 0.3	2.4 ^a ± 0.8	3.4 ^a ± 0.2
Decanoic acid	1.8 ^A ± 0.8	1.9 ^A ± 0.3	0.5 ^{Ba} ± 0.1	0.5 ^a ± 0.2	0.5 ^a ± 0.1

TSD wine: wine with typical sulphur dioxide (35 mg/L); LSD wine: wine with low sulphur dioxide (25 mg/L); LSDA wine: wine with low sulphur dioxide (25 mg/L) plus a mixture of antioxidants (glutathione 20 mg/L, caffeic acid 60 mg/L and gallic acid 20 mg/L).

Values in mg/L as 4-methyl-1-pentanol are the means of three trials along with standard deviations.

Different capital letters indicate significant differences among TSD wine samples stored for 0, 18 and 36 months.

Different lowercase letters indicate significant differences among TSD, LSD and LSDA wine samples after 36 months of storage.

aroma volatiles –esters, alcohols and fatty acids – by sulphur dioxide during wine storage has been reported by others (Garde-Cerdan & Ancin-Azpilicueta, 2007; Roussis *et al.*, 2007; Roussis & Sergianitis, 2008).

After 36 months of storage, the LSDA wine exhibited higher levels of most volatiles in comparison to the LSD wine. These volatiles were ethyl acetate, ethyl butanoate, isoamyl acetate, 5-methyl-3-heptanoate, ethyl hexanoate, ethyl octanoate, ethyl 9-decanoate, ethyl decanoate, 1-hexanol, 2-phenylethanol, 1,3-butanediol and 2,3-butanediol. These results indicate that the addition of the mixture of antioxidants to red wine with low sulphur dioxide protected these volatiles during long storage of the wine.

After 36 months, the LSDA and TSD wines exhibited similar levels of most volatiles, such as ethyl acetate, ethyl butanoate, isoamyl acetate, 5-methyl-3-heptanoate, ethyl hexanoate, ethyl 9-decanoate, 1-hexanol, 2-phenylethanol and 1,3-butanediol. Ethyl octanoate, ethyl decanoate and 2,3-butanediol were higher in the LSDA wine, and hexanoic acid in the TSD wine. These results indicate that the addition of the mixture of antioxidants to red wine with low sulphur dioxide (25 mg/L) at bottling replaced the addition of sulphur dioxide and its control at typical levels (35 mg/L) as far as the protection of aroma volatiles is concerned.

The Merlot-Cabernet Sauvignon blend wine studied is a red wine with floral and fruity notes recommended for consumption within three years. As indicated by two of the

judges, the wine with low sulphur dioxide exhibited lower sensory quality, aroma and taste than the control wine. On the other hand, the wine with low sulphur dioxide plus the mixture of antioxidants retained its sensory quality and was comparable to the wine with typical sulphur dioxide.

Several chemical reactions influence the composition of the volatile constituents of wine during ageing. Ester changes can be due to hydrolysis and esterification phenomena (Ramey & Ough, 1980), and also to their oxidation initiated by hydroxyl radicals (Patrianakou & Roussis, 2013). Changes in the concentration of fatty acids and alcohols may be explained by their generation due to the hydrolysis of esters and by their oxidation to yield aldehydes (Nykanen, 1986; Perez-Prieto *et al.*, 2003). Subsequently, the protective effect of sulphur dioxide and also of glutathione, caffeic acid and gallic acid may be attributed to their antioxidant properties.

A significant part of the basic odour of wines is attributed to the esters ethyl acetate, isoamyl acetate, ethyl hexanoate and ethyl octanoate. Acetates and ethyl esters are important contributors to the aroma of young wine and they exhibit floral and fruity odours. Alcohols have intense odours that play a role in wine aroma, while fatty acids contribute a fresh flavour to wine (Rapp & Mandery, 1986). Acetates and ethyl esters, 2-phenylethanol, 1-hexanol and butanediols, and hexanoic and octanoic acids have been reported as significant aroma volatiles of Merlot and Cabernet Sauvignon wines

(Ferreira *et al.*, 2000; Kotseridis & Baumes, 2000; Tao & Li, 2009; Welke *et al.*, 2012).

Taking the above into account, the present results indicate that the control of sulphur dioxide, and alternatively the increase in the concentrations of glutathione, caffeic acid and gallic acid along with low sulphur dioxide, are critical for the retention of aroma volatiles during the storage of red wines exhibiting floral and fruity aroma, such as the Merlot-Cabernet Sauvignon blend used in this study.

CONCLUSIONS

The present results indicate that a mixture of glutathione, caffeic acid and gallic acid can protect esters and other volatiles in red wine with a low sulphur dioxide content and can replace part of the sulphur dioxide typically used.

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