Protective Effect of Thiols on Wine Aroma Volatiles

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Abstract: The ability of glutathione, oxidized glutathione, *N*-acetyl-cysteine, and *N*-acetyl-serine to protect wine aroma volatiles was examined.

Muscat-white and Xinomavro-red wine were stored in open bottles at 20 °C and aroma volatiles were determined using solid phase microextraction along with GC-MS. Glutathione and *N*-acetyl-cysteine inhibited the decrease of several acetate esters, ethyl esters and terpenols, while oxidized glutathione and *N*-acetyl-serine did not.

Present results show that the free –SH is responsible for the ability of glutathione and *N*-acetyl-cysteine to protect white and red wine aroma volatiles. Consequently, the protective action of any thiol in any wine may be taken into account.

Keywords: Glutathione, *N*-acetyl-cysteine, volatiles, wine.

INTRODUCTION

The oxidative spoilage of white as well as red wines is characterized by the transformation of aroma compounds. It leads to a loss of characteristic aromas of wines, and subsequently leads to the formation of new aromas characteristic of older wines or atypical aromas associated with wine deterioration. Several wine compounds such as esters and terpenes are transformed during wine storage, and the loss of wine aroma may occur [1-8]. SH-containing amino acids and peptides, especially glutathione and *N*-acetyl-cysteine, are good inhibitors of both enzymatic and non-enzymatic browning in a wide variety of foods. Glutathione is a naturally occurring tripeptide, while *N*-acetyl-cysteine is an excellent nutritional source of cysteine for humans [9].

As regards wine aroma compounds, inhibition of the decrease of several acetate esters, ethyl esters and terpenols during storage of white and red wines by glutathione and *N*-acetyl-cysteine has been reported [3-8].

The effort of present study was to find out if the protective effect of *N*-acetyl-cysteine and glutathione on wine aroma volatiles is due to their –SH group.

MATERIALS AND METHODS

Reagents and Wines

Glutathione, oxidized glutathione, *N*-acetyl-cysteine and *N*-acetyl-serine were purchased from Sigma (St. Louis, MO, USA).

Both wines used are Greek wines protected by Appellation of Origin. The dry white Muscat wine used was from Lemnos (Chonas, Lemnos) and the dry Xinomavro wine from Naoussa (Ktima Kyr-Yianni, Naoussa).

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Wine Storage and Analysis of Volatiles

One mL of each amino acid/peptide aqueous solution was added to 20 mL of Muscat or Xinomavro wine at a final concentration of 20 mg/L. Control samples were also prepared by adding 1 mL of distilled water to 20 mL of wine. The bottles (D=3.2 cm, h=10.6 cm, 60 mL capacity) were kept open at 20 °C. After 0, 3 or 6 days of storage for Muscat wine and 0, 2.5 or 5 days for Xinomavro wine, bottles were taken and wine samples were examined.

Wine volatile esters and terpenes were determined by SPME along with GC-MS analysis, as described previously with minor modifications [10]. The fiber used for the absorption of volatiles was a Carbowax^{TM}-Divinylbenzene 65 μm (Supelco, Bellefonte, PA, USA). Two mL of each wine sample were transferred into a 4 mL screw-capped glass vial with a Teflon-rubber septum (12 mm, Red TFE/SIL, USA). The contents were stirred for 15 min at 25 °C. Then, a constant length of the fiber was exposed to the headspace for another 30 min, under the same conditions.

Desorption of volatiles took place at 250 °C using a 0.75 mm ID liner (Supelco, Bellefonte, PA, USA) for 5 min. Split/splitless mode was used, splitless for 4 min and split ratio was 1:20.

GC-MS analysis was carried out on an HP 5973 quadrupole mass spectrometer directly coupled to an HP 6890 gas chromatograph (Hewllet Packard, USA). MS was operated in the electron impact mode with the electron energy set at 70 eV. For the period 0-5.20 min, mass range 50-370 m/z and 2.33 scan s^{-1} were applied, following by mass range 29-350 m/z and 2.32 scan s^{-1}. A G1701BA Chemstation was employed. Source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. The transfer line was kept at 220 °C. A non polar column, the Solgel-1 fused-silica column (30 m x 0.25 mm, 0.25 μm film thickness, SGE Analytical Science International) was used. The carrier gas was helium at a constant flow rate of 0.7 ml/min and average velocity 30 cm/sec. The oven temperature was programmed

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from 35 °C for 8 min and then raised to 45, 150, 180 and 210 °C at rates of 1.5, 3.0, 4.0 and 3.6 °C / min, respectively. It was held at 210 °C for 14.51 min.

All peaks were identified by comparing mass spectra to those obtained from Wiley 275 and NIST 98 libraries. Moreover, the identification of many peaks was confirmed with mass spectra and retention times of standard compounds determined in the same analysis conditions. Authentic standards used were: ethyl lactate, ethyl hexanoate, diethyl succinate, ethyl octanoate, ethyl decanoate, ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, limonene (Merck, Darmstadt, Germany), linalool and α-terpineol (Aldrich, Milwaukee, USA).

Semiquantitative relative data were determined using external standards. Phenylethyl acetate was used for acetate esters and ethyl esters, and linalool for terpenes.

Statistical Analysis

Each experiment was repeated three times, and results reported here are the means of the three runs. The one way analysis of variance (ANOVA), using the Duncan test at a level of significance $p<0.05$ was used for the statistical analysis (SPSS 11.5).

RESULTS AND DISCUSSION

The effect of *N*-acetyl-cysteine, *N*-acetyl-serine, glutathione and oxidized glutathione on aroma volatiles of a white and a red wine stored in open bottles at 20 °C was examined.

The effect of each amino acid/peptide on several ethyl esters, acetate esters and terpenes of Muscat-white and Xinomavro-red wine is reported in Tables **1** and **2**, respectively.

Table 1. Effect of *N*-Acetyl-Cysteine, *N*-Acetyl-Serine, Glutathione and Oxidized Glutathione on the Relative Concentrations of Some Aroma Volatiles During Oxidative Storage of Muscat-White Wine at 20 °C

Volatiles	Control	NAC	NAS	GSH	GSSG
			0 days		
Ethyl lactate	0.06 ^{Aa} ± 0.01	0.05 ^{a} ± 0.02	0.06 ^{a} ± 0.01	0.06 ^{a} ± 0.01	0.06 ^{a} ± 0.01
Diethyl succinate	0.24 ^{Aa} ± 0.08	0.24 ^{a} ± 0,07	0.23 ^{a} ± 0.06	0.23 ^{a} ± 0.07	0.23 ^{a} ± 0.07
Ethyl hexanoate	1.68 ^{Aa} ± 0.74	1.55 ^{a} ± 0.51	1.62 ^{a} ± 0.61	1.65 ^{a} ± 0.66	1.65 ^{a} ± 0.57
Ethyl octanoate	9.83 ^{Aa} ± 3.63	9.39 ^{a} ± 3.57	9.75 ^{a} ± 3.61	9.57 ^{a} ± 3.44	9.19 ^{a} ± 3.25
Ethyl 9-decanoate	0.89 ^{Aa} ± 0.41	0.94 ^{a} ± 0.41	0.91 ^{a} ± 0.40	0.86 ^{a} ± 0.38	0.97 ^{a} ± 0.41
Ehtyl decanoate	4.49 ^{Aa} ± 2.11	4.75 ^{a} ± 1.67	4.32 ^{a} ± 1.36	4.54 ^{a} ± 1.25	4.26 ^{a} ± 1.54
Ethyl acetate	0.13^{Aa*} ± 0,03**	0,13^{a} ± 0,03	0,14^{a} ± 0,03	0,14^{a} ± 0,03	0,13^{a} ± 0,02
Isoamyl acetate	0.50 ^{Aa} ± 0.19	0.50 ^{a} ± 0.04	0.50 ^{a} ± 0.03	0.52 ^{a} ± 0.03	0.48 ^{a} ± 0.02
2-phenylethylacetate	0.09 ^{Aa} ± 0.01	0.10 ^{a} ± 0.01	0.09 ^{a} ± 0.01	0.09 ^{a} ± 0.01	0.10 ^{a} ± 0.01
Linalool	0.88 ^{Aa} ± 0.20	0.88 ^{a} ± 0.23	0.91 ^{a} ± 0.08	0.92 ^{a} ± 0.05	0.94 ^{a} ± 0.13
α-terpineol	0.17 ^{Aa} ± 0.01	0.17 ^{a} ± 0.02	0.18 ^{a} ± 0.01	0.18 ^{a} ± 0.01	0.19 ^{a} ± 0.01
Limonene	0.12 ^{Aa} ± 0.03	0.09 ^{a} ± 0.01	0.12 ^{a} ± 0.02	0.10 ^{a} ± 0.01	0.11 ^{a} ± 0.01
			3 days		
Ethyl lactate	0.05 ^{Aa} ± 0.01	0.05 ^{a} ± 0.02	0.05 ^{a} ± 0.01	0.05 ^{a} ± 0.01	0.05 ^{a} ± 0.01
Diethyl succinate	0.21 ^{Aa} ± 0.07	0.23 ^{a} ± 0.06	0.20 ^{a} ± 0.05	0.21 ^{a} ± 0.06	0.21 ^{a} ± 0.07
Ethyl hexanoate	0.10 ^{Ba} ± 0.06	0.25 ^{b} ± 0.06	0.08 ^{a} ± 0.03	0.22 ^{b} ± 0.01	0.10 ^{a} ± 0.02
Ethyl octanoate	0.78 ^{Ba} ± 0.19	1.83 ^{b} ± 0.33	0.80 a ± 0.14	1.51 ^{b} ± 0.32	0.74 ^{a} ± 0.12
Ethyl 9-decanoate	0.16 ^{Ba} ± 0.02	0.39 ^{c} ± 0.08	0.14 ^{a} ± 0.02	0.30 ^{b} ± 0.05	0.15 ^{a} ± 0.03
Ehtyl decanoate	1.00 ^{Ba} ± 0.17	2.01 ^{c} ± 0.10	0.91 ^{a} ± 0.13	1.94 ^{c} ± 0.27	0.83 ^{a} ± 0.11
Ethyl acetate	0.04 ^{Ba} ± 0.02	0.09 ^{b} ± 0.01	0.04 ^{a} ± 0.01	0.07 ^{b} ± 0.01	0.04 ^{a} ± 0.01
Isoamyl acetate	0.04 ^{Ba} ± 0.01	0.11 ^{c} ± 0.02	0.05 ^{a} ± 0.01	0.08 ^{b,c} ± 0.00	0.04 ^{a} ± 0.01
2-phenylethylacetate	0.06 ^{Ba} ± 0.01	0.09 ^{b} ± 0.01	0.06 ^{a} ± 0.01	0.07 ^{a,b} ± 0.01	0.06 ^{a} ± 0.01
Linalool	0.65 ^{Ba} ± 0.15	0.83 ^{a,b} ± 0.22	0.62 ^{a} ± 0.23	0.86 ^{b} ± 0.04	0.64 ^{a} ± 0.15
α-terpineol	0.11 ^{Ba} ± 0.02	0.15 ^{c} ± 0.02	0.11 ^{a,b} ± 0.01	0.14 ^{b,c} ± 0.03	0.11 ^{a,b} ± 0.00
Limonene	0.00 ^{Ba} ± 0.00	0.02 ^{c} ± 0.00	0.00 ^{a} ± 0.00	0.01 ^{b} ± 0.00	0.00 ^{a} ± 0.00

(Table 1). Contd…..

Volatiles	Control	NAC	NAS	GSH	GSSG
			6 days		
Ethyl lactate	0.05 ^{Aa} ± 0.01	0.04 ^{a} ± 0.01	0.05 ^{a} ± 0.02	0.05 ^{a} ± 0.02	0.05 ^{a} ± 0.01
Diethyl succinate	0.20 ^{Aa} ± 0.07	0.22 ^{a} ± 0.06	0.18 ^{a} ± 0.05	0.20 ^{a} ± 0.06	0.19 ^{a} ± 0.08
Ethyl hexanoate	0.04 ^{Ba} ± 0.00	0.13 ^{b} ± 0.04	0.04 ^{a} ± 0.01	0.10 ^{b} ± 0.03	0.05 ^{a} ± 0.02
Ethyl octanoate	0.49 ^{Ba} ± 0.04	1.21 ^{c} ± 0.25	0.47 ^{a} ± 0.07	0.84 ^{b} ± 0.15	0.45 ^{a} ± 0.06
Ethyl 9-decanoate	0.08 ^{Ba,b} ± 0.02	0.20 ^{c} ± 0.03	0.06 ^{a} ± 0.01	0.17 ^{c} ± 0.03	0.09 ^{a,b} ± 0.02
Ehtyl decanoate	0.49 ^{Bb} ± 0.09	0.98 ^{c} ± 0.05	0.36 ^{a} ± 0.03	0.86 ^{c} ± 0.05	0.46 ^{a,b} ± 0.04
Ethyl acetate	0.03 ^{Ba} ± 0.01	0.06 ^{b} ± 0.01	0.02 ^{a} ± 0.00	0.05 ^{b} ± 0.01	0.03 ^{a} ± 0.01
Isoamyl acetate	0.02 ^{Ba} ± 0.00	0.04 ^{b} ± 0.01	0.02 ^{a} ± 0.01	0.03 ^{a} ± 0.00	0.02 ^{a} ± 0.00
2-phenylethylacetate	0.04 ^{Ca} ± 0.00	0.06 ^{b} ± 0.01	0.03 ^{a} ± 0.00	0.05 ^{b} ± 0.00	0.03 ^{a} ± 0.00
Linalool	0.48 ^{Ca,b} ± 0.12	0.76 ^{c} ± 0.23	0.45 ^{a,b} ± 0.11	0.66 ^{b,c} ± 0.03	0.36 ^{a} ± 0.00
α-terpineol	0.08 ^{Ca} ± 0.02	0.14 ^{b} ± 0.02	0.08 ^{a} ± 0.00	0.10 ^{a} ± 0.02	0.07 ^{a} ± 0.00
Limonene	0.00 ^{Ba} ± 0.00	0.01 ^{b} ± 0.00	0.00 ^{a} ± 0.00	0.00 ^{a} ± 0.00	0.00 ^{a} ± 0.00

Esters are in mg/L as phenylethyl acetate, and terpenes are in mg/L as linalool.
Means are given with their standard deviation.
A, B, C: They were used in the comparison of volatiles of control wine at 0, 3 and 6 days of storage. Means that do not bear a common superscript differ significantly at $p<0.05$.
a, b, c: They were used in the comparison of volatiles of control wine and those containing each amino acid/peptide at the same sampling time (0, 3 or 6 days). Means that do not bear a common superscript differ significantly at $p<0.05$.
NAC=*N*-acetyl-cysteine; NAS=*N*-acetyl-serine; GSH=Glutathione; GSSG=Oxidized glutathione.

Table 2. Effect of *N*-Acetyl-Cysteine, *N*-Acetyl-Serine, Glutathione, Oxidized Glutathione on the Relative Concentrations of Some Aroma Volatiles During Oxidative Storage of Xinomavro-Red Wine at 20 ^{o}C

Volatiles	Control	NAC	NAS	GSH	GSSG
			0 days		
Ethyl lactate	0.21^{Aa} ± 0.04	0.19 ^{a} ± 0.05	0.20 ^{a} ± 0.03	0.18 ^{a} ± 0.04	0.19 ^{a} ± 0.02
Dimethyl succinate	1.38 ^{Aa} ± 0.38	1.30 ^{a} ± 0.21	1.29 ^{a} ± 0.13	1.34 ^{a} ± 0.10	1.35 ^{a} ± 0.24
Ethyl hexanoate	0.55 ^{Aa} ± 0.22	0.52 ^{a} ± 0.13	0.58 ^{a} ± 0.19	0.55 ^{a} ± 0.18	0.53 ^{a} ± 0.13
Ethyl octanoate	2.78 ^{Aa} ± 1.21	2.84 ^{a} ± 1.39	2.78 ^{a} ± 1.18	2.72 ^{a} ± 1.02	2.31 ^{a} ± 0.78
Ethyl decanoate	0.72 ^{Aa} ± 0.29	0.67 ^{a} ± 0.26	0.72 ^{a} ± 0.16	0.75 ^{a} ± 0.30	0.74 ^{a} ± 0.22
Ethyl acetate	0.19^{Aa*}±0.12**	0.19 ^{a} ± 0.07	0.19 ^{a} ± 0.08	0.18 ^{a} ± 0.10	0.20 ^{a} ± 0.11
Isoamyl acetate	0.14 ^{Aa} ± 0.05	0.14 ^{a} ± 0.02	0.15 ^{a} ± 0.03	0.15 ^{a} ± 0.03	0.14 ^{a} ± 0.03
			2.5 days		
Ethyl lactate	0.19 ^{Aa} ± 0.06	0.17 ^{a} ± 0.05	0.18 ^{a} ± 0.02	0.16 ^{a} ± 0.04	0.18 ^{a} ± 0.03
Dimethyl succinate	1.29 ^{Aa} ± 0.36	1.28 ^{a} ± 0.23	1.22 ^{a} ± 0.11	1.34 ^{a} ± 0.10	1.29 ^{a} ± 0.22
Ethyl hexanoate	0.03 ^{Ba} ± 0.01	0.12 ^{b} ± 0.03	0.03 ^{a} ± 0.01	0.12 ^{b} ± 0.01	0.04 ^{a} ± 0.01
Ethyl octanoate	0.12 ^{Ba} ± 0.09	0.69 ^{b} ± 0.32	0.13 ^{a} ± 0.04	0.55 ^{a,b} ± 0.59	0.10 ^{a} ± 0.04
Ethyl decanoate	0.06 ^{Ba} ± 0.01	0.20 ^{b} ± 0.08	0.06 ^{a} ± 0.01	0.16 ^{b} ± 0.03	0.07 ^{a} ± 0.01
Ethyl acetate	0.05 ^{Ba} ± 0.02	0.11 ^{b} ± 0.04	0.05 ^{a} ± 0.02	0.07 ^{a,b} ± 0.03	0.05 ^{a} ± 0.02
Isoamyl acetate	0.01 ^{Ba,b} ± 0.01	0.03 ^{c} ± 0.00	0.01 ^{a,b} ± 0.00	0.02 ^{b,c} ± 0.00	0.01 ^{a} ± 0.00
			5 days		
Ethyl lactate	0.16 ^{Aa} ± 0.04	0.16 ^{a} ± 0.04	0.17 ^{a} ± 0.04	0.16 ^{a} ± 0.04	0.16 ^{a} ± 0.03
Dimethyl succinate	1.33 ^{Aa} ± 0.39	1.16 ^{a} ± 0.20	1.17 ^{a} ± 0.10	1.31 ^{a} ± 0.11	1.25 ^{a} ± 0.23
Ethyl hexanoate	0.01 ^{Ba} ± 0.01	0.09 ^{b} ± 0.02	0.01 ^{a} ± 0.00	0.08 ^{b} ± 0.01	0.02 ^{a} ± 0.01
Ethyl octanoate	0.07 ^{Ba} ± 0.08	0.51 ^{b} ± 0.14	0.08 ^{a} ± 0.05	0.41 ^{a,b} ± 0.41	0.06 ^{a} ± 0.04
Ethyl decanoate	0.01 ^{Ba} ± 0.00	0.13 ^{c} ± 0.03	0.03 ^{a,b} ± 0.00	0.10 ^{c} ± 0.01	0.02 ^{a,b} ± 0.00
Ethyl acetate	0.01 ^{Ca} ± 0.01	0.07 ^{b} ± 0.04	0.02 ^{a} ± 0.01	0.04 ^{a,b} ± 0.03	0.02 ^{a} ± 0.00
Isoamyl acetate	0.01 ^{Ba} ± 0.01	0.01 ^{a} ± 0.01	0.00 ^{a} ± 0.00	0.01 ^{a} ± 0.00	0.00 ^{a} ± 0.00

Volatiles are in mg/L as phenylethyl acetate.
Means are given with their standard deviation.
A, B, C: They were used in the comparison of volatiles of control wine at 0, 3 and 6 days of storage. Means that do not bear a common superscript differ significantly at $p<0.05$.
a, b, c: They were used in the comparison of volatiles of control wine and those containing each amino acid/peptide at the same sampling time (0, 3 or 6 days). Means that do not bear a common superscript differ significantly at $p<0.05$.
NAC=*N*-acetyl-cysteine; NAS=*N*-acetyl-serine; GSH=Glutathione; GSSG=Oxidized glutathione.

The addition of each amino acid/peptide in each wine did not change the concentration of any volatile acetate, ethyl ester or terpene at t=0. In control wines, all volatiles determined decreased during wine storage, at a statistically significant level, with the exceptions of ethyl lactate and dimethyl succinate which did not change. *N*-acetyl-cysteine and glutathione inhibited the decrease of volatiles which decreased during storage of wines, while they did not affect the concentration of ethyl lactate and dimethyl succinate. In Muscat wine, both thiols inhibited the decrease of ethyl hexanoate, ethyl octanoate, ethyl-9-decanoate, ethyl decanoate, ethyl acetate, isoamyl acetate, 2-phenylethyl acetate, linalool, a-terpineol and limonene. In Xinomavro wine, they inhibited the decrease of ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl acetate and isoamyl acetate. On the contrary, *N*-acetyl-serine and oxidized glutathione did not protect any volatile of Muscat or Xinomavro wine at any sampling time.

In present work, the protection of volatiles during wine storage by amino acids/peptides was studied. The main difference between *N*-acetyl-serine and *N*-acetyl-cysteine is that *N*-acetyl-serine contains the –OH group instead of the –SH group. Similarly, the main difference between oxidized glutathione and (reduced) glutathione is the absence of free –SH group in the former. Consequently, it can be concluded that the –SH group is essential for the protective effect of *N*-acetyl-cysteine and glutathione on wine aroma volatiles.

Volatile losses may be because of oxidation and other chemical reactions. For example, ester content may be changed because of hydrolysis and esterification [11]. Moreover, ester oxidation by hydroxyl radical oxidation-related processes has also been postulated by some authors [2]. Most monoterpene alcohols are replaced by terpene oxides, and linalool may be replaced by a-terpineol [12]. The inhibitory action of glutathione and *N*-acetyl-cysteine may be related to their antioxidant properties.

Glutathione is a naturally occurring tripeptide and a strong antioxidant. Except its protective effect on wine aroma volatiles, glutathione plays a crucial role in must oxidation. Glutathione increases with maturation of grape berries [13]. In must, the enzymatic oxidation product of caftaric acid can react with glutathione forming what is termed as grape reaction product. The grape reaction product terminates the oxidation process and subsequently limits oxidation [14]. Glutathione is assimilated by yeast during the beginning of fermentation and released at the end of alcoholic fermentation. Thirty days-post fermentation, glutathione levels can be as high or higher than in the initial juice. When lees are eliminated, the glutathione concentration diminishes rapidly. In new barrels, this reduction is even greater due to the oxidation effect of new wood. Glutathione levels in wine may be 2.5-5.0 mg/L or much higher 20-30 mg/L [4, 15-17]. It has been reported that the impact of glutathione on limiting the decrease of volatile esters and terpenes during aging is concentration dependent in the range 0-20 mg/L [7]. Moreover, it is estimated that 20 mg/L of glutathione at the end of aging period is optimum for aroma protection [18]. All the above indicate that the management of glutathione may be highly important for wine quality, especially of white wine, by helping to preserve aroma.

Previously, it was reported that glutathione and *N*-acetyl-cysteine protect several wine aroma volatiles [2-8]. Present results show that the protective effect of glutathione and *N*-acetyl-cysteine is due to their free –SH. Wines contain glutathione and other thiols too [15]. Based on present results, it may be said that any thiol present in wine may protect several wine aroma volatiles.

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