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Food Chemistry 93 (2005) 485–492
Food Chemistry
www.elsevier.com/locate/foodchem

Inhibition of the decline of volatile esters and terpenols during oxidative storage of Muscat-white and Xinomavro-red wine by caffeic acid and *N*-acetyl-cysteine

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Received 23 April 2004; received in revised form 20 October 2004; accepted 21 October 2004

Abstract

Caffeic acid and *N*-acetyl-cysteine were tested as inhibitors of the decline of volatile aroma compounds during oxidative storage of Muscat-white and Xinomavro-red wine. Caffeic acid was added at 100 ppm while *N*-acetyl-cysteine at 20 ppm. Both wines were stored in open bottles at 20 °C, and samples were analysed using solid phase microextraction along with GC-MS analysis at 0, 1, and 2 days.

No effect on the concentration of any volatile was observed as a result of adding caffeic acid or *N*-acetyl-cysteine in each wine. Many ethyl esters – such as hexanoate, octanoate, decanoate – and acetate esters – such as isoamyl acetate – decreased during storage of both white and red wine. Caffeic acid and *N*-acetyl-cysteine significantly inhibited the decline of the volatile esters. Linalool and α-terpineol decreased during storage of Muscat wine. Their decline was significantly inhibited by caffeic acid and *N*-acetyl-cysteine.

Present results indicate that caffeic acid and *N*-acetyl-cysteine may be taken into account as potent inhibitors of the disappearance of aromatic volatile esters and terpenols in wines.

Keywords: Wine; Volatiles; Aroma; Ethyl esters; Acetate esters; Terpenols; Phenolic acids; Caffeic acid; Thiols; *N*-acetyl-cysteine

1. Introduction

The oxidation of white as well as red wines is a well-known problem in winemaking, particularly the oxidative spoilage of young white wines. Wines retain their organoleptic characteristics until the oxidation starts, and then begin to lose those characteristics that define their quality. The first step of wine oxidation 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 the deterioration of the product. The oxidative browning is a later step of wine oxidation (Escudero, Asensio, Cacho, & Ferreira, 2002; Escudero, Cacho, & Ferreira, 2000a, Escudero, Hernadez-Orte, Cacho, & Ferreira, 2000b; Fernadez-Zurbano et al., 1995; Silva Ferreira, Guedes De Pinho, Rodrigues, & Hogg, 2002; Singleton, 1987).

Wine volatiles, which include ethyl esters, acetate esters, terpenols, higher alcohols, fatty acids, aldehydes, ketones, hydrocarbons and others, have a major impact on wine aroma. Ethyl esters and acetates are formed enzymically during fermentation and are important wine aroma compounds. Ethyl esters of hexanoic, octanoic and decanoic acids, and isoamyl and isobutyl acetates

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doi:10.1016/j.foodchem.2004.10.025

are often considered to give wine much of its vinous fragrance. The low molecular weight esters, often called 'fruity esters', have distinctly fruity fragrances (Jackson, 1994). Terpenes are an important group of aromatic compounds, giving fragrance to several grape varieties and wines. Among them, the Muscat cultivars are characterized by their terpene content and monoterpene alcohols are primarily responsible for the fragrance of Muscat wines (Jackson, 1994).

Phenolic compounds participate in wine oxidation phenomena. The intensity of brown color caused by enzymic browning depends on the type of phenolic compounds involved. Certain phenolic acids inhibit the polyphenoloxidase activity, whereas others can promote the browning reaction. Wines are rich in phenolic compounds that impart an antioxidant activity to wine that is its natural preservative. Among them, benzoic and cinnamic acid derivatives exhibit antioxidant activity in different systems (Jackson, 1994; Natella, Nardini, De Felice, & Scaccini, 1999).

SH-containing amino acids and peptides are good inhibitors of both enzymic and non-enzymic browning in fruit juices and other foods (Friedman, 1994, 1996). *N*-acetyl-cysteine is an excellent nutritional source of cysteine for humans and animals and acts as an antimutagen and anticarcinogen. Moreover, it is used as a drug to reduce lung congestion (Friedman, 1997). It has been reported that *N*-acetyl-cysteine is an excellent antibrowning agent in fruits and vegetables (Molnar-Perl & Friedman, 1990). As regards wine aroma compounds, inhibition of the decline of linalool and α-terpineol in Muscat wines by *N*-acetyl-cysteine has been previously reported (Papadopoulou & Roussis, 2001).

The present study was undertaken to determine the ability of caffeic acid and *N*-acetyl-cysteine to inhibit the decline of volatile aroma compounds of Muscat-white and Xinomavro-red wine during oxidative storage.

2. Materials and methods

Caffeic acid and *N*-acetyl-cysteine were purchased from Sigma (St. Louis, USA). The dry Muscat wine used was produced on the island of Lemnos (Filonoi 2002, Lemnos Greece), using the white Muscat variety. The dry Xinomavro wine used was produced in the region of Naoussa (Ktima Kyr-Yianni 2000, Naoussa Greece), using the Xinomavro variety. Both wines used are of Appelation of Origin.

Gross composition of wine samples was determined by the classic methods (Ough & Amerine, 1988). Alcohol was determined with a hydrometer, reducing sugars by Lane-Eynon method, pH with a pH-meter, total acidity by volumetric analysis, and free sulphide by the Ripper method. The total phenolic contents of wines were determined according to the Folin–Ciocalteu method (Singleton & Rossi, 1965) using gallic acid as a standard.

One millilitre of caffeic acid or *N*-actetyl-cysteine aqueous solution was added to 20 mL of Muscat or Xinomavro wine at a final concentration of 100 and 20 mg/L, respectively. 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, 1 or 2 days of storage, bottles were taken and wine samples were examined.

The fiber used for the absorption of volatiles was a Carbowax™-Divinylbenzene 65 μm (Supelco, Bellefonte, PA, USA). Two millilitre of wine sample and 50 μL of internal standard in 10% ethanol (4-methyl-1-pentanol, 5 mg/L in final solution) 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 10 min at 35 °C. Then, a constant length of the fiber was exposed to the headspace for another 5 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 10 min. Split/splitless mode was used for 2 min.

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. Mass range, 29–400 *m/z*, and 2.35 scan s^{-1} were applied, and a G1701BA Chemstation was employed. Source and quadrupole temperatures were set at 230 and 150 °C, respectively. The transfer line was kept at 220 °C.

An Innowax fused-silica column was used (30 m × 0.32 mm, 0.5 μm film thickness, J&W. Scientific, Folsom, USA). The carrier gas was helium at a flow rate of 0.7 ml/min and average velocity 30 cm/s. The oven temperature was programmed from 35 °C for 5 min and then raised to 60, 220, and 250 °C at rates of 2.0, 5.0 and 15 °C/min, respectively. It was held at 250 °C for 5.5 min.

Peak identification was carried out by comparing mass spectra to those obtained from Wiley 275 and NIST 98 libraries. Moreover, the identification of several peaks was confirmed with the retention indices of standard compounds determined in the same analysis conditions.

Semiquantitative data were expressed in milligrams per liter ((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 Duncan test at level of significance $P < 0.05$ was used for statistical analysis (SPSS 11.5).

3. Results

3.1. Muscat wine

The average composition of the dry white Muscat wine was: alcohol content 11.0%, pH 3.26, total acidity 5.0 g/L as tartaric acid, and free SO_2 33 mg/L. Total phenolic content of Muscat wine was 232 mg/L gallic acid equivalents.

The effect of caffeic acid and *N*-acetyl-cysteine on the sum of the relative concentrations of ethyl esters and acetate esters of Muscat wine during oxidative storage at 20 °C is presented in Figs. 1 and 2, respectively. At $t = 0$, the sum of ethyl esters and acetate esters was statistically equal in control and samples containing caffeic acid or *N*-acetyl-cysteine. In the control, the sum of ethyl esters and acetate esters decreased during wine storage, at a statistically significant level. The total content of ethyl esters dropped by 71% at $t = 1$ and 83% at $t = 2$, compared to $t = 0$. Similarly, the total content of acetate esters dropped by 47% at $t = 1$ and 60% at $t = 2$, compared to $t = 0$. The decrease of both ethyl esters and acetate esters was significantly less in the presence of caffeic acid or *N*-acetyl-cysteine.

The relative concentrations of each volatile ethyl ester and acetate ester of Muscat wine kept at 20 °C are reported in Tables 1 and 2, respectively. Many ethyl esters decreased during wine oxidative storage for 1–2 days. Among them were ethyl hexanoate, ethyl octanoate and ethyl decanoate. In contrast, ethyl lactate and diethyl succinate were stable during wine storage. At $t = 1$ and 2, wine samples containing caffeic acid or *N*-acetyl-cysteine had higher contents of the ethyl esters which decreased during wine storage. For example, at $t = 2$ samples containing caffeic acid or *N*-acetyl-cysteine contained an ethyl octanoate content about 2 and 3 times higher than the control, respectively.

With the exception of 2-phenyl ethyl acetate, acetate esters decreased during wine storage. The decline of ethyl acetate and isoamyl acetate was inhibited by the addition of caffeic acid or *N*-acetyl-cysteine.

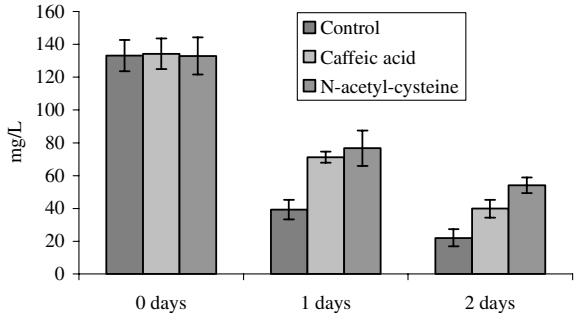

Fig. 1. Sum of the relative concentrations of volatile ethyl esters during oxidative storage at 20 °C of Muscat wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations.

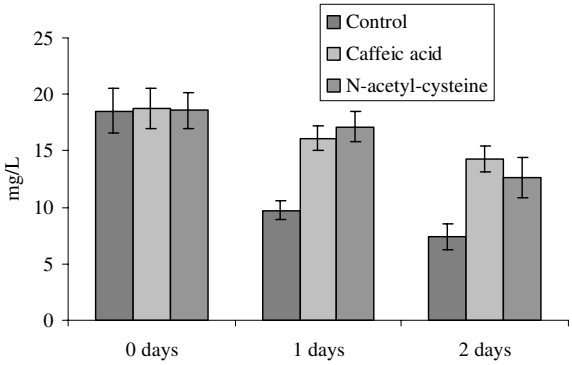

Fig. 2. Sum of the relative concentrations of volatile acetate esters during oxidative storage at 20 °C of Muscat wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations.

The effect of caffeic acid and *N*-acetyl-cysteine on the sum of the relative concentrations of terpenols of Muscat wine during oxidative storage at 20 °C is presented in Fig. 3. At $t = 0$, the sum of terpenols was statistically equal in control and samples containing caffeic acid or *N*-acetyl-cysteine. In the control, the sum of terpenols decreased during wine storage, at a statistically significant level. The decrease of terpenols was significantly less in the presence of caffeic acid or *N*-acetyl-cysteine.

The relative concentrations of each volatile terpenol of Muscat wine during oxidative storage are reported in Table 3. Linalool and α-terpineol decreased during wine storage. At $t = 1$ or 2, wine samples containing caffeic acid or *N*-acetyl-cysteine contained higher concentrations of linalool and α-terpineol than control ones.

The effect of caffeic acid and *N*-acetyl-cysteine on the sum of the relative concentrations of alcohols and fatty acids of Muscat wine during oxidative storage at 20 °C is presented in Figs. 4 and 5, respectively. Caffeic acid or *N*-acetyl-cysteine had no statistically significant effect on the sum of alcohols and fatty acids at any time of sampling. The sum of alcohols and fatty acids did not decrease at a statistically significant level during wine storage.

3.2. Xinomavro wine

The average composition of the dry red Xinomavro wine was: alcohol content 14.1%, pH 3.25, total acidity 5.5 g/L as tartaric acid, and free SO_2 53 mg/L. Total phenolic content of Xinomavro wine was 3920 mg/L gallic acid equivalent.

The effect of caffeic acid and *N*-acetyl-cysteine on the sum of the relative concentrations of ethyl esters and acetate esters of Xinomavro wine during oxidative storage at 20 °C is presented in Figs. 6 and 7, respectively.

Table 1
Relative concentrations of volatile ethyl esters during oxidative storage at 20 °C of Muscat wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L)

Compounds	Control (days)			Caffeic acid (days)			*N*-acetyl cysteine (days)		
	0	1	2	0	1	2	0	1	2
Ethyl butanoate	1.56^{A}	0.38^{Ba}	0.00^{Ca}	1.57	0.49^{a}	0.19^{b}	1.52	1.20^{b}	0.69^{c}
Ethyl isovalerate	0.18^{A}	0.00^{B}	0.00^{B}	0.18	0.00	0.00	0.19	0.00	0.00
Ethyl hexanoate	22.59^{A}	7.01^{Ba}	2.32^{Ca}	22.76	11.66^{b}	6.35^{b}	22.11	11.40^{b}	5.99^{b}
Ethyl lactate	1.50^{A}	1.33^{A}	1.30^{A}	1.51	1.46	1.44	1.48	1.46	1.32
Ethyl octanoate	78.94^{A}	16.81^{Ba}	8.82^{Ca}	79.36	36.23^{b}	17.15^{b}	78.90	39.57^{b}	27.69^{c}
Ethyl pelargonate	0.09^{A}	0.00^{Ba}	0.00^{Ba}	0.09	0.06^{b}	0.01^{b}	0.11	0.09^{c}	0.01^{b}
Ethyl decanoate	16.30^{A}	6.79^{Ba}	4.03^{Ca}	16.64	11.86^{b}	8.02^{b}	17.23	14.18^{b}	11.57^{c}
Isoamyl octanoate	0.42^{A}	0.00^{Ba}	0.00^{B}	0.40	0.06^{b}	0.00	0.37	0.05^{b}	0.00
Diethyl succinate	3.97^{A}	3.98^{A}	3.86^{A}	4.06	4.00	3.89	3.92	3.90	3.90
Ethyl-9-decanoate	6.19^{A}	2.12^{Ba}	1.36^{B}	6.16	4.54^{b}	2.28	5.74	3.99^{b}	2.17
Ethyl laurate	0.40^{A}	0.22^{B}	0.10^{Ba}	0.41	0.25	0.18^{b}	0.40	0.36	0.25^{c}
Ethyl-3-methylbutyl-butanedioate	0.25^{A}	0.00^{B}	0.00^{B}	0.21	0.00	0.00	0.14	0.00	0.00
Ethyl myristate	0.33^{A}	0.19^{AB}	0.15^{B}	0.33	0.26	0.19	0.33	0.27	0.21
Ethyl palmitate	0.49^{A}	0.35^{A}	0.14^{B}	0.51	0.38	0.18	0.47	0.39	0.21

Values (mg/L) are the means of three trials.
A, B, C: They were used in the comparison of each volatile of control wine at 0, 1 and 2 days of storage. Means that do not bear a common superscript differ significantly.
a, b, c: They were used in the comparison of each volatile of control wine and those containing caffeic acid or *N*-acetyl-cysteine at the same sampling time (0, 1 or 2 days). Among means bearing superscripts, those that do not bear a common superscript differ significantly.

Table 2
Relative concentrations of volatile acetate esters during oxidative storage at 20 °C of Muscat wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L)

Compounds	Control (days)			Caffeic acid (days)			*N*-acetyl cysteine (days)		
	0	1	2	0	1	2	0	1	2
Ethyl acetate	13.30^{A}	7.88^{Ba}	6.23^{Ba}	13.66	12.07^{b}	11.37^{b}	13.29	12.89^{b}	10.02^{b}
Isoamyl acetate	4.34^{A}	1.38^{Ba}	0.83^{Ba}	4.46	3.55^{b}	2.52^{b}	4.41	3.61^{b}	$2.18^{a,b}$
Hexyl acetate	0.29^{A}	0.00^{B}	0.00^{B}	0.31	0.02	0.00	0.31	0.10	0.00
Ethyl phenyl acetate	0.16^{A}	0.09^{ABa}	0.07^{B}	0.17	0.11^{ab}	0.08	0.16	0.14^{a}	0.11
2-phenyl ethyl acetate	0.44^{A}	0.38^{A}	0.29^{A}	0.44	0.38	0.31	0.40	0.37	0.31

Values (mg/L) are the means of three trials.
A, B, C: They were used in the comparison of each volatile of control wine at 0, 1 and 2 days of storage. Means that do not bear a common superscript differ significantly.
a, b, c: They were used in the comparison of each volatile of control wine and those containing caffeic acid or *N*-acetyl-cysteine at the same sampling time (0, 1 or 2 days). Among means bearing superscripts, those that do not bear a common superscript differ significantly.

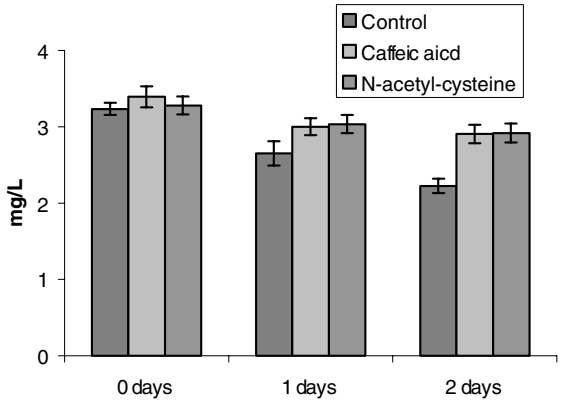

Fig. 3. Sum of the relative concentrations of volatile terpenols during oxidative storage at 20 °C of Muscat wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations.

At $t = 0$, the sum of ethyl esters and acetate esters was statistically equal in control and samples containing caffeic acid or *N*-acetyl-cysteine. In the control, the sum of ethyl esters and acetate esters decreased during wine storage, at a statistically significant level. The total content of ethyl esters dropped by 50% at $t = 1$ and 58% at $t = 2$, compared to $t = 0$. Similarly, the total content of acetate esters dropped by 52% at $t = 1$ and 67% at $t = 2$, compared to $t = 0$. The decrease of both ethyl esters and acetate esters was significantly less in the presence of caffeic acid or *N*-acetyl-cysteine.

The relative concentrations of each volatile ethyl ester and acetate ester of Xinomavro wine kept at 20 °C are reported in Tables 4 and 5, respectively. Most ethyl esters decreased during wine oxidative storage for 1 or 2 days, notably ethyl hexanoate, ethyl octanoate and ethyl

Table 3
Relative concentrations of volatile terpene alcohols during oxidative storage at 20 °C of Muscat wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L)

Compounds	Control (days)			Caffeic acid (days)			*N*-acetyl cysteine (days)		
	0	1	2	0	1	2	0	1	2
Linalool	1.74^{A}	1.55^{AB}	1.32^{Ba}	1.75	1.68	1.63^{b}	1.76	1.70	1.67^{b}
α-terpineol	1.36^{A}	1.10^{Ba}	0.90^{Ca}	1.37	1.32^{b}	1.28^{b}	1.38	1.33^{b}	1.25^{b}
Citronellol	0.07^{A}	0.00^{B}	0.00^{B}	0.09	0.00	0.00	0.07	0.00	0.00
Geraniol	0.06^{A}	0.00^{B}	0.00^{B}	0.28	0.00	0.00	0.06	0.01	0.00

Values (mg/L) are the means of three trials.
A, B, C: They were used in the comparison of each volatile of control wine at 0, 1 and 2 days of storage. Means that do not bear a common superscript differ significantly.
a, b, c: They were used in the comparison of each volatile of control wine and those containing caffeic acid or *N*-acetyl-cysteine at the same sampling time (0, 1 or 2 days). Among means bearing superscripts, those that do not bear a common superscript differ significantly.

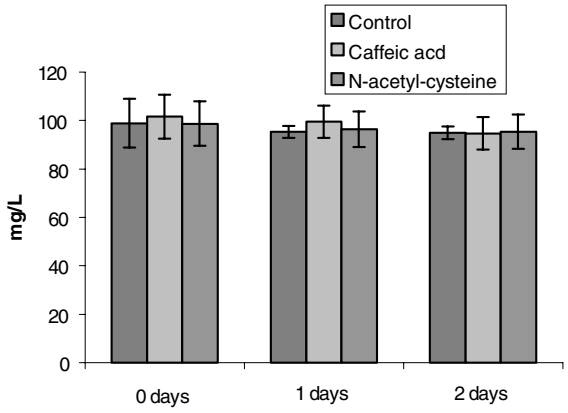

Fig. 4. Sum of the relative concentrations of volatile alcohols during oxidative storage at 20 °C of Muscat wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations. The alcohols determined are: 1-propanol, isobutyl alcohol, isoamyl alcohol, hexanol, *cis*-3-hexenol, 2-ethyl-hexanol, 2,6-dimethyl-4-heptanol, octanol, nonanol, 3-(methylthio)-1-propanol, decanol, and 2-phenylethanol.

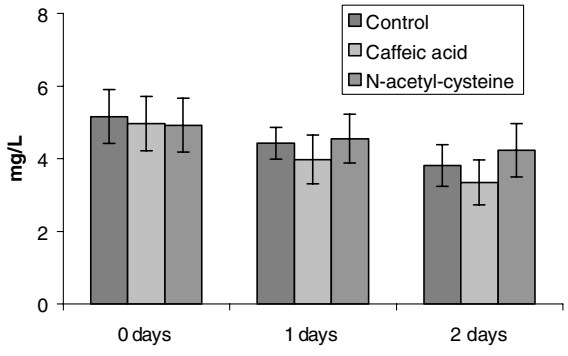

Fig. 5. Sum of the relative concentrations of volatile fatty acids during oxidative storage at 20 °C of Muscat wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations. The acids determined are: hexanoic, octanoic, decanoic, and palmitic.

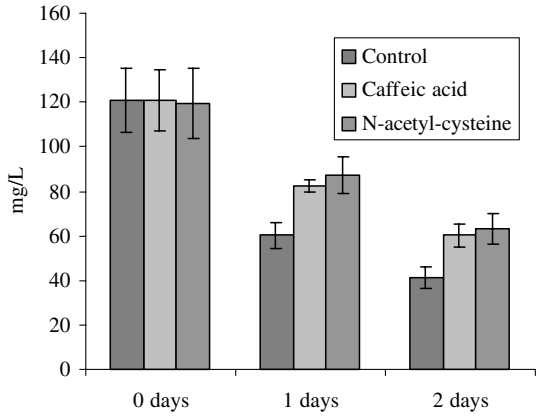

Fig. 6. Sum of the relative concentrations of volatile ethyl esters during oxidative storage at 20 °C of Xinomavro wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations.

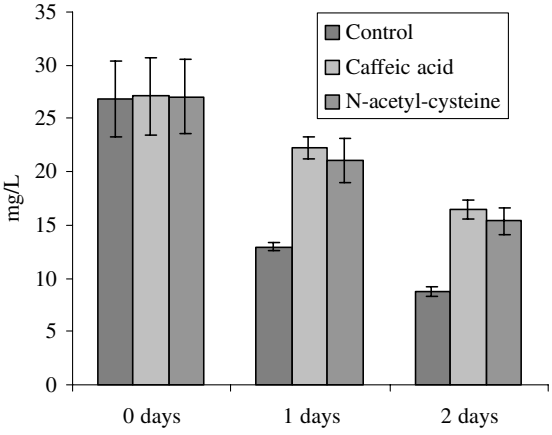

Fig. 7. Sum of the relative concentrations of volatile acetate esters during oxidative storage at 20 °C of Xinomavro wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations.

decanoate. Diethyl succinate was stable during wine storage. Ethyl lactate dropped by about 15% after 1 or 2 days of storage. At $t = 1$ and 2, wine samples containing caffeic acid or *N*-acetyl-cysteine had higher contents of most of the ethyl esters which decreased during wine storage. For example, at $t = 2$ samples containing caffeic

Table 4
Relative concentrations of volatile ethyl esters during oxidative storage at 20 °C of Xinomavro wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L)

Compounds	Control (days)			Caffeic acid (days)			*N*-acetyl cysteine (days)		
	0	1	2	0	1	2	0	1	2
Ethyl butanoate	0.90^{A}	0.59^{AB}	0.46^{B}	0.93	0.73	0.67	0.92	0.74	0.66
Ethyl isovalerate	0.92^{A}	0.21^{Ba}	0.03^{Ca}	0.89	0.23^{a}	0.03^{a}	0.93	0.64^{b}	0.12^{b}
Ethyl-2-methylbutanoate	0.84^{A}	0.29^{B}	0.00^{Ca}	0.84	0.38	0.16^{b}	0.90	0.52	0.09^{b}
Ethyl hexanoate	11.01^{A}	2.86^{Ba}	1.13^{Ca}	10.98	5.70^{b}	2.62^{b}	10.73	4.99^{b}	2.47^{b}
Ethyl lactate	8.54^{A}	7.23^{B}	7.22^{B}	8.62	7.58	7.56	8.46	8.23	8.04
Methyl octanoate	0.18^{A}	0.00^{B}	0.00^{B}	0.17	0.00	0.00	0.16	0.00	0.00
Ethyl octanoate	48.46^{A}	6.76^{Ba}	3.72^{Ba}	48.26	22.44^{b}	9.41^{b}	48.24	28.46^{c}	13.23^{c}
Ethyl pelargonate	0.18^{A}	0.00^{Ba}	0.00^{B}	0.16	0.02^{b}	0.00	0.16	0.04^{b}	0.00
Ethyl decanoate	12.22^{A}	4.81^{Ba}	2.66^{C}	12.33	9.20^{b}	4.35^{b}	12.21	8.43^{b}	5.25^{b}
Isoamyl octanoate	0.24^{A}	0.00^{B}	0.00^{B}	0.25	0.00	0.00	0.24	0.00	0.00
Diethyl succinate	35.95^{A}	36.99^{A}	35.64^{A}	36.11	35.54	35.17	33.15	34.17	32.80
Ethyl-9-decanoate	0.26^{A}	0.03^{B}	0.00^{B}	0.26	0.08	0.01	0.26	0.12	0.04
Ethyl laurate	0.33^{A}	0.00^{Ba}	0.00^{Ba}	0.32	0.07^{b}	0.02^{b}	0.32	0.14^{b}	0.05^{b}
Ethyl-3-methylbutyl-butanedioate	0.30^{A}	0.23^{B}	0.21^{B}	0.30	0.25	0.22	0.28	0.25	0.23
Ethyl myristate	0.22^{A}	0.03^{Ba}	0.00^{B}	0.21	0.04^{a}	0.00	0.20	0.12^{b}	0.06
Ethyl palmitate	0.17^{A}	0.08^{AB}	0.06^{B}	0.17	0.13	0.09	0.16	0.14	0.12

Values (mg/L) are the means of three trials.
A, B, C: They were used in the comparison of each volatile of control wine at 0, 1 and 2 days of storage. Means that do not bear a common superscript differ significantly.
a, b, c: They were used in the comparison of each volatile of control wine and those containing caffeic acid or *N*-acetyl-cysteine at the same sampling time (0, 1 or 2 days). Among means bearing superscripts, those that do not bear a common superscript differ significantly.

Table 5
Relative concentrations of volatile acetate esters during oxidative storage at 20 °C of Xinomavro wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L)

Compounds	Control (days)			Caffeic acid (days)			*N*-acetyl cysteine (days)		
	0	1	2	0	1	2	0	1	2
Ethyl acetate	23.96^{A}	11.63^{Ba}	7.98^{Ca}	24.22	20.18^{b}	15.09^{b}	23.26	19.22^{b}	14.17^{b}
Isoamyl acetate	2.26^{A}	0.83^{Ba}	0.33^{Ba}	2.27	1.51^{b}	0.90^{b}	2.24	1.35^{b}	0.72^{b}
Ethyl phenyl acetate	0.25^{A}	0.20^{A}	0.18^{A}	0.25	0.22	0.19	0.22	0.20	0.17
2-phenyl ethyl acetate	0.37^{A}	0.31^{A}	0.28^{A}	0.38	0.35	0.29	0.34	0.32	0.31

Values (mg/L) are the means of three trials.
A, B, C: They were used in the comparison of each volatile of control wine at 0, 1 and 2 days of storage. Means that do not bear a common superscript differ significantly.
a, b, c: They were used in the comparison of each volatile of control wine and those containing caffeic acid or *N*-acetyl-cysteine at the same sampling time (0, 1 or 2 days). Among means bearing superscripts, those that do not bear a common superscript differ significantly.

acid or *N*-acetyl-cysteine contained an ethyl octanoate content about 2.5 and 3.5 times higher than the control, respectively. Among acetate esters, ethyl acetate and isoamyl acetate decreased during wine storage. Their decline was inhibited by the addition of caffeic acid or *N*-acetyl-cysteine. Ethyl phenyl acetate and 2-phenyl ethyl acetate did not significantly decrease during wine storage.

The effect of caffeic acid and *N*-acetyl-cysteine on the sum of the relative concentrations of alcohols and fatty acids of Xinomavro wine during oxidative storage at 20 °C is presented in Figs. 8 and 9, respectively. Caffeic acid or *N*-acetyl-cysteine had no statistically significant effect on the sum of alcohols and fatty acids at any time of sampling. In the control, the sum of alcohols was statistically equal at any time of sampling. The sum of fatty acids in control at $t = 0$ was significantly higher than the respective at $t = 1$ or 2 days. With the exception of decanoic acid, all volatile acids declined during wine oxidative storage.

4. Discussion

We studied the effect of caffeic acid and *N*-acetyl-cysteine on the concentration of several volatiles during oxidative storage of Muscat-white and Xinomavro-red wine. In both wines, the results were similar.

Many ethyl esters and acetate esters decreased to a large extent during oxidative storage of both wines. Some ethyl esters, such as diethyl succinate, were stable. These results are similar to results observed by others (Ferreira, Escudero, Fernadez, & Cacho, 1997) and

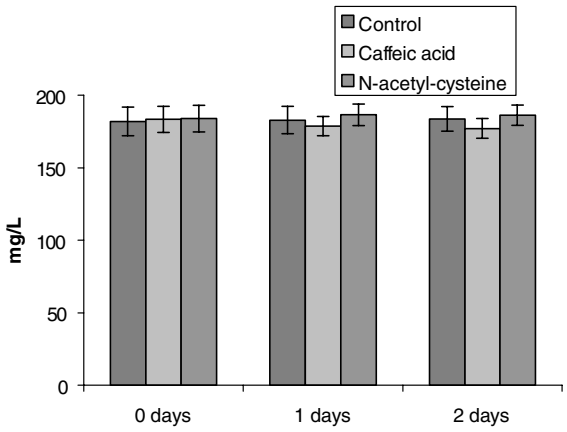

Fig. 8. Sum of the relative concentrations of volatile alcohols during oxidative storage at 20 °C of Xinomavro wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations. The alcohols determined are: 1-propanol, isobutyl alcohol, 1-butanol, isoamyl alcohol, hexanol, 2-ethyl-hexanol, 2,6-dimethyl-4-heptanol, octanol, nonanol, 3-(methylthio)-1-propanol, decanol, and 2-phenylethanol, 4-methyl phenol, and 3-ethyl phenol.

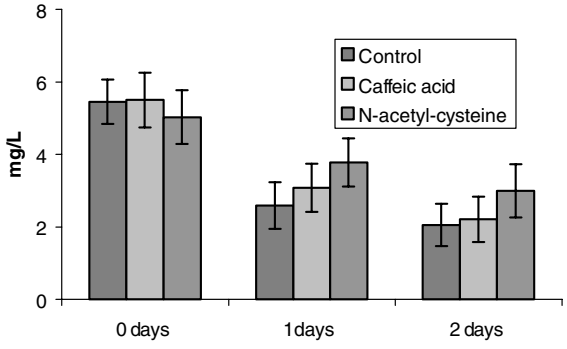

Fig. 9. Sum of the relative concentrations of volatile fatty acids during oxidative storage at 20 °C of Xinomavro wine in the presence of caffeic acid (100 mg/L) or *N*-acetyl-cysteine (20 mg/L). Values (mg/L) are the means of three trials and error bars indicate standard deviations. The acids determined are: hexanoic, octanoic, decanoic, lauric, myristic, and palmitic.

can be explained by different hydrolysis-esterification equilibria of the esters (Ramey & Ough, 1980).

The terpenols linalool and α-terpineol declined during the oxidative storage of white Muscat wine. It is known that in Muscat wines, losses of monoterpene alcohol may occur due to oxidation or other transformations and a marked loss of aroma can result (Jackson, 1994; Marais, 1983). During oxidative storage of another white wine linalool and α-terpineol also decreased (Ferreira et al., 1997).

In both white and red wine, the volatile alcohols were stable during oxidative storage. Similarly, no effect of oxidative storage of other wines on alcohol concentration has been reported by others (Ferreira et al., 1997). It seems that alcohol oxidation to aldehydes only accounts for a small proportion of their concentration.

In white wine, the volatile acids were stable during oxidative storage. Moreover, several acids declined during oxidative storage of red wine. The behaviour of fatty acids during oxidative storage may be explained by their generation due to hydrolysis of ethyl esters and by their autooxidation to yield aldehydes (Nykanen, 1986). Decreases of fatty acids during oxidative storage of wines have also been reported by others (Ferreira et al., 1997).

Caffeic acid at 100 ppm or *N*-acetyl-cysteine at 20 ppm inhibited the decline of several ethyl esters and acetate esters of both white and red wine.

Ethyl hexanoate, ethyl octanoate, ethyl decanoate, and isoamyl acetate and ethyl acetate decreased to a large extent during oxidative storage of both wines. Caffeic acid and *N*-acetyl-cysteine significantly inhibited the decline of all these volatile esters. Ethyl hexanoate, ethyl octanoate and ethyl decanoate are the most important wine ethyl esters and play a key role in the fruity notes of wines (Jackson, 1994). Isoamyl acetate is one of the most important acetate esters and has a distinctly banana-like fragrance. All the above esters are considered to give wine much of its vinous fragrance. Ethyl acetate contributes to the fruity character and adds to general fragrance complexicity of wine, while it is undesirable if present at concentrations >150–200 mg/L giving a sour-vinegar off-odor (Jackson, 1994). Preventing the loss of these esters during oxidative storage of white and red wine may protect their aroma.

Caffeic acid and *N*-acetyl-cysteine significantly inhibited the decline of linalool and α-terpineol in Muscat wine. Muscat wines derive much of their fragrance from monoterpene alcohols, especially linalool. However, a marked loss of aroma can result during aging. Most monoterepene alcohols are replaced by terpene oxides that have sensory thresholds about 10 times higher than their precursors. Linalool may be progressively replaced by α-terpineol. All the above indicate that the inhibition of the decline of linalool and α-terpineol during oxidative storage of Muscat wine may be related to their ability to restrict the disappearance of the specific aroma of Muscat wines.

The ability of caffeic acid to inhibit the decline of aroma compounds during oxidative storage of wines emphasizes its significance for wine quality. In grapes, caffeic acid and other cinnamic acids exist as esters of tartaric acid. However, they are susceptible to hydrolysis in the aqueous acidic solution of wine (Waterhouse, 2002). Subsequently, the simple caffeic acid is possibly active in inhibiting the decline of wine volatile esters and terpenols. It is possible that other wine cinnamic acids may also be active. This is important in making wines of high quality, since wines contain significant amounts of cinnamic acids i.e 60–130 mg/L (Waterhouse, 2002).

The ability of *N*-acetyl-cysteine to inhibit the decline of aroma compounds during oxidative storage of wines

may be useful in keeping wine quality. It is possible that other SH-containing amino acids and peptides may also be active. It is of interest in winemaking, since wines contain several mg/L of such compounds (Park, Boulton, & Noble, 2000).

5. Conclusions

Present results indicate that caffeic acid and *N*-acetyl-cysteine inhibit the decline of several aromatic ethyl esters and acetate esters during oxidative storage of Muscat-white and Xinomavro-red wine. Moreover, both of them inhibit the decline of linalool and α-terpineol during storage of Muscat wine. Caffeic acid and *N*-acetyl-cysteine may be used to prevent the loss of aromatic volatile esters and terpenols in wines.

Acknowledgement

For this work, the GC-MS facilities of the Food Quality Certification Unit of the University of Ioannina have been used.

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