

Inhibition of the Decrease of Volatile Esters and Terpenes During Storage of Wines and a Model Wine Medium by Wine Phenolic Extracts

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Summary

The effect of red wine phenolic extracts on the stability of wine volatile esters and terpenes was examined. Muscat (white) and Xinomavro (red) wines were enriched with each extract at 120 or 200 mg/L, and stored in open bottles at 20 °C for 3 and 2.5 days, respectively. Moreover, a model wine medium containing isoamyl acetate, ethyl hexanoate and linalool was enriched with each extract at 100 mg/L, and stored in sealed bottles at 20 °C for 45–90 days. All samples were analysed for volatiles using SPME along with GC-MS analysis. Phenolic composition of wine extracts was determined using HPLC-DAD. No effect on the concentration of any volatiles was observed as a result of the addition of each extract in each wine or the model medium. A wine extract rich in phenolic acids and another one rich in anthocyanins and flavanols inhibited the decrease of volatile esters and terpenes in one or both wines and the model medium. Among them were several important for the aroma of wine such as ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and linalool. The results presented here indicate that wine phenolic acids, and anthocyanins or flavanols may be taken into account as potent inhibitors of the disappearance of volatile esters and terpenes in wines.

Key words: wine, volatiles, aroma, acetate esters, ethyl esters, terpenes, phenolic acids, anthocyanins, flavanols

Introduction

The oxidative spoilage of white as well as red wines is characterized by transformation of aroma compounds. It leads to the loss of characteristic aroma of wines, and subsequently to the formation of new aromas characteristic of older wines or atypical aromas associated with wine deterioration (1–4). Several wine compounds such as esters and terpenes are transformed during wine storage, and the loss of wine aroma may occur (5).

Phenolic compounds impart an antioxidant quality to wine. Several phenolics, such as anthocyanins, flavonols, flavanols and phenolic acids, exhibit antioxidant activity and act as natural preservatives (5). As regards

wine aroma compounds, inhibition of linalool decrease during oxidative storage of Muscat wine by caffeic acid or gallic acid has previously been reported (6). Similarly, inhibition of the decrease of volatile esters and linalool during oxidative storage of white and red wines by caffeic acid has also been reported (7). Moreover, wine anthocyanin extract or malvidin-3-glucoside attenuated the decrease of the wine odorous compound 3-mercaptohexan-1-ol in a model wine medium (8).

The present study was undertaken to determine the ability of red wine phenolic extracts to inhibit the decrease of volatile aroma compounds during storage of a white wine (Muscat), red wine (Xinomavro) and a model wine medium.

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Materials and Methods

Chemicals and wines

Ethyl hexanoate and linalool were purchased from Sigma-Aldrich (St. Louis, MO, USA) and isoamyl acetate from Merck (Darmstadt, Germany). The water used in the experiments was of HPLC grade (LabScan, Dublin, Ireland).

Wines used are Greek wines protected by the Appellation of Origin, the dry white Muscat wine from Lemnos and the dry red Xinomavro wine from Naoussa.

Gross composition of wine samples was determined by the classic methods (9). Alcohol was determined with a hydrometer, reducing sugars by Lane-Eynon method, pH with a pH-meter, total acidity by volumetric analysis, and volatile acidity by steam distillation. Total and free sulphides were determined by the Ripper method. Total phenol contents of wines were determined by the Folin-Ciocalteu method (10), using gallic acid as a standard.

The average composition of Muscat wine was: alcohol content 11.7 % (by volume), pH=3.32, total acidity 5.1 g/L as tartaric acid, volatile acidity 0.48 g/L as acetic acid, total SO₂ 132 mg/L and free SO₂ 58 mg/L. Total phenolic content of Muscat wine was 223 mg/L of gallic acid equivalent. Similarly, the average composition of Xinomavro wine was: alcohol content 14.6 % (by volume), pH=3.15, total acidity 5.4 g/L as tartaric acid, volatile acidity 0.30 g/L as acetic acid, total SO₂ 61 mg/L and free SO₂ 32 mg/L. Total phenolic content of Xinomavro wine was 3802 mg/L of gallic acid equivalent.

Wine extracts

Liquid/liquid extractions of dealcoholized Xinomavro (red) wine were performed to obtain three extracts (11).

Dealcoholized wine was obtained by evaporation; wine added to an equal volume of distilled water was concentrated to the original volume (25 °C, 80 mbar). The dealcoholized wine (pH=2.0) was first extracted with ethyl acetate. Aqueous phase was the first extract (extract 1). Organic phase after the evaporation was redissolved in water at pH=7.0, and extracted again with ethyl acetate. This organic phase was the second extract (extract 2). The aqueous phase was adjusted at pH=2.0 and extracted again with ethyl acetate. This extract was the third extract (extract 3). The three extracts in 10 % ethanol were used. The total phenol contents of the samples were determined by the Folin-Ciocalteu method (10), using gallic acid as a standard.

The wine extracts were analysed by high performance liquid chromatography and diode array detector (HPLC-DAD) for individual phenolic compounds. Samples were filtered using syringe filter (PTFE 0.45, Altech Associates, Deerfield, IL, USA) prior to the injection. Waters 600E system with a 996-photodiode array detector and a 600E pump was used. Chromatograms were treated using the Millennium 32 program. The column was a C18 reversed phase Spherisorb (4.0 × 250 mm) with 5-µm packing (Waters, Milford, MA, USA). The mobile phases were: A, water/glacial acetic acid (98:2); B, methanol/water/glacial acetic acid (60:38:2) and C,

methanol/glacial acetic acid (98:2). The gradient was 0–30 min, 100 % A at 0.20 mL/min; 30–40 min, from 58.3 % A to 41.7 % B at 0.60 mL/min; 40–120 min, from 41.7 % A to 58.3 % B at 0.20 mL/min; 120–155 min, from 25 % A to 75 % B at 0.30 mL/min; 155–165 min, 100 % C at 0.60 mL/min and 165–180 min, 100 % C at 0.90 mL/min.

All peaks were classified using absorbance characteristics of the phenolic classes derived from the literature (12,13) and from our observations using several standards. The maximal absorbance of phenolic classes was at the following wavelengths: benzoic acids 250–280 nm; cinnamic acids 305–330 nm, and several of them 290–300 nm; anthocyanins 450–560 and 240–280 nm, and some of them 315–325 nm; flavanols 270–280 nm and around 230 nm; flavonols 350–380 and 250–270 nm, and some of them around 300 nm; flavones and isoflavones 300–350 and 245–270 nm; flavanones 270–295 nm, and some of them 300–320 nm. Unclassified peaks that exhibited maximum absorbance at 280–305 nm were expressed as unclassified 280 nm. Unclassified peaks that exhibited maximum absorbance at around 230 nm were expressed as unclassified 230 nm. As main phenolic peaks were taken those exhibiting high absorbance at 280, 255, 320, 360 or 520 nm.

Muscat and Xinomavro wines

One milliliter of each wine phenolic extract of Xinomavro wine in 10 % ethanol solution was added to 20 mL of Muscat or Xinomavro wine. Phenolic extract 1 was not used in Muscat white wine because of its red colour. In Muscat wine, the final concentration of each phenolic extract was 120 mg/L, while in Xinomavro wine it was 200 mg/L. Control samples were also prepared by adding 1 mL of 10 % ethanol to 20 mL of wine. The bottles ($d=3.2$ cm, $h=10.6$ cm, $V=60$ mL) were kept open at 20 °C. After 0 and 3 days of storage of Muscat wine and 0 and 2.5 days of Xinomavro wine, bottles were taken and wine samples were examined. We used higher phenolics in red (200 mg/L) than in white wine (120 mg/L), since the natural phenolic content of red wines is much higher than that of white ones. Wines were stored in open bottles in order to quickly get valuable results, even though this is not typical wine storage.

Samples of Muscat and Xinomavro wines were tested organoleptically by two judges familiar with these wines. Samples were compared to each other and to wines from the unopened bottles.

Wine volatile esters and terpenes were determined by SPME along with GC-MS analysis. The fiber used for the absorption of volatiles was a CarbowaxTM/divinylbenzene 65 µm (Supelco, Bellefonte, PA, USA). A volume of 2 mL of each wine sample was transferred into 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 25 °C. Then, a constant length of the fiber was exposed to the headspace for 30 min, under the same conditions. Desorption of volatiles took place at 250 °C using a 0.75-mm i.d. 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 (Agilent Technologies, Santa Clara, CA, USA). MS was operated in the electron impact mode with the electron energy set at 70 eV. A G1701BA Chemstation was employed. Source and quadrupole temperatures were set at 230 and 150 °C, respectively. A non-polar column, the Solgel-1 fused-silica (30 m × 0.25 mm, 0.25 µm film thickness, SGE Analytical Science, Austin, TX, USA), was used. The oven temperature was programmed at 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. The transfer line was kept at 220 °C. The carrier gas was helium at a constant flow rate of 0.7 mL/min and average velocity 30 cm/s. For the period of 0–5.2 min, mass range of 50–370 *m/z* and 2.33 scan/s were applied, followed by mass range of 29–350 *m/z* and 2.32 scan/s.

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

All peaks were identified by comparing mass spectra with 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 under the same analytical conditions. Authentic standards used were: ethyl acetate, isoamyl acetate, hexyl acetate, 2-phenylethyl acetate, ethyl butanoate, ethyl lactate, ethyl hexanoate, diethyl succinate, ethyl octanoate, ethyl decanoate, ethyl laurate, limonene (Merck, Darmstadt, Germany), linalool, α -terpineol, nerol, citronellol and geraniol (Sigma-Aldrich, St. Louis, MO, USA).

Model wine medium

The model wine medium consisted of 12 % (by volume) ethanol and 5 g/L of tartaric acid in water with the pH adjusted to 3.5 using 1M NaOH. The medium contained isoamyl acetate 50 mg/L, ethyl hexanoate 50 mg/L, and linalool 10 mg/L. K₂S₂O₅ was also added to give 20 mg/L of free SO₂, as determined by the Ripper method (9). Samples of the model medium, 40 mL, were put in small wine bottles of 50 mL capacity. Solution of each wine extract in 10 % ethanol, 0.3 mL, was added to the model wine medium at a final concentration of 100 mg/L of total phenolics. Control samples were also prepared by adding 0.3 mL of 10 % ethanol to 40 mL of the model wine. The bottles were sealed using cork and sealing wax and stored in a dark room at 20 °C. After 0, 45 and 90 days of storage, bottles were taken and model wines were analysed.

In the model wine medium, we used phenolics (100 mg/L) closer to those existing in wines, since we had good results using higher phenolics in wines (120 or 200 mg/L). We stored model wine media in sealed bottles with significant headspace in order to get valuable results within some months under conditions comparable to typical wine storage.

The volatiles were determined by SPME along with GC-MS analysis. For their absorption, the same fiber and glass vials were used. A volume of 2 mL of each sample and 50 µL of internal standard in 10 % ethanol (4-methyl-1-pentanol, 5 mg/L in final solution) were trans-

ferred into a 4-mL screw-capped glass vial. The contents were stirred for 15 min at 35 °C. Then, a constant length of the fiber was exposed to the headspace for 15 min, under the same conditions. Desorption of volatiles took place at 240 °C using a 0.75-mm i.d. liner (Supelco, Bellefonte, PA, USA) for 5 min. Split/splitless mode was used, splitless for 2 min and split ratio was 1:20. GC-MS analysis was carried out using the same system as described above. A polar column, the Innowax fused-silica, was used (30 m × 0.32 mm, 0.5 µm film thickness, J&W Scientific, Folsom, CA, USA). The oven temperature was programmed at 40 °C for 5 min and then raised to 60, 90, and 240 °C at the rates of 3, 10 and 30 °C/min, respectively. It was held at 240 °C for 4 min. The transfer line was kept at 260 °C. The carrier gas was helium at a constant flow rate of 1.5 mL/min and average velocity of 30 cm/s. Mass range of 29–300 *m/z* and 2.73 scan/s were applied beginning at 5 min in order to avoid ethanol.

Semiquantitative data were expressed in milligrams per liter [(area of compound/area of internal standard) × concentration of internal standard].

Statistical analysis

Each 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's test at a level of significance $p < 0.05$ was used for statistical analysis. When two groups were compared (control wines at 0 and 3 or 2.5 days of storage), the paired test was used instead of the ANOVA (SPSS 11.5).

Results and Discussion

The effect of red wine phenolic extracts on aroma volatiles of a white and red wine stored in open bottles was examined.

Muscat (white) and Xinomavro (red) wine samples clearly retained less aroma after storage for 3 and 2.5 days, respectively. On the other hand, samples of each wine containing extract 3 appeared to be more aromatic. Samples of Xinomavro wine containing extract 1 also retained some aroma.

Wine samples were analysed for volatile esters and terpenes using SPME along with GC-MS analysis. Typical GC chromatograms of Muscat and Xinomavro wines are presented in Fig. 1 and Fig. 2, respectively.

The effect of red wine extracts 2 and 3 on volatiles of Muscat wine is reported in Table 1. At $t=0$, the sum of acetate esters, ethyl esters and terpenes was statistically equal in control and the samples containing wine extracts 2 or 3. In the control, the sum of acetate esters, ethyl esters and terpenes decreased during wine storage at a statistically significant level. The total content of acetate esters, ethyl esters and terpenes dropped by 81, 86 and 64 %, respectively. Their decrease was significantly lower in the presence of wine extract 3. Ethyl acetate, isobutyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl 9-decanoate, ethyl decanoate, ethyl laurate, linalool, α -terpineol were protected by wine extract 3. This extract was rich in phenolic acids, as revealed by HPLC-DAD analysis (Fig. 3). This indicates that wine

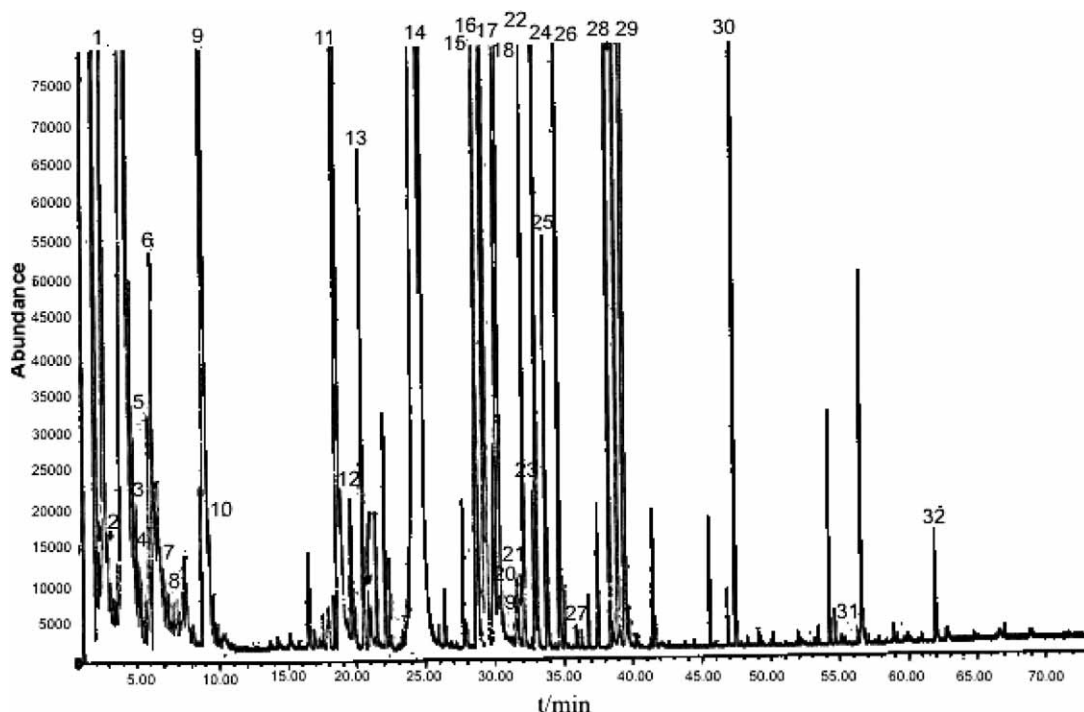


Fig. 1. Typical GC chromatogram of volatile ester and terpene determination in Muscat (white) wine

1. ethyl acetate, 2. ethyl propanoate, 3. ethyl isobutanoate, 4. isobutyl acetate, 5. ethyl butanoate, 6. ethyl lactate, 7. ethyl-2-methyl butanoate, 8. ethyl-3-methyl butanoate, 9. isoamyl acetate, 10. 2-methyl-1-butyl acetate, 11. ethyl hexanoate, 12. hexyl acetate, 13. limonene, 14. linalool, 15. diethyl succinate, 16. α -terpineol, 17. hotrienol, 18. ethyl octanoate, 19. nerol, 20. phenylethyl acetate, 21. citronellol, 22. 2-phenylethyl acetate, 23. geraniol, 24. terpinolene, 25. geranyl acetate, 26. geranyl ether 1 and 2, 27. ethyl pelargonate, 28. ethyl-9-decanoate, 29. ethyl decanoate, 30. ethyl laurate, 31. ethyl myristate, 32. ethyl palmitate

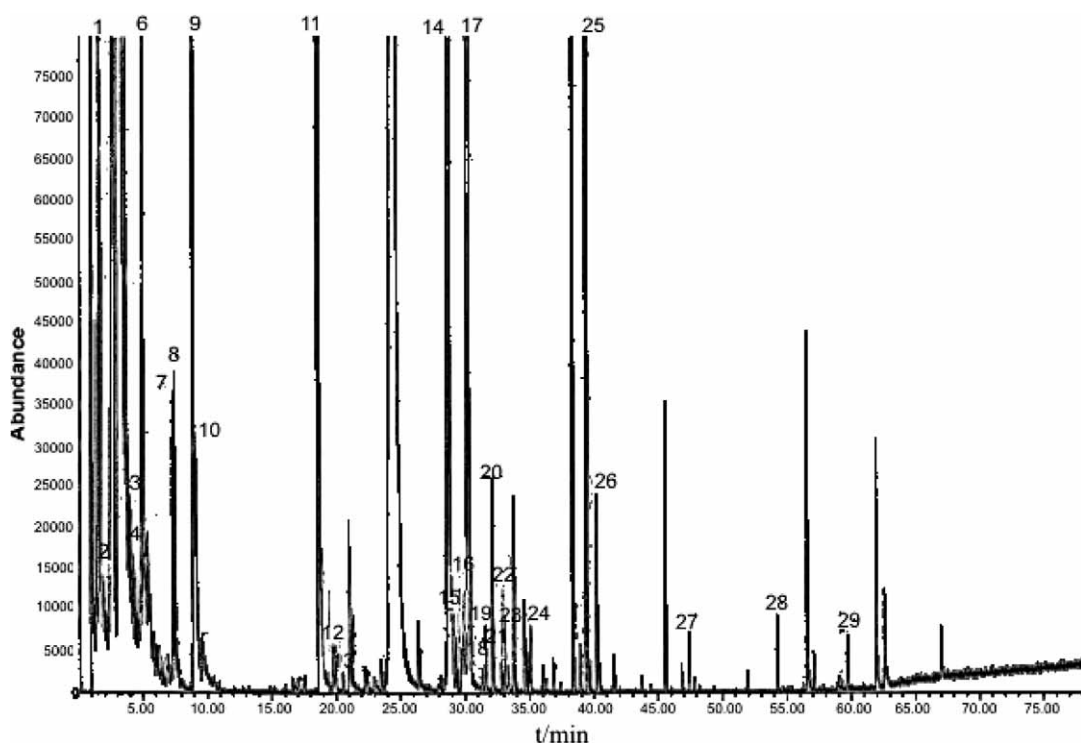


Fig. 2. Typical GC chromatogram of volatile ester and terpene determination in Xinomavro (red) wine

1. ethyl acetate, 2. ethyl propanoate, 3. ethyl isobutanoate, 4. isobutyl acetate, 5. ethyl butanoate, 6. ethyl lactate, 7. ethyl-2-methyl butanoate, 8. ethyl-3-methyl butanoate, 9. isoamyl acetate, 10. 2-methyl-1-butyl acetate, 11. ethyl hexanoate, 12. hexyl acetate, 13. limonene, 14. linalool, 15. α -terpineol, 16. hotrienol, 17. ethyl octanoate, 18. nerol, 19. phenylethyl acetate, 20. 2-phenylethyl acetate, 21. geraniol, 22. terpinolene, 23. geranyl ether 1 and 2, 24. ethyl pelargonate, 25. ethyl decanoate, 26. ethyl-3-methyl butyl butanedioate, 27. ethyl laurate, 28. ethyl myristate, 29. ethyl palmitate

Table 1. Effect of red wine extracts 2 and 3 at 120 mg/L on the relative concentration of volatile acetate esters, ethyl esters and terpenes of Muscat (white) wine after storage at 20 °C for 3 days in open bottles

Volatiles	0 days			3 days		
	Control	Extract 2	Extract 3	Control	Extract 2	Extract 3
Ethyl acetate	0.131 ^A	0.140	0.139	0.039 ^{Ba}	0.040 ^a	0.062 ^b
Isobutyl acetate	0.029 ^A	0.024	0.027	0.011 ^{Ba}	0.008 ^a	0.018 ^b
Isoamyl acetate	0.502 ^A	0.490	0.545	0.045 ^{Ba}	0.047 ^a	0.117 ^b
2-methyl-1-butyl acetate	0.058 ^A	0.061	0.065	0.000 ^B	0.000	0.000
Hexyl acetate	0.031 ^A	0.033	0.035	0.004 ^B	0.006	0.006
Phenylethyl acetate	0.006 ^A	0.006	0.006	0.002 ^{Ba}	0.003	0.003
2-phenylethyl acetate	0.094 ^A	0.092	0.095	0.057 ^B	0.064	0.066
SUM OF ACETATE ESTERS	0.851 ^A	0.846	0.912	0.158 ^{Ba}	0.168 ^a	0.271 ^b
Ethyl propanoate	0.012 ^A	0.010	0.012	0.000 ^B	0.003	0.004
Ethyl isobutanoate	0.043 ^A	0.044	0.049	0.020 ^B	0.018	0.028
Ethyl butanoate	0.037 ^A	0.040	0.035	0.014 ^B	0.013	0.013
Ethyl lactate	0.058 ^A	0.057	0.064	0.048 ^A	0.052	0.048
Ethyl-2-methyl butanoate	0.015 ^A	0.016	0.015	0.000 ^B	0.000	0.000
Ethyl isovalerate	0.025 ^A	0.025	0.028	0.000 ^B	0.000	0.000
Ethyl hexanoate	1.678 ^A	1.670	1.731	0.103 ^{Ba}	0.095 ^a	0.199 ^b
Diethyl succinate	0.236 ^A	0.220	0.244	0.212 ^A	0.226	0.202
Ethyl octanoate	9.834 ^A	9.944	9.846	0.784 ^{Ba}	0.748 ^a	1.232 ^b
Ethyl pelargonate	0.010 ^A	0.011	0.011	0.001 ^B	0.002	0.002
Ethyl-9-decanoate	0.892 ^A	0.902	0.951	0.160 ^{Ba}	0.188 ^a	0.256 ^b
Ethyl decanoate	4.489 ^A	3.584	4.825	1.002 ^{Ba}	1.038 ^a	1.353 ^b
Ethyl laurate	0.151 ^A	0.154	0.163	0.023 ^{Ba}	0.027 ^a	0.051 ^b
Ethyl myristate	0.035 ^A	0.033	0.041	0.010 ^B	0.006	0.016
Ethyl palmitate	0.015 ^A	0.016	0.015	0.006 ^B	0.002	0.005
SUM OF ETHYL ESTERS	17.531 ^A	16.726	18.040	2.384 ^{Ba}	2.418 ^a	3.409 ^b
Limonene	0.119 ^A	0.123	0.127	0.000 ^B	0.001	0.003
Linalool	0.882 ^A	0.911	0.921	0.654 ^{Ba}	0.667 ^a	0.747 ^b
Hotrienol	0.241 ^A	0.248	0.251	0.001 ^B	0.003	0.002
α -terpineol	0.173 ^A	0.177	0.184	0.107 ^{Ba}	0.116 ^a	0.152 ^b
Nerol	0.009 ^A	0.009	0.010	0.005 ^B	0.003	0.006
Citronellol	0.017 ^A	0.016	0.016	0.006 ^B	0.004	0.009
Geraniol	0.037 ^A	0.036	0.039	0.009 ^{Ba}	0.009 ^a	0.017 ^b
Terpinolene	0.376 ^A	0.380	0.388	0.001 ^B	0.000	0.000
Geranyl acetate	0.092 ^A	0.090	0.092	0.001 ^B	0.001	0.001
Geranyl ether 1 and 2	0.271 ^A	0.268	0.270	0.007 ^B	0.011	0.009
SUM OF TERPENES	2.217 ^A	2.258	2.298	0.789 ^{Ba}	0.815 ^a	0.946 ^b

Values are the means of three trials. Acetate esters and ethyl esters are in mg/L as phenylethyl acetate and terpenes in mg/L as linalool

^{A, B} were used in the comparison of volatiles of control wine at 0 and 3 days of storage

^{a, b} were used in the comparison of control wine and those containing the wine extract 2 or 3 at the same sampling time (0 or 3 days) Means that bear different superscripts differ significantly

phenolic acids may be active in inhibiting the decrease of acetate esters, ethyl esters and terpenes in Muscat wine.

Wine extract 2 had no protective effect on acetate esters, ethyl esters and terpenes. This extract contained mainly flavanols, flavonols and tyrosol (Fig. 3), indicating that such phenolics may not be active.

The effect of wine extracts 1, 2 and 3 on volatile acetate esters and ethyl esters of Xinomavro wine is reported in Table 2. At $t=0$, the sum of acetate esters and

ethyl esters was statistically equal in control and the samples containing wine extracts 1, 2 or 3. In the control, the sum of acetate esters and ethyl esters decreased during wine storage at a statistically significant level. The total content of acetate esters and ethyl esters dropped by 77 and 70 %, respectively. Their decrease was significantly lower in the presence of wine extract 1 or 3. Ethyl acetate, isobutyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate and ethyl decanoate were protected by wine extracts 1 or 3. Wine extract 1 was rich in an-

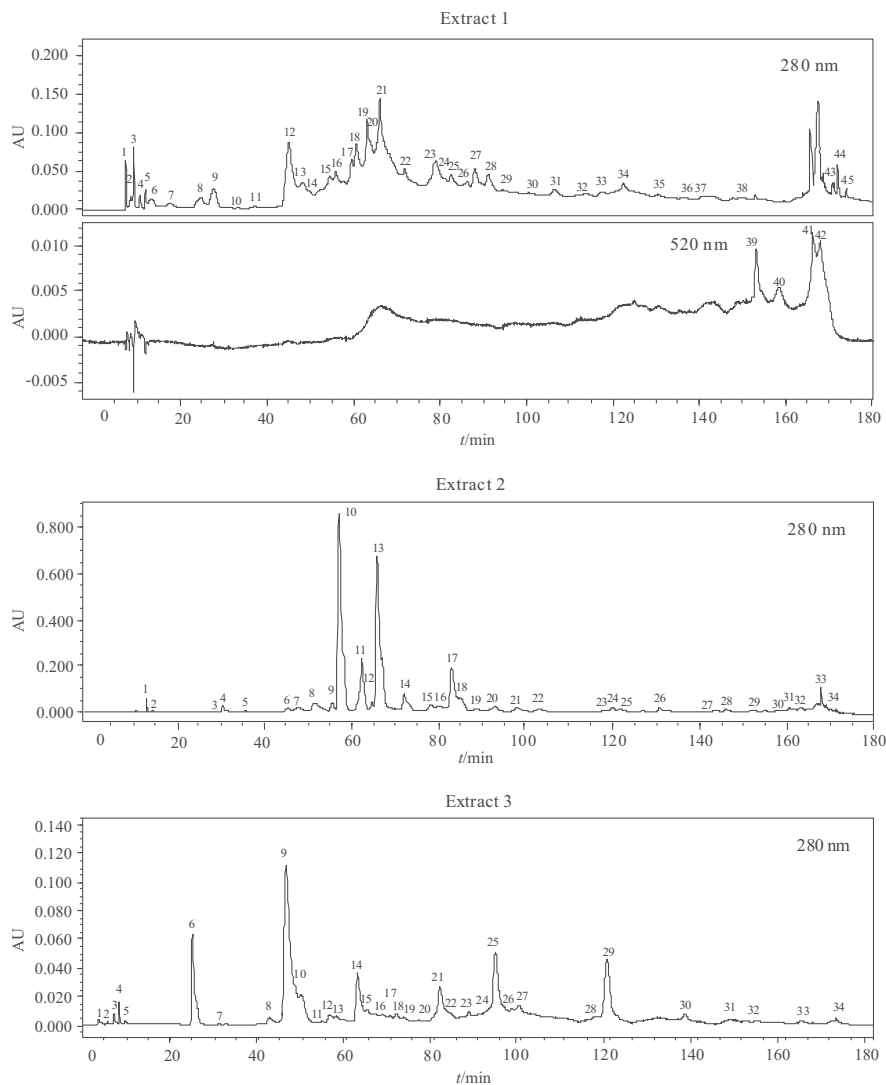


Fig. 3. HPLC chromatograms of Xinomavro (red) wine extracts 1, 2 and 3

Extract 1: 1–3. unclassifieds 230 nm, 4. benzoic acid, 5. unclassified 230 nm, 6–11. benzoic acids, 12–13. cinnamic acids, 14–22. flavanols, 23–24. cinnamic acids, 25. flavanol, 26–28. cinnamic acids, 29–30. unclassifieds 280 nm, 31. cinnamic acids, 32. benzoic acids, 33. unclassified 280 nm, 34. cinnamic acid, 35. unclassified 280 nm, 36–38. flavonols, 39–42. anthocyanins, 43. benzoic acid, 44. cinnamic acid, 45 benzoic acid

Extract 2: 1. benzoic acid, 2–3. unclassifieds 230 nm, 4. unclassified 280 nm, 5. benzoic acid, 6. unclassified 280 nm, 7–9. cinnamic acids, 10. tyrosol, 11–17. flavanols, 18. unclassified 280 nm, 19. flavanol, 20–22. benzoic acids, 23–25. unclassifieds 280 nm, 26. cinnamic acid, 27–29. flavonols, 30. unclassified 280 nm, 31–32. cinnamic acids, 33–34. flavonols

Extract 3: 1. unclassified 230 nm, 2–6. benzoic acids, 7. cinnamic acid, 8. benzoic acid, 9–10. cinnamic acids, 11. benzoic acid, 12. cinnamic acid, 13. benzoic acid, 14–17. cinnamic acid, 18–19. benzoic acids, 20–22. cinnamic acids, 23. benzoic acid, 24–26. cinnamic acids, 27–28. benzoic acids, 29. cinnamic acid, 30–33. flavonols, 34. benzoic acid

thocyanins and flavanols, while wine extract 3 in phenolic acids, as revealed by HPLC-DAD analysis (Fig. 3). This indicates that wine anthocyanins or flavanols, and phenolic acids may be active in inhibiting the decrease of acetate esters and ethyl esters in Xinomavro wine.

Wine extract 2 had no protective effect on acetate esters, ethyl esters and terpenes. As reported above, this extract contained mainly flavanols, flavonols and tyrosol (Fig. 3), indicating that such phenolics may not be active.

Xinomavro wine contained only very low amounts of terpenes. The sum of terpenes was initially 0.023 mg/L as linalool and dropped to 0.004 mg/L after 2.5 days of storage.

Both wines were stored in open bottles. This storage shows the effects mainly of evaporation and oxidation. During wine storage several other chemical changes may also occur. Ester content may be changed because of hydrolysis and esterification (14). Geraniol and nerol can interconvert and then form α -terpineol (15), and linalool may be replaced by α -terpineol (5).

The effect of red wine extracts on the relative concentrations of isoamyl acetate, ethyl hexanoate and linalool during storage of the model wine medium at 20 °C is presented in Fig. 4.

No matrix effect was observed as a result of the addition of extract 1 or extract 3. At $t=0$, the concentration

Table 2. Effect of red wine extracts 1, 2 and 3 at 200 mg/L on the relative concentration of volatile acetate esters and ethyl esters of Xinomavro (red) wine after storage at 20 °C for 2.5 days in open bottles

Volatiles	0 days				2.5 days			
	Control	Extract 1	Extract 2	Extract 3	Control	Extract 1	Extract 2	Extract 3
Ethyl acetate	0.193 ^A	0.196	0.183	0.194	0.054 ^{Ba}	0.090 ^b	0.062 ^a	0.094 ^b
Isobutyl acetate	0.029 ^A	0.032	0.029	0.030	0.015 ^{Ba}	0.019 ^b	0.015 ^a	0.020 ^b
Isoamyl acetate	0.144 ^A	0.144	0.139	0.166	0.014 ^{Ba}	0.033 ^b	0.018 ^a	0.028 ^b
2-methyl-1-butyl acetate	0.057 ^A	0.060	0.052	0.058	0.000 ^B	0.000	0.000	0.000
Hexyl acetate	0.004 ^A	0.004	0.004	0.004	0.000 ^B	0.001	0.002	0.001
Phenylethyl acetate	0.004 ^A	0.005	0.005	0.005	0.003 ^B	0.004	0.002	0.003
2-phenylethyl acetate	0.010 ^A	0.015	0.011	0.010	0.008 ^B	0.012	0.007	0.008
SUM OF ACETATE ESTERS	0.411 ^A	0.456	0.423	0.467	0.095 ^{Ba}	0.135 ^b	0.106 ^a	0.154 ^b
Ethyl propanoate	0.014 ^A	0.017	0.015	0.014	0.008 ^A	0.006	0.006	0.011
Ethyl isobutanoate	0.068 ^A	0.073	0.066	0.071	0.033 ^B	0.035	0.031	0.038
Ethyl butanoate	0.031 ^A	0.023	0.020	0.026	0.013 ^B	0.010	0.009	0.013
Ethyl lactate	0.215 ^A	0.208	0.212	0.224	0.190 ^A	0.179	0.183	0.200
Ethyl-2-methyl butanoate	0.038 ^A	0.039	0.038	0.035	0.000 ^B	0.000	0.000	0.000
Ethyl isovalerate	0.052 ^A	0.056	0.055	0.054	0.000 ^B	0.000	0.000	0.000
Ethyl hexanoate	0.548 ^A	0.573	0.585	0.543	0.034 ^{Ba}	0.054 ^b	0.040 ^a	0.071 ^b
Diethyl succinate	1.384 ^A	1.685	1.133	1.303	1.291 ^A	1.373	1.239	1.243
Ethyl octanoate	2.783 ^A	2.420	2.882	2.836	0.124 ^{Ba}	0.227 ^b	0.151 ^{ab}	0.406 ^c
Ethyl pelargonate	0.004 ^A	0.004	0.003	0.004	0.000 ^B	0.000	0.000	0.001
Ethyl decanoate	0.716 ^A	0.729	0.702	0.716	0.061 ^{Ba}	0.110 ^{bc}	0.064 ^{ab}	0.121 ^c
Ethyl-3-methylbutyl butanedioate	0.007 ^A	0.007	0.006	0.006	0.006 ^A	0.006	0.005	0.006
Ethyl laurate	0.005 ^A	0.005	0.003	0.005	0.001 ^B	0.002	0.003	0.004
Ethyl myristate	0.002 ^A	0.002	0.001	0.002	0.000 ^A	0.001	0.000	0.001
Ethyl palmitate	0.005 ^A	0.005	0.005	0.005	0.001 ^A	0.002	0.000	0.003
SUM OF ETHYL ESTERS	5.872 ^A	5.846	5.726	5.844	1.762 ^{Ba}	2.005 ^b	1.580 ^a	2.118 ^b

Values are the means of three trials. Acetate esters and ethyl esters are in mg/L as phenylethyl acetate

^{A, B} were used in the comparison of volatiles of the control wine at 0 and 2.5 days of storage

^{a, b, c} were used in the comparison of control wine and those containing the wine extracts 1, 2 or 3 at the same sampling time (0 or 2.5 days)

Means that bear different superscripts differ significantly

of each of the three volatiles was statistically equal in control and the samples containing extracts 1 or 3.

In the control, the concentration of the three volatiles decreased during storage at a statistically significant level. After 45 and 90 days of storage, isoamyl acetate dropped by 35 and 57 %, respectively, while ethyl hexanoate dropped by 52 and 78 % and linalool by 29 and 39 %, respectively. Model media were stored in sealed bottles that had a significant headspace. Under these conditions, volatile losses may be due to oxidation and to other chemical reactions.

Extracts 1 and 3 inhibited the decrease of the three volatiles at a statistically significant level. The inhibitory action of extract 3 was statistically higher than that of extract 1 at any sampling time. At $t=90$, in samples containing the extract 3 isoamyl acetate dropped by 42, ethyl hexanoate by 65 and linalool by 29 %. In the presence of the extract 1 their drop was 48, 70 and 33 %, respectively.

The phenolic compositions of extracts 3 and 1 indicate that some wine phenolic acids and anthocyanins or flavanols may be active in inhibiting the three volatiles.

Samples containing extract 2 exhibited statistically equal concentrations of isoamyl acetate and linalool to those of the control at any sampling time. This indicates that some wine flavanols, flavonols and tyrosol may not be so active.

We studied the effect of red wine phenolic extracts on the concentration of volatile esters and terpenes of Muscat and Xinomavro wines stored in open bottles. Wine extract rich in phenolic acids inhibited the decrease of several acetate esters and ethyl esters of both wines and the decrease of terpenes in Muscat wine. Moreover, wine extract rich in anthocyanins and flavanols inhibited the decrease of several acetate esters and ethyl esters in Xinomavro wine. We also studied the effect of wine extracts on the concentration of isoamyl acetate, ethyl hexanoate and linalool in a model wine medium stored in sealed bottles with significant headspace. One extract rich in phenolic acids and another rich in anthocyanins and flavanols inhibited the decrease of the three volatiles.

The results presented here indicate that wine phenolic extracts may protect several wine volatiles such as ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and linalool.

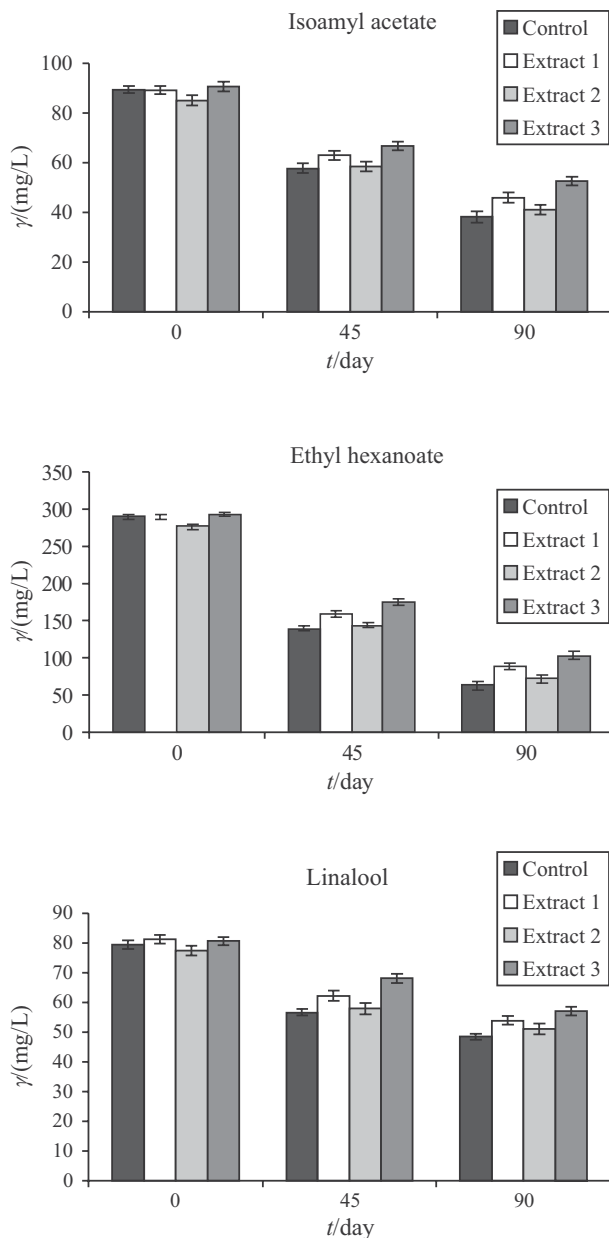


Fig. 4. Effect of red wine phenolic extracts 1, 2 and 3 at 100 mg/L on the relative concentrations of isoamyl acetate, ethyl hexanoate and linalool during storage of a model wine medium at 20 °C

Values, in mg/L as 4-methyl-1-pentanol, are the means of three trials

Ethyl acetate contributes to the fruity character and adds to general fragrance complexity of wine, while it is undesirable if present at concentrations >150–200 mg/L giving a sour-vinegar off-odour. Isoamyl acetate is one of the most important acetate esters and has a distinctly bananalike fragrance. 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. All the above esters are considered to give wine much of its vinous fragrance. Monoterpene alcohols, especially linalool, contribute to wine fragrance. In Muscat wines, a significant loss of aroma can occur during aging due to linalool transformation to linalool oxide, which has

much higher sensory threshold (5). Preventing the loss of these aroma volatile compounds during storage of wines may protect their aroma.

Levels of total hydroxycinnamates in wine are typically 130 mg/L in whites and 60 mg/L in reds. Levels of benzoic acids are 10 and 60 mg/L in white and red wines, respectively (16).

In the present study, wine extract rich in phenolic acids, at 120, 200 or 100 mg/L, inhibited the decrease of several wine aroma volatiles. So, present results emphasize the significance of wine phenolics for wine aroma.

Levels of anthocyanins in red wines are more than 90 mg/L while of flavanols are 40 mg/L in white and 1100 mg/L in red wine (16). In the present study, wine extract rich in anthocyanins and flavanols, at 120, 200 or 100 mg/L, inhibited the decrease of several wine aroma volatiles. Thus, present results emphasize the significance of wine anthocyanins or flavanols for wine aroma.

Conclusion

The results presented here indicate that wine phenolic acids and anthocyanins or flavanols inhibit the decrease of several volatile esters and terpenes in wines and a model wine medium stored under the conditions of abundant or limited air, respectively. Consequently, wine phenolic acids and anthocyanins or flavanols may be taken into account as potent inhibitors of the disappearance of volatile aroma during typical wine storage.

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References

1. A. Escudero, E. Asensio, J. Cacho, V. Ferreira, Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants, *Food Chem.* 77 (2002) 325–331.
2. A. Escudero, J. Cacho, V. Ferreira, Isolation and identification of odorants generated in wine during its oxidation: A gas chromatography-olfactometric study, *Eur. Food Res. Technol.* 211 (2000) 105–110.
3. A.C. Silva Ferreira, P. Guedes De Pinho, P. Rodrigues, T. Hogg, Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters, *J. Agric. Food Chem.* 50 (2002) 5919–5924.
4. V.L. Singleton, Oxygen with phenols and related reactions in musts, wines and model systems: Observations and practical implications, *Am. J. Enol. Vitic.* 38 (1987) 69–77.
5. S.R. Jackson: *Wine Science. Principles and Applications*, Academic Press, San Diego, USA (1994).
6. I.G. Roussis, K. Soulti, P. Tzimas, Inhibition of the decrease of linalool in Muscat wine by phenolic acids, *Food Technol. Biotechnol.* 43 (2005) 389–392.
7. I.G. Roussis, I. Lambropoulos, D. Papadopoulou, 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, *Food Chem.* 93 (2005) 485–492.
8. M.L. Murat, T. Tominaga, C. Saucier, Y. Glories, D. Dubour-dieu, Effect of anthocyanins on stability of a key odorous

- compound, 3-mercaptohexan-1-ol, in Bordeaux rose wines, *Am. J. Enol. Vitic.* 54 (2003) 135–138.
9. C.S. Ough, M.A. Amerine: *Methods for Analysis of Musts and Wines*, John Wiley & Sons, Davis, CA, USA (1988).
 10. V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Vitic.* 16 (1965) 144–158.
 11. A. Ghiselli, M. Nardini, A. Baldi, C. Scaccini, Antioxidant activity of different phenolic fractions separated from an Italian red wine, *J. Agric. Food Chem.* 46 (1998) 361–367.
 12. H.S. Lee, B.W. Widmer: Phenolic Compounds. In: *Handbook of Food Analysis, Vol. 1*, L.M. Nollet (Ed.), Marcel Dekker, New York, USA (1996).
 13. K. Robards, P.D. Prenzler, G. Tucker, P. Swatsitang, W. Glover, Phenolic compounds and their role in oxidative processes in fruits, *Food Chem.* 66 (1999) 401–436.
 14. D.D. Ramey, C.S. Ough, Volatile ester hydrolysis or formation during storage of model solutions and wines, *Food Chem.* 28 (1980) 928–934.
 15. D.S. Pedersen, D.L. Capone, G.K. Skouroumounnis, A.P. Pollnitz, M.A. Sefton, Quantitative analysis of geraniol, linalool, nerol and α -terpineol in wine, *Anal. Bioanal. Chem.* 375 (2003) 517–522.
 16. A.L. Waterhouse: Wine Phenolics. In: *Handbook of Antioxidants*, L. Packer, E. Cadenas (Eds.), Marcel Dekker, New York, USA (2002).