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Inhibition of the Decrease of Linalool in Muscat Wine by Phenolic Acids

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Summary

Two white wine extracts rich in phenolic acids, caffeic acid and gallic acid were tested as inhibitors of the decrease of linalool in Muscat wine. Each wine extract was added at 60 ppm and each phenolic acid at 40 ppm. Immediately after the addition of each wine extract or phenolic acid, no effect on the concentration of linalool was observed, but it decreased from the initial 470.9 to 223.3 μg/L after storage in open bottles at 20 °C for 4 days. Its decrease was significantly inhibited by each wine extract or phenolic acid.

Key words: aroma, linalool, muscat wine, oxidation, phenolic acids

Introduction

The oxidation of white as well as red wine is a well-known problem in wine-making. 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, while oxidative browning is a later step of their oxidation (*1–3*). Wines are rich in phenolic compounds, natural preservatives of wines due to their antioxidant activity. Among them, benzoic and cinnamic acid derivatives exhibit antioxidant activity in different physicochemical systems (*4,5*). 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. Monoterpene alcohols are primarily responsible for the fragrance of Muscat wines. In Muscat wines, losses of monoterpene alcohol may occur owing to oxidation or other transformations resulting in significant loss of aroma. Most monoterpene alcohols are replaced by terpene oxides that have sensory thresholds about 10 times higher than the precursors (*4,6*).

The present study was undertaken to determine the ability of caffeic acid, gallic acid and white wine extracts rich in phenolic acids to inhibit the decrease of linalool in Muscat wine.

Materials and Methods

Reagents and wines

Caffeic acid and gallic acid were purchased from Sigma (St. Louis, USA). Linalool, α-terpineol, citronellol, nerol, and geraniol were purchased from Aldrich (Steinheim, Germany). The dry white Muscat wine used is protected by Appellation of Origin (Lemnos, Greece). The wine extracts examined were from Roditis wine, a well-known Greek dry white wine.

Experimental procedure

Tested wine extracts and phenolic acids were added to Muscat wine as 10 % solutions. A volume of 2 mL of each solution was added to 60 mL of wine. Wine extracts were added at a final concentration of 60 mg/L, while phenolic acids were added at a final concentration

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of 40 mg/L. Control samples were also prepared by adding 2 mL of 10 % ethanol to 60 mL of wine. The bottles (4.9 cm in diameter, 9.9 cm high, 80 mL capacity) were kept open in a dark place at 20 °C. After 4 days of storage wine samples were analysed. The whole experiment was repeated three times, and the results reported are the means of the three trials. The one-way analysis of variance using the Duncan test at level of significance $P<0.05$ was used for statistical analysis (SPSS 11.5).

Analytical methods and organoleptic evaluation

Gross composition of wine samples was determined by classic methods (*7*). Total and free sulphite were determined by the Ripper method, and total acidity by volumetric analysis. Alcohol was determined with a hydrometer, and reducing sugars by the Lane-Eynon method. Samples of Muscat wine were tested organoleptically by two judges, familiar with Muscat wines. Samples were compared to each other and to wines from unopened bottles.

Terpene alcohols in Muscat wine were determined by gas chromatographic analysis after the extraction procedure. A volume of 50 mL of wine samples was taken and the pH was adjusted to 8.0 using 2 M NaOH. Terpene alcohols were obtained by three extractions using 5, 3 and 2 mL of 1:1 volume ratio of ether:hexane. The total organic phase was dehydrated using Na_2SO_4, and concentrated in graduated tubes to 1.0 mL using nitrogen. The sample was put into 1.8 mL Snap/Crimp Stepvials, capped with 11-mm Snap caps with PTFE/silicone septa (SRI) and analysed by gas chromatography.

A Fisons model 9000 series gas chromatograph (Fisons Instruments, Milano, Italy) equipped with a flame-ionization detector was used. The following column and conditions were used: column – capillary AT-1000, 50 m × 0.25 mm, 0.2 μm film thickness (Alltech Associates, Inc., Deerfield, USA); carrier gas – helium, flow rate 0.36 mL/min; temperature programme (column oven) – 60 °C for 5 min, 60 to 200 °C at a rate of 3 °C/min, 30 min held at 200 °C; temperature of injector 200 °C, temperature of detector 250 °C; volume of sample 1.6 μL. Split/splitless mode was used, splitless for 30 s and split ratio was 1:30.

The recovery of each of the terpene alcohols was determined by adding known quantities of each to the wine samples. Quantification of the terpene alcohols in the wine samples was made using different concentrations of each of the terpene alcohols as external standards. The processing of the chromatograms was done with the Chrom-card for Windows version 1.8 software system (Fisons Instruments, Italy).

Wine extracts used were prepared by extraction of the Roditis wine total extract. Total wine extract was dealcoholized wine concentrated by rotary evaporation at 25 °C and 80 mbar. Dealcoholized wine was obtained by evaporation; wine added to an equal volume of distilled water was concentrated to the original volume (at 25 °C and 80 mbar). The total wine extract (pH=2.0) was first extracted with ethyl acetate. Aqueous phase was the first extract (R1). 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 (R2). The aqueous phase was adjusted to pH=2.0 and extracted again with ethyl acetate. This extract was the third extract (R3). The three extracts in 10 % ethanol were initially tested. The R2 extract was not used further since it resulted in turbidity and gave an undesirable odour to Muscat wine. The total phenol contents of the samples were determined by the Folin-Ciocalteau method (*8*), 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 as described previously (*9*). Samples were filtered using syringe filter (PTFE 0.45, Alltech) prior to the injection. Waters 600E system with a 996-photodiode array detector and a 600E pump was used. Chromatograms were treated using the Millenium 32 program. The column was a C18 reversed phase Spherisorb (4.0 × 250 mm) with 5-μm packing. 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 0.90 mL/min.

All peaks were classified using absorbance characteristics of the phenolic classes derived from the literature (*10,11*) and from our observations using several standards. The absorbance wave lengths of phenolic classes were as follows: benzoic acids at 250–280 nm; cinnamic acids at 305–330 nm, and several of them also at 290–300 nm; anthocyanins at 450–560 and 240–280 nm, and some of them at 315–325 nm; flavanols at 270–280 nm and at around 230 nm; flavonols at 350–380 and 250–270 nm, and some of them at around 300 nm; flavones and isoflavones at 300–350 and 245–270 nm; flavanones at 270–295 nm, and some of them at 300–320 nm. Unclassified peaks which exhibited maximum absorbance at 280–305 nm were expressed as unclassified 280 nm. Unclassified peaks that exhibited maximum absorbance at around 230 nm, and also absorbed at around 280 nm, were expressed as unclassified 230 nm. As main phenolic peaks were taken those exhibiting high area at 280, 255, 320, 360 or 520 nm.

Results and Discussion

The average composition of the dry (<1 g/L of reducing sugars) white Muscat wine used was: alcohol content 12.0 %, total acidity 6.4 g/L as tartaric acid, total SO_2 144 mg/L, and free SO_2 22 mg/L. Total phenolic content of Muscat wine was 232 mg/L of gallic acid equivalents.

The dry Muscat wine was platinum-green with a fairly rich Muscat aroma. The ability of wine extracts and phenolic acids to prevent the disappearance of Muscat wine aroma was first evaluated organoleptically. It appeared that all wine extracts and phenolic acids were effective. Since control wines retained clearly less muscat aroma after 4 days of storage, wine samples were analysed after that period of storage.

Muscat wine had an average content of 470.9 μg/L of linalool, 560.6 μg/L of α-terpineol, 37.4 μg/L of citronellol, 34.8 μg/L of nerol, and 46.1 μg/L of geraniol. Similar concentrations of terpene alcohols in Muscat wines had been reported previously (*12*).

The effectiveness of white wine extracts and phenolic acids was evaluated by determining the content of linalool in Muscat wine, since the concentration of α-terpineol was constant during 4 days of storage and the concentration of the three other terpene alcohols was much lower. The α-terpineol stayed constant possibly because linalool may be progressively replaced by α-terpineol (*4*). Moreover, it is known that the flavour of Muscat wines is especially dependent on the content of linalool and that the aroma threshold value of linalool is low, *i.e.* 100 μg/L (*6*,*13*).

The content of linalool in Muscat wine treated with wine extracts or phenolic acids and kept at 20 °C for 4 days is reported in Table 1. At t=0 day, the linalool concentration was statistically equal in control and in samples containing each wine extract or phenolic acid. In the control, linalool content dropped by 50 % at t=4 day compared to t=0 day. At t=4 day, samples containing the wine extract R1 or R3 exhibited statistically higher linalool concentration compared to the control samples. HPLC analysis of the wine extracts R1 and R3 revealed that most of their main phenolics were phenolic acids (Fig. 1). The above results indicate that some phenolic acids may have a protective effect on linalool in Muscat wine. Indeed, at t=4 day, samples containing caffeic acid or gallic acid exhibited statistically higher linalool concentration compared to the control samples. In samples containing caffeic acid, linalool content was statistically equal at t=0 and t=4 day. On the other hand, in samples containing gallic acid linalool content was statistically lower at t=4 day, compared to t=0 day. The above indi-

Table 1. Linalool content (in μg/L) of Muscat wine samples treated with wine extracts, caffeic acid or gallic acid and kept at 20 °C

Wine (Sample treatment)	Storage time	
	0 day	4 day
Control	$470.9^{a,b,c}$±76.5	223.2^{e}±71.7
Wine extract R1 (γ=60 mg/L)	$492.1^{a,b}$±48.8	$413.0^{b,c,d}$±32.8
Wine extract R3 (γ=60 mg/L)	$451.3^{b,c}$±58.0	$417.4^{b,c,d}$±34.0
Caffeic acid (γ=40 mg/L)	$377.0^{c,d}$±11.5	323.5^{d}±3.9
Gallic acid (γ=40 mg/L)	488.6^{a}±73.0	$385.9^{c,d}$±37.4

Means in every column and row with different superscript differ significantly at P<0.05
± standard deviation

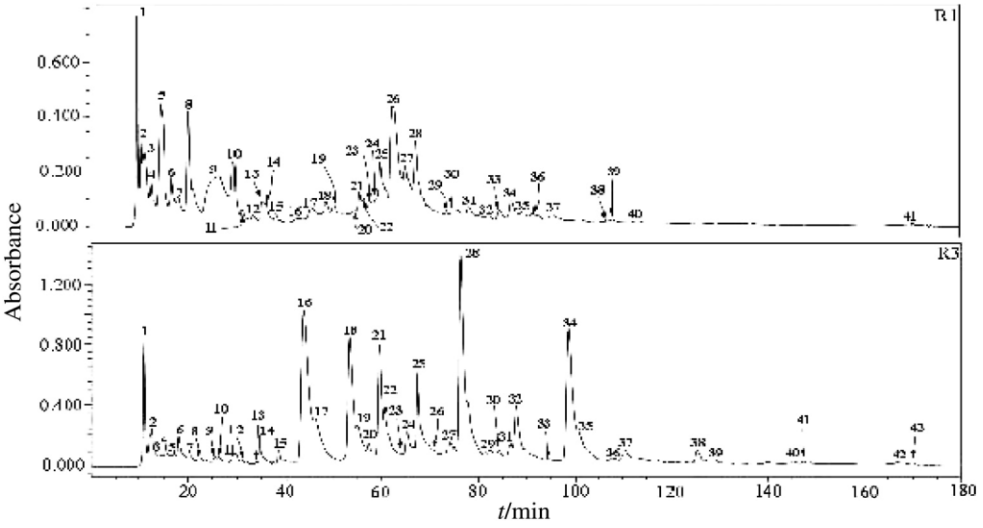

Fig. 1. HPLC chromatograms of wine extracts R1 and R3 at 280 nm
Peaks of R1: 1–4, unclassified with maximum absorbance (max. abs.) at 230 nm; 5, cinnamic acid; 6–7, flavones; 8–15, benzoic acids; 16, flavanol; 17, cinnamic acid; 18–19, flavanols; 20, unclassified with max. abs. at 280 nm; 21–22, benzoic acids; 23–24, flavanones; 25, benzoic acid; 26–27, flavones; 28, benzoic acid; 29, flavanone; 30, benzoic acid; 31, unclassified with max. abs. at 230 nm; 32, flavanol; 33–34, benzoic acids; 35–36, flavanones; 37–39, flavanols; 40–41, benzoic acids
Peaks of R3: 1, unclassified with max. abs. at 230 nm; 2, flavanone; 3–4, unclassified with max. abs. at 230 nm; 5–6, benzoic acids; 7, unclassified with max. abs. at 230 nm; 8–10, flavanols; 11, benzoic acid; 12, unclassified with max. abs. at 280 nm; 13, flavanone; 14, cinnamic acid; 15, benzoic acid; 16–22, cinnamic acids; 23, flavanone; 24–25, cinnamic acids; 26, flavanone; 27, flavone; 28, cinnamic acid; 29–30, benzoic acids; 31, unclassified with max. abs. at 280 nm; 32, cinnamic acid; 33, flavone; 34–35, cinnamic acids; 36, benzoic acid; 37, unclassified with max. abs. at 280 nm; 38–41, flavonols; 42, flavanol; 43, unclassified with max. abs. at 230 nm

cates that the cinnamic acid (caffeic acid) is more effective than the benzoic acid (gallic acid).

It is concluded that some phenolic acids, such as caffeic acid and gallic acid, prevent the decline of linalool in Muscat wine. Subsequently, they can be taken into account as potential inhibitors of the disappearance of aroma in Muscat wines.

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Inhibicija snizivanja količine linaloola u muškatu s pomoću fenolnih kiselina

Sažetak

Dva ekstrakta bijelih vina bogatih fenolnim kiselinama, te kafeinska i galna kiselina, ispitani su kao inhibitori snizivanja linaloola u muškatu. Dodano je po 60 ppm svakoga vinskog ekstrakta i po 40 ppm svake fenolne kiseline. Dodatkom svakoga vinskog ekstrakta ili fenolne kiseline nije opaženo snizivanje koncentracije linaloola, dok je nakon 4 dana skladištenja vina u otvorenim bocama na 20 °C njegova koncentracija smanjena od početnih 470,9 na 223,3 μg/L. Snizivanje koncentracije bitno inhibiraju bilo vinski ekstrakt bilo fenolne kiseline.