Interfering Substances in the Colorimetric Quantitation of Grape Monoterpenes

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Abstract. The potential for interference by specific C₆ compounds in the colorimetric quantitation of grape (*Vitis vinifera* L.) monoterpenes was investigated in model solutions and muscat and neutral-flavored grape cultivars. The unsaturated C₆ aldehyde 2-hexen-1-al (2HX) showed color absorption at 608 nm in distilled water after reaction with an acidified vanillin solution. Absorbance also increased significantly when 2HX was added to a series of linalool solutions; ≈ 2.5 mg 2HX per liter of a 1-mg-liter⁻¹ linalool solution increased the absorbance by >10%. Adding 2.5 mg 2HX per kilogram of 'Gewürztraminer' berry homogenate significantly increased apparent free volatile terpene (FVT) concentrations to 121% of unadulterated control treatments but did not affect potentially volatile terpenes (PVT). Adding 2HX also increased apparent FVT concentration in 'Perlette' and 'Flame Seedless'. Both neutral-flavored table grape cultivars contained some FVT and PVT as a consequence of their muscat ancestries. FVT and PVT quantitation by colorimetric methods may be subject to significant error if the concentration of 2HX and other unsaturated C₆ compounds in grape berries or must are >5 mg-liter⁻¹. However, low concentrations of unsaturated C₆ compounds (<80 μ g-liter⁻¹) in British Columbia wines suggest that there is a low probability of significant interference with this method.

Monoterpenes are important aroma compounds in determining the varietal character of many grape cultivars (Dimitriadis and Williams, 1984). Some C₆ compounds may interfere with the colorimetric reaction used in the Dimitriadis and Williams's (1984) method for grape monoterpene quantitation. C₆ compounds may distill over in the free volatile terpene (FVT) distillate, creating elevated readings. Because our program uses a modification of the aforementioned procedure (Reynolds and Wardle, 1989a) for assessing viticultural and enological treatments, any sources of error must be identified.

Sulfur dioxide (Dimitriadis and Williams, 1984) and isoamyl alcohol (Williams, personal communication, 1988) create slightly elevated FVT readings if the original procedure is applied to wines. For berry homogenates and unsulfited juices, the only interfering compound specifically mentioned is trans-hex-2-en-1-ol (Dimitriadis and Williams, 1984), which gave false high FVT readings, although the color absorbance of this

compound at 608 nm following reaction with acidified vanillin was only $\approx 60\%$ of the absorbance of the least reactive terpenes, the furan linalool oxides. Compounds other than C₆ aldehydes also may be interfering substances. Dimitriadis and Williams (1984) mentioned

that the C₁₃ allylic alcohols, α - and β -ionols, and hydroxymethyl furan may be interfering substances. Other common volatile aldehydes present in grapes also may lead to false high readings, but currently we are not aware of other compounds that have significant interfering capabilities.

The purpose of our investigation was to 1) quantitate the potential for interference in the colorimetric method by specific C₆ compounds in muscat and neutral grape cultivars to assess their interference capabilities within a background of terpene compounds and a "neutral" background and 2) examine absorbance and concentration curves of the interfering substances in those cultivars.

Materials and Methods

Effect of adulteration of 'Gewürztraminer' berry homogenates by 2-hexene-1-al (2HX). Choice of compounds for adulteration of grape samples was contingent on identifying those compounds (prepared at 1.0 to 15.0 mg-liter⁻¹) in distilled water that showed color absorption at 608 nm after reaction with an acidified vanillin solution and significantly increased absorbance when added to a series of linalool solutions (1.0 to 8.0 mg-liter⁻¹). Hexanol and hexanal produced no colorimetric reactions. Absorbance curves for 2HX in distilled water were linear [$2\text{HX} = (26.09 \times \text{absorbance}) + 0.022$], and ≈ 2.5 mg 2HX per liter of a 1-g-liter⁻¹ linalool solution increased the absorbance by >10% (Fig. 1). Reynolds et al. (1994b) found concentrations of *cis*- and *trans*-3-hexen-1-ol in British Columbia 'Riesling' wines at 60 to 70 μ g-liter⁻¹, but higher concentrations of these compounds may exist in berries and musts.

Fig. 1. Absorbance at 608 nm of various 2HX per linalool solutions. Concentrations ranged from 0 to 15.0 and from 0 to 8.0 mg-liter⁻¹ for 2HX and linalool, respectively. Vertical bars represent SD.

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Next, two adulteration ("spiking") levels of 0 and 2.5 mg 2HX per kilogram of 'Gewürztraminer' berry homogenate were created by adding 1 ml of a 250-mg-liter⁻¹ stock solution of 2HX to 100 g of berry homogenate immediately before adding the NaOH used to neutralize the homogenate. There were three canopy treatments (control, hedge, leaf removal) combined with two sites and four blocks (Reynolds and Wardle, 1994) for each spiking level. Distillation and quantitation of FVT and potentially volatile terpenes (PVT) proceeded as per the standard method (Dimitriadis and Williams, 1984; Reynolds and Wardle, 1989a). Data analysis was performed with the SAS statistical package (SAS Institute, Cary, N.C.) using the general linear models procedure and site $\times$ treatment $\times$ 2HX $\times$ block as the error term.

Effect of adulteration of neutral grape cultivars by 2HX. About 2 kg of 'Perlette' and 'Flame Seedless' grapes were obtained from the produce section of a local supermarket. Berries were removed from the rachis, washed in tap water, and placed into $\approx$ 120-g samples. Six 2HX concentrations (0, 1.0, 2.5, 5.0, 8.0, and 10.0 mg $\cdot$ kg⁻¹) were created by adding appropriate volumes of stock solution. Distillations were performed three times to provide replications (15 samples total). Distillation and FVT and PVT quantitation were performed using the standard method (Dimitriadis and Williams, 1984; Reynolds and Wardle, 1989a). Analysis of variance was performed with the SAS statistical package using linear and quadratic contrasts and 2HX $\times$ replication as the error term.

Results and Discussion

'Gewürztraminer' berry homogenates. Adding 2.5 mg 2HX per kilogram of 'Gewürztraminer' berry homogenate significantly increased apparent FVT concentrations to 121% of that of the controls ($P \leq 0.01$; data not shown) but did not increase PVT. Respective mean FVT and PVT concentrations of the controls were 1.48 and 2.21 mg $\cdot$ kg⁻¹. This result is significant because PVT are considered to be most responsive to vineyard treatments (Eschenbruch et al., 1987; McCarthy and Coombe, 1985; McCarthy et al., 1987; Reynolds and Wardle, 1989a, 1989b, 1991; Reynolds et al., 1994a). No interactions occurred between 2HX and either site or canopy treatment.

Neutral grape cultivars. Despite its relatively neutral flavor, 'Perlette', owing to its ancestry ('Muscat Reine des Vignes' $\times$ 'Thompson Seedless'), contained mean FVT and PVT concentrations of 0.96 and 0.87 mg $\cdot$ kg⁻¹, respectively. Adding 2HX linearly increased apparent FVT concentration ($P = 0.0085$; Fig. 2). Treating 'Flame Seedless' (Weinberger and Harmon, 1974) produced a similar response insofar as apparent FVT increased linearly with increasing 2HX addition ($P = 0.0011$). Apparent PVT displayed a slight linear decrease in response to 2HX ($P = 0.0361$; Fig. 2). Also, note that this neutral-flavored cultivar contained some FVT and PVT (0.59 and 0.36 mg $\cdot$ kg⁻¹, respectively), perhaps as a consequence of one of its paternal grandparents ('Muscat of Alexandria' $\times$ 'Thompson Seedless').

Our results indicate that quantitation of FVT and PVT by the Dimitriadis and Williams method (1984) may be subject to significant error, depending on the concentration of 2HX and other unsaturated C₆ compounds in grape berries or must. Concentrations of these compounds >5 mg $\cdot$ liter⁻¹ are likely to cause significant elevation in absorbance, which may bias or otherwise confound results based on spectrophotometric measurements. However, because of low concentrations of unsaturated C₆ compounds (<80 μ g $\cdot$ liter⁻¹) in British Columbia wines (Reynolds et al., 1994b), there is a low probability of significant interference with this method.

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Fig. 2. Apparent FVT and PVT readings from 'Perlette' and 'Flame Seedless' berry homogenates spiked with several concentrations of 2HX (concentration range from 0 to 10.0 mg $\cdot$ liter⁻¹). Vertical bars represent SD.