JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008**, *56*, 3745–3748 **3745**

Determination of Rotundone, the Pepper Aroma Impact Compound, in Grapes and Wine

TRACEY E. SIEBERT,[†] CLAUDIA WOOD,[†,§] GORDON M. ELSEY,[†] AND ALAN P. POLLNITZ*[,†]

The Australian Wine Research Institute, P.O. Box 197, Glen Osmond (Adelaide), SA 5064, Australia, and School of Agriculture, Pontificia Universidad Católica de Chile, Casilla 306 Correo 22, Santiago, Chile

Shiraz, also known as Syrah or Hermitage, is one of Australia's most popular red wine varieties both domestically and internationally. Black pepper aroma and flavor are important to some Australian Shiraz red wine styles. Recently, rotundone (a bicyclic sesquiterpene) was identified as the potent aroma compound responsible for pepper aromas in grapes, wine, herbs, and spices, including peppercorns. Here the development, optimization, and validation of the analytical method for the quantitative determination of rotundone in grapes and wine are described and discussed. The method is precise, accurate, robust, and sensitive with a subpart per trillion limit of quantitation. The method uses stable isotope dilution analysis with d_5-rotundone as internal standard, solid-phase extraction and microextraction, and gas chromatography–mass spectrometry.

KEYWORDS: GC-MS; SPE; SPME; grape; wine; rotundone; pepper; aroma; analysis

INTRODUCTION

One of the world's most widely grown and successful red wine grape varieties is Syrah, also called Shiraz or Hermitage, especially in Australia, where Shiraz constitutes about 40% of all red wines made each year. Some of Australia's most famous "icon" wines are made from Shiraz grapes. Black pepper aroma and flavor are distinctive positive attributes important to some Australian Shiraz red wine styles as discussed in Parker et al. (*1*). Recently, we identified rotundone (a bicyclic sesquiterpene) as the aroma compound responsible for pepper aromas in grapes and wine and in herbs and spices, especially black and white pepper (*2*), where it has a high odor activity value (OAV) (*3*) of up to 250000! Rotundone is extremely potent, with aroma detection thresholds of 8 ng/L in water and 16 ng/L in red wine (*2*). Here we describe the development, optimization, and validation of the analytical method for the quantitative determination of rotundone in grapes and wine.

MATERIALS AND METHODS

Rotundone (**Figure 1**) was synthesized as described previously (*2*): EIMS, *m/z* (rel intensity) 219 (17), 218 ([M]$^+$, 100), 204 (14), 203 (89), 189 (10), 177 (11), 175 (27), 163 (38), 162 (28), 161 (50), 147 (37), 137 (39), 135 (19), 133 (25), 121 (18), 120 (27), 119 (37), 107 (21), 105 (32), 95 (17), 93 (20), 91 (30), 79(18), 77 (18), 67 (15), 55 (11), 41 (17).

d_5-Rotundone (**Figure 1**) was prepared by deuterium exchange on synthetic rotundone under alkaline conditions. Synthetic rotundone (4.7 mg) was dissolved in a 1.0 M solution of sodium ethoxide in ethanol-*d*, sealed in a glass ampule, and allowed to stand at 25 °C for 24 h. After this time, the product was isolated and purified by normal-phase high-performance liquid chromatography (HPLC) using an Agilent 1100 series HPLC fitted with a Luna 5 μ silica 100A column of dimensions 250 × 2.00 mm and hexane/ethyl acetate elution as described by Wood et al. (*2*), yielding d_5-rotundone: EIMS *m/z* (rel intensity) 223 ([M]$^+$, 100), 208 (55), 207 (39), 167 (42), 166 (34), 165 (37), 164 (36), 142 (38), 123 (29), 122 (35), 107 (27), 95 (30), 93 (29), 82 (28); ^{1}H NMR (600 MHz, $CDCl_3$), δ 4.72 (1H, app quint, J = 0.9, H_{12a}), 4.69 (1H, app quint, J = 1.8, H_{12b}), 2.97 (1H, ddq, J = 11.2, 3.4, and 7.3, H_{10}), 2.00 (1H, m, H_7), 1.81–1.74 (3H, overlapping m, $H_{8,9\alpha}$), 1.75 (3H, br s, H_{13}), 1.51 (1H, m, $H_{9\beta}$), 1.10 (3H, s, H_{14}), 0.99 (3H, d, J = 7.2, H_{15}). The signals associated with the protons on H_3, H_4, and H_6 (*2*) are absent, confirming the deuterium exchange at these positions. Also, the signals associated with protons H_7 and especially H_{14} have been modified, reflecting the absence of the neighboring protons.

Samples. The red wine used as nonpeppery control (and for standard additions for method validation) was a young (<12 months old) cask ("bag-in-box") wine of predominantly Cabernet Sauvignon variety. No rotundone was detected by the method (i.e., <0.5 ng/L) in this control red wine.

Shiraz grape samples, both nonpeppery controls (as used for standard additions for method validation) and samples of potentially 'spicy'/'peppery' grapes, were obtained from vineyards in South Australia and Victoria over two vintages. No rotundone was detected by the method (i.e., <0.5 ng/kg) in the control grapes.

Blanks, model wines, buffer solutions, etc., were checked regularly by the method below to ensure there was no carry-over or cross-contamination occurring at any stage. All solvents were verified for purity by GC-MS prior to use. Water was purified by the Milli-Q system.

Grape Sampling and Subsampling. After picking, grape samples were immediately frozen and kept below –18 °C for a minimum of 24 h prior to transport on dry ice (or refrigerated transport) to the

* Corresponding author (fax +61 8 8303 6601; e-mail ajpollni@senet.com.au).

[†] The Australian Wine Research Institute.

[§] Pontificia Universidad Católica de Chile.

10.1021/jf800184t CCC: $40.75 © 2008 American Chemical Society
Published on Web 05/08/2008

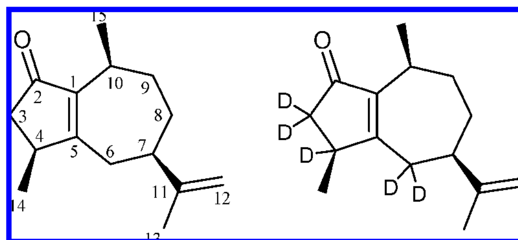

Figure 1. Structures of (−)-rotundone and d_5-rotundone.

Australian Wine Research Institute. These larger samples (5–200 kg each) were each further randomized prior to taking smaller subsamples (typically 250 g). Grape samples were frozen and kept at –20 °C until immediately prior to extraction and analysis.

Stable Isotope Dilution Assay (SIDA). Rotundone concentration was determined by SIDA SPE-SPME-GCMS. The method was optimized as discussed under Results and Discussion. The optimum method was as follows.

Sample Preparation. For juice analysis, 250 g of destemmed grapes was first homogenized using a hand-held blender (Breville wizz stick). The blender has stainless steel blades, and grapes were blended with minimal seed breakage in a glass beaker to avoid contact with plastic and potential "scalping" of volatiles of interest (*4*). Ethanol (20 mL) and water (30 mL) were added. The sample was shaken for 24 h, sonicated, centrifuged, and filtered (0.45 μm glass fiber). One hundred milliliters of the filtrate was used for the analysis. Wine samples (100 mL) were used directly. d_5-Rotundone (12.5 ng in 100 μL of ethanol) was added as internal standard (IS) to each sample prior to SPE.

SPE. SPE cartridges (Phenomenex styrene−divinylbenzene (SDB-L) 500 mg/6 mL) were conditioned with *n*-pentane/ethyl acetate (4:1), then methanol, and finally model wine (12% aqueous ethanol, buffered to pH 3.2 with potassium hydrogen tartrate and tartaric acid). The wine (or juice/water) sample (100 mL) was percolated through the SPE cartridge, which was then washed with water and eluted with *n*-pentane (2 mL, discarded), followed by *n*-pentane/ethyl acetate (9:1) (10 mL). The organic solvent was removed and the residue dissolved in ethanol (0.5 mL). Aqueous tartrate buffer (pH 3.2, 9.5 mL) was added. This model wine solution was analyzed by the SPME-GCMS method (following).

SPME-GC-MS Analysis. An Agilent Technologies 6890 gas chromatograph (GC) was equipped with a Gerstel MPS2 multipurpose sampler and coupled to an Agilent 5973N mass selective detector. The instrument was controlled with Agilent G1701CA ChemStation software in conjunction with Gerstel MASter software (version 1.81). The GC was fitted with a J&W DB-Wax capillary column of dimensions approximately 30 m × 0.25 mm, 0.25 μm film df. The carrier gas was helium (ultrahigh purity), and the flow rate was 1.0 mL/min in constant flow mode. The Agilent GC inlet was fitted with a resilanized borosilicate glass SPME inlet liner (6.5 mm o.d., 0.75 mm i.d., 78.5 mm long) and was held at 240 °C. A Supelco "blue" polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm fiber was immersed in the sample for 60 min at 35 °C, with agitation. The SPME fiber was desorbed in the pulsed splitless mode and left in the injector for 5 min. The splitter, at 30:1, was opened after 36 s. The oven temperature was started at 80 °C, held at this temperature for 1 min, then increased to 220 at 3 °C/min, then increased to 240 at 40 °C/min, and held at 240 °C for 20 min. The MS transfer line was held at 240 °C. The ions monitored via positive ion electron impact selected ion monitoring (SIM) at 70 eV for determination of rotundone with d_5-rotundone as internal standard were m/z 147, 161, 203, 208, 218, and 223, dwell time = 30 ms each. The target ions were typically m/z 218 for rotundone and m/z 223 for d_5-rotundone, with the other ions used as qualifiers. The data were analyzed with Agilent G1701CA ChemStation software.

Validation. The precision of the assay was validated by a series of duplicate standard addition experiments to red wine and red grape juice matrices as follows.

Wine. Duplicate standard additions of rotundone to give concentrations of 0, 0.5, 2.5, 5, 14, 27, 54 and 138 ng/L ($N = 8 \times 2$) were made to the control red wine samples and then analyzed by the method. The resultant calibration function was linear throughout the calibration range with excellent correlation ($R^2 = 0.999$) and a limit of quantitation of 0.5 ng/L. The repeatability of the method was also demonstrated by seven replicate additions at concentrations of 3 and 30 ng/L. The relative standard deviation (RSD) was <2% at both of these levels.

Grapes. Very low 'pepper' aroma intensity Shiraz grapes (8.4 kg, containing <0.5 ng/kg rotundone) were blended, sonicated, centrifuged, and filtered as described above to prepare 5.6 L of juice. Replicate subsamples (75 mL) of this clarified juice plus water (25 mL) were spiked with standard additions of rotundone to obtain duplicate grape juice samples of concentrations 0, 0.7, 4, 7, 18, 36, 73, and 184 ng/L ($N = 8 \times 2$). The resultant calibration function was linear throughout the calibration range with excellent correlation ($R^2 = 0.999$) and a limit of quantitation (LOQ) of 0.5 ng/L, determined as the lowest concentration at which a signal-to-noise ratio of greater than 3 to 1 could be consistently achieved in spiked red wine samples for both target and qualifier ions of unlabeled rotundone. The repeatability of the method was also demonstrated by several replicate additions at concentrations of 4 and 40 ng/L. The RSD was 3% at both of these levels.

RESULTS AND DISCUSSION

Liquid–liquid extraction or SPME analysis directly on the wine or grape juice samples gave poor LOQs of rotundone of the order of 100–200 ng/L or worse, depending on the sample. As we needed a LOQ below the sensory threshold of rotundone of 8 ng/L in water (*2*), an optimized procedure was required to enhance the selective extraction of rotundone from the many other components present. We also needed to load aqueous samples and elute with nonaqueous solvents to avoid extraction of sugars, anthocyanins, and polymeric material present in the grapes or wine. To achieve this, we employed SPE followed by SPME as discussed below.

Normally the use of such selective procedures, especially in series, could be of concern regarding possible matrix effects on the recovery of rotundone from different grape or wine samples or the precision of this recovery. We addressed these issues by the appropriate use of a suitable stable isotope labeled internal standard (added prior to SPE and SPME), d_5-rotundone, that we synthesized as described earlier. The advantages of stable isotope dilution assays (SIDA) in grape and wine analysis have been reviewed recently (*5*).

SPE. SPE has been used in the extraction and concentration of a variety of volatile aroma compounds from wine using a range of solid-phase materials (e.g., refs *6* and *7*), which can exhibit different extraction efficiencies depending on the sorbent and solvent system used (*8*). C-8, C-18, phenyl, and styrene/divinylbenzene (SDB) solid phases were initially considered. Following these preliminary experiments two solid phases were selected for further tests, Supelclean ENVI-Carb (Supelco) and Strata SDB-L (Phenomenex), both with 0.5 g packed into the SPE tube in a 6 mL reservoir. These were evaluated with different solvent systems (dichloromethane/methanol and pentane/ethyl acetate) with all samples being extracted and analyzed in triplicate. For selective extraction of rotundone from spiked Shiraz red wine samples, we achieved the optimum results using the Phenomenex Strata SDB-L solid phase, mainly because the rotundone eluted with pentane/ethyl acetate quantitatively in the one fraction (as detailed under Materials and Methods). The Supelclean ENVI-Carb phase loaded and preconditioned fine, but upon elution would "tail" across fractions (or be incompletely extracted) with typically 74% eluting in the first 10 mL fraction, 21% in the second 10 mL fraction, and 5% in the third. Our observation that the SDB phase was more suited for the analysis of terpenoid compounds in wine is supported in the literature (*7*). The other solid phases were less suitable for some samples, with partial loss of rotundone (up to 50%) in the sam-

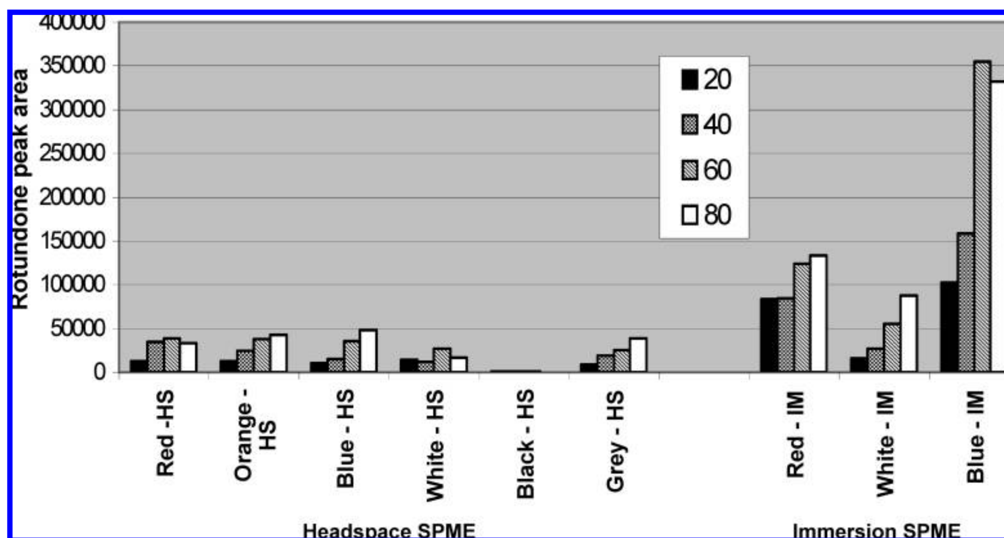

Figure 2. Solid-phase microextraction of rotundone from wine over time (20, 40, 60, and 80 min) with several different fibers. HS, headspace solid-phase microextraction (SPME); IM, immersion (liquid) SPME. The colors are consistent with the Supelco fiber colors: red, 100 μm polydimethylsiloxane (PDMS); orange, 70 μm Carbowax/divinylbenzene (CW/DVB); blue, 65 μm PDMS/DVB; white, 85 μm polyacrylate (PA); black, 75 μm Carboxen (CAR)/PDMS; gray, 50/30 μm DVB/CAR/PDMS.

ple loading step, presumably due to the SPE column becoming saturated too early. Recoveries were assessed by quantitative GC-MS comparison of the spiked red wine samples with model wine samples and pure reference solutions.

SPME-GCMS Analysis. The SPME method for the determination of rotundone in wine was optimized for the following parameters: salt addition, agitation, fiber choice, time of exposure, headspace or immersion SPME, desorption time, injector block temperature, and GC temperature programming conditions. The optimum SPME conditions required a 60 min immersion extraction at 35 °C with agitation, using a 65 μm polydimethylsiloxane (PDMS)/divinylbenzene (DVB) (blue) fiber desorped for 5 min at 240 °C with the GC conditions given under Materials and Methods. Salt addition (as sodium chloride) had little effect on the recovery of rotundone. Agitation improved recoveries by an order of magnitude or more. The optimum time for SPME was found to be 60 min. Longer extraction times usually gave slightly better recovery but were considered to be impractical. The fibers investigated were as follows: 65 μm PDMS/DVB (blue) was optimal, 70 μm Carbowax/DVB (orange), 100 μm PDMS (red), 85 μm polyacrylate (PA) (white), and 50/30 μm DVB/Carboxen(CAR)/PDMS (gray) were adequate; 75 μm CAR/PDMS (black) was very poor (0.2% recovery at best). The relative recovery of rotundone in red wine obtained with these fibers for different extraction times is shown in **Figure 2**. We investigated immersion for only the red, white, and blue fiber compositions as the others were not suitable for dipping. Although there were some differences between fibers and extraction times, the recovery of rotundone was generally several times higher with immersion SPME as compared to headspace SPME. This is consistent with an expected relatively low volatility of rotundone, as evident from its long GC retention time (*2*). This was also in agreement with our experience with ortho-amino acetophenone (*11*) but in contrast to the majority of published work on SPME analysis (see, for example, refs *5*, *10*, and *12*), which generally deals with compounds of significantly higher volatility. This apparent low volatility of rotundone would also explain why headspace recovery with the black CAR/PDMS fiber was so poor, as this fiber is designed for analysis of gases and other very volatile molecules. In a single immersion SPME application about 85% of the total rotundone present (at concentrations of around 50−100 ng/L) was recovered from the wine or grape juice extract, although this percentage varied depending on the SPME conditions used, the concentration of rotundone, and the matrix passed through the SPE cartridge (wine or juice, ethanol content, ionic strength, etc.). However, these potential sources of variation had no significant effect on the accuracy or precision of the quantitation, as the stable isotopically labeled standard (d_5-rotundone) was affected by any or all of these factors in effectively the same way.

The optimum injector block temperature for desorption of the SPME fiber was found to be 240 °C. Lower temperatures gave less signal, and higher temperatures gave more background noise and decreased fiber longevity (data not shown). As discussed previously (*9, 10*) the SPME conditions that give the highest signal are not necessarily the best for wine analysis, where the signal-to-noise ratio is more important.

To decrease method cycle time we settled for an initial GC oven temperature of 80 °C. It was not necessary to start the GC program at a lower temperature as 80 °C was more than adequate for condensation and effective band formation of rotundone, which elutes relatively late (*2*). The slow oven ramp of 3 °C/min was necessary to achieve baseline resolution of rotundone (and the d_5-rotundone internal standard) from the numerous other oxygenated sesquiterpenes and other compounds present, especially around the trace levels at which rotundone occurs in grapes and wine. The final oven hold time of 20 min was needed to ensure no carry-over of semivolatile components from the wine matrix, whereas shorter hold times decreased the number of samples able to be run before system maintenance was required for optimum chromatography.

Recovery of Rotundone from Grape Extracts. To ensure optimum recovery of rotundone from grapes we investigated several methods of grape sample preparation. **Table 1** shows the relative recovery of rotundone from subsamples (250 g) of the same batch of Shiraz grapes (2 kg), which contained at least 88 ng/kg of rotundone (the amount measured by the optimum method, method 4). As can be seen in **Table 1**, the relative recovery of rotundone from grapes can vary from 16 to 100%, depending on the extraction method used. We repeated this recovery trial on another 2 kg batch of Shiraz grapes rich in naturally occurring rotundone, which gave good agreement in the relative rotundone recoveries obtained (data not shown).

Table 1. Relative Recovery of Rotundone from Shiraz Grapes (Containing at Least 88 ng/kg of Rotundone) Using Five Sample Preparation Methods[a]

method	recovery (%)
1: grapes were thawed, quickly blended, frozen flat in a clip-lock bag overnight at –20 °C, thawed again, sonicated for 10 min, centrifuged, and filtered; ethanol and water were added; SPE then SPME-GCMS	16
2: grapes were thawed, quickly blended, left to sit overnight at room temperature (20 °C), sonicated for 20 min, centrifuged, and filtered; ethanol and water were added; SPE then SPME-GCMS	28
3: grapes were thawed, quickly blended with ethanol and aqueous tartrate buffer (pH 3.2), left to sit at room temperature (20 °C) for 24 h, sonicated for 20 min, centrifuged, and filtered; SPE then SPME-GCMS	83
4: grapes were deseeded, blended finely with ethanol and aqueous tartrate buffer (pH 3.2), left to sit at room temperature (20 °C) for 24 h, sonicated for 20 min, centrifuged, and filtered; SPE then SPME-GCMS	100
5: grapes were blended finely with ethanol and aqueous tartrate buffer (pH 3.2), left to sit at room temperature (20 °C) for 24 h, sonicated for 20 min, centrifuged, and filtered; SPE then SPME-GCMS	45

[a] Following the individual preparations outlined, all five of these methods used the same optimum solid-phase extraction (SPE) and solid-phase microextraction–gas chromatography–mass spectrometry (SPME-GCMS) method as detailed under Materials and Methods.

Applying the method developed in this paper, we have determined the levels of rotundone in grapes, wine, and other matrices (e.g., spices, especially pepper) and shown that rotundone is important to the 'black pepper' aroma of grapes and wine (*2*). We have also confirmed that rotundone concentration, and the corresponding 'black pepper' character, is more dominant in 'peppery' wineries and 'peppery' vintages (*2*), in agreement with previous anecdotal observations (*1, 13*). A wide range of further possible viticultural and enological studies is envisaged utilizing this validated, accurate, robust, and precise methodology to determine rotundone in grapes and wine with a subpart per trillion level of quantification.

ACKNOWLEDGMENT

We are indebted to our collaborators (M. Eggers, M. Meier, T. Vössing, S. Widder, and G. Krammer) at Symrise, Holzminden, Germany, for the valuable reference sample of pure rotundone. We thank our colleagues at AWRI, especially M. A. Sefton and M. J. Herderich, for valuable comments and encouragement. We are grateful to our collaborators in the Australian Grape and Wine Industry for their ongoing interest in this project, for the supply of grape samples, and for interesting discussions, especially N. Scarlett, D. Buckle, and D. Sheehan at Mt. Langi Ghiran, A. and A. Hart at Fosters Wines, I. Pearce and M. Wirper at Orlando Wyndham, and S. Hodder at Wynns Coonawarra Estate.

LITERATURE CITED

(1) Parker, M.; Pollnitz, A. P.; Cozzolino, D.; Francis, I. L.; Herderich, M. J. Identification and quantification of a marker compound for 'pepper' aroma and flavor in Shiraz grape berries by combination of chemometrics and gas chromatography−mass spectrometry. *J. Agric. Food Chem.* **2007**, *55*, 5948–5955.

(2) Wood, C.; Siebert, T. E.; Parker, M.; Capone, D. L.; Elsey, G. M.; Pollnitz, A. P.; Eggers, M.; Meier, M.; Vössing, T.; Widder, S.; Krammer, G.; Sefton, M. A.; Herderich, M. J. From wine to pepper: rotundone, an obscure sesquiterpene, is a potent spicy aroma compound. *J. Agric. Food Chem.* **2008**, *56*, xxxx–xxxx.

(3) Grosch, W. Detection of potent odorants in food by aroma extract dilution analysis. *Trends Food Sci. Technol.* **1993**, *4* (3), 68–72.

(4) Pollnitz, A. P.; Capone D. L. ; Caldersmith, M. C.; Sefton, M. A. The effect of various wine bottle closures and fining agents on flavour and aroma compounds in wine. In *Grapegrowing at the Edge; Managing the Wine Business; Impacts on Wine Flavour: Proceedings of a Seminar, Barossa Convention Centre, Tanunda, Australia*, July 10–11; Bell, S. M., de Garis, K. A., Dundon, C. G., Hamilton, R. P., Partridge, S. J., Wall, G. S., Eds.; Australian Society of Viticulture and Oenology: Adelaide, Australia, 2003; pp 59–63.

(5) Hayasaka, Y.; Baldock, G. A.; Pollnitz, A. P. Contributions of mass spectrometry in the Australian Wine Research Institute to advances in knowledge of grape and wine constituents. *Aust. J. Grape Wine Res.* **2005**, *11*, 188–204.

(6) Lopez, R.; Aznar, M.; Cacho, J.; Ferreira, V. Determination of minor and trace volatile compounds in wine by solid-phase extraction and gas chromatography with mass spectrometric detection. *J. Chromatogr., A* **2002**, *966*, 167–177.

(7) Piñeiro, Z.; Palma, M.; Barroso, C. G. Determination of terpenoids in wines by solid phase extraction and gas chromatography. *Anal. Chim. Acta* **2004**, *513*, 209–214.

(8) Ferreira, V.; Ortega, L.; Escudero, A.; Cacho, J. A comparative study of the ability of different solvents and adsorbents to extract aroma compounds from alcoholic beverages. *J. Chromatogr. Sci.* **2000**, *38*, 469–476.

(9) Pollnitz, A. P.; Jones, G. P.; Sefton, M. A. Determination of oak lactones in barrel-aged wines and in oak extracts by stable isotope dilution analysis. *J. Chromatogr., A* **1999**, *857*, 239–246.

(10) Siebert, T. E.; Smyth, H. E.; Capone, D. L.; Neuwöhner, C.; Pardon, K. H.; Skouroumounis, G. K.; Herderich, M. J.; Sefton, M. A.; Pollnitz, A. P. Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. *Anal. Bioanal. Chem.* **2005**, *381*, 937–947.

(11) Siebert, T.; Herderich, M.; Francis, L.; Pollnitz, A. No evidence of 'atypical ageing taint' in Australian white wine. *Aust. N. Z. Wine Ind. J.* **2003**, *18* (5), 55–56–58.

(12) Yang, X.; Peppard, T. Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem.* **1994**, *42*, 1925–1930.

(13) Iland, P.; Gago, P. *Australian Wine: Styles and Tastes*; Patrick Iland Wine Promotions: Adelaide, Australia, 2002.

Received for review January 18, 2008. Revised manuscript received March 5, 2008. Accepted March 8, 2008. The funding by Vicerrectoría Adjunta de Investigación y Doctorado (VRAID) of the Pontificia Universidad Católica de Chile to C.W. is acknowledged. This project was supported by Australia's grapegrowers and winemakers through their investment body The Grape and Wine Research and Development Corporation, with matching funds from the Australian government.

JF800184T