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Monitoring Acetaldehyde Concentrations during Micro-oxygenation of Red Wine by Headspace Solid-Phase Microextraction with On-Fiber Derivatization

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An analytical method was developed to quantify levels of acetaldehyde in wine samples. The method utilizes headspace solid-phase microextraction with on-fiber derivatization using *O*-(pentafluorobenzyl)-hydroxylamine and quantification by gas chromatography with flame ionization detection. The technique showed good sensitivity and reproducibility in samples of Chardonnay, Petite Sirah, and Merlot wines containing acetaldehyde at levels below the sensory threshold (40−100 ppm). The method was used to monitor acetaldehyde concentrations during the micro-oxygenation of Merlot wine in a 141 L pilot-plant experiment and a 2400 L full-scale study. In both experiments, levels of acetaldehyde remained constant for several weeks before increasing at rates of the order of 1 ppm/day. Variations in the levels of acetaldehyde present are discussed within the context of the underlying chemical reactions.

KEYWORDS: Wine aging; wine oxidation; GC-MS; volatile analysis; acetaldehyde

INTRODUCTION

Since its introduction in the early 1990s, micro-oxygenation has become widely used in the wine industry as an alternative to oak barrel aging (*1*). In the micro-oxygenation process, oxygen gas is slowly bubbled through the wine, initiating a sequence of chemical reactions that can lead to improved attributes of the wine. These include color stability, softer tannins, reduced astringency, and improved mouthfeel (*2*, *3*). The same chemical reactions also occur within oak wine barrels as air slowly diffuses through the barrel and into the wine, but on a much longer time scale. Thus, the micro-oxygenation process may result in some of the same improvements in wine quality, but in a fraction of the time and at a lower cost.

The oxidation processes occurring within wines have been the subject of many studies (*2*, *4*−*17*). The broad features of the chemistry are widely accepted and are shown schematically in **Figure 1**. Evidence for this chemistry is based on mechanistic studies of wine model systems and the identification of ethyl-linked oligomers in a variety of wines. Wines contain hydroquinones such as catechol derivatives that undergo redox reactions, reducing oxygen to hydrogen peroxide (R1 and R2) (*2*, *4*, *15*, *17*). In this process, hydroquinones are oxidized to quinones in a two-electron process via a semiquinone intermediate. Iron(II) species present may then react with hydrogen peroxide to form hydroxyl radicals in the Fenton reaction (R3) (*17*, *18*). The iron(III) formed in reaction R3 may regenerate iron(II) by oxidizing the semiquinone intermediates to quinones (not shown). These radicals can then react with alcohols to form aldehydes (R4). Because ethanol is the predominant alcohol present in wine, acetaldehyde is the major product of this reaction. Acetaldehyde can undergo condensation reactions with anthocyanins and flavanols to form ethyl-linked oligomers (R5) (*4*−*14*, *16*, *19*). These may then react with additional acetaldehyde, anthocyanins, and flavanols to generate polymeric-type structures. Although other chemical reactions are also likely to play a role in the oxygenation process (*18*, *19*), these reactions involving acetaldehyde are believed to be the most significant.

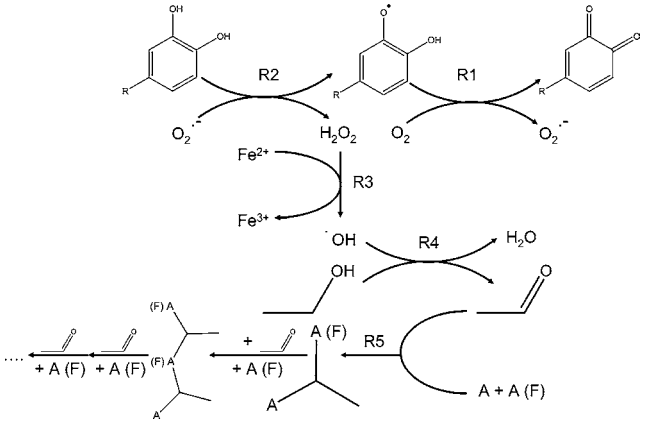

Figure 1. Simplified reaction mechanism for the reaction of oxygen with wine components (A, anthocyanin; F, flavanol).

There are several practical problems associated with the implementation of micro-oxygenation. If oxygen is introduced

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too quickly, aroma compounds may be oxidized, browning may occur, and precipitates may form (*1*, *3*, *20*). Excessive oxidation may also result in increased levels of acetaldehyde, a compound that at sensory threshold levels adversely affects wine flavor and aroma. Currently, the process is monitored via dissolved oxygen measurements, turbidity measurements, spectrophotometry, and tasting (*20*). Sensory detection limits for red wines are typically in the range of 40−100 ppm (*21*, *22*), and by the time that acetaldehyde reaches these levels, consumers may consider the wine to have spoiled. Thus, a practical analytical method capable of monitoring acetaldehyde levels during micro-oxygenation at concentrations below the sensory threshold is highly desirable.

Numerous methods for the quantification of trace aroma components in wine samples including carbonyl compounds have been reported in the literature. Typically, samples are analyzed using gas chromatography (*23*−*40*) or high-performance liquid chromatography (*25*, *41*) following analyte concentration by solid phase (*26*, *28*, *30*, *31*, *33*−*36*, *40*) or solvent extraction (*23*, *27*, *29*, *35*). Acetaldehyde is generally difficult to chromatograph because of its short retention time, and so several studies have employed derivatization reagents such as cisteamine (*23*, *27*) and *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) (*30*, *31*, *33*, *36*) to increase the specificity and accuracy of the analytical method.

Recently, Wang and co-workers (*33*) described the use of headspace extraction and derivatization of C1−C10 aldehydes from various media using solid-phase microextraction (SPME) fibers coated with PFBHA. The carbonyls react with the derivatization reagent bound to the poly(dimethylsiloxane)−divinylbenzene (PDMS−DVB) SPME fibers to form the corresponding oxime. These are then desorbed from the fiber and analyzed by gas chromatography with flame ionization detection (GC-FID). The authors report detection limits of 0.5 ppt for aqueous acetaldehyde solutions saturated with sodium chloride.

Flamini and co-workers (*36*) applied a similar method to follow the evolution of acetaldehyde, diacetyl, and acetoin during malolactic fermentation of Merlot wines. A solution containing PFBHA was added directly to the wine samples, and the resulting oximes were extracted from the headspace using a poly(ethylene glycol)−divinylbenzene (PEG−DVB) SPME fiber. Quantification was performed by gas chromatography with mass spectrometry (GC-MS) using chemical ionization. The technique was used to quantify these carbonyls in 375 mL of wine undergoing malolactic fermentation using two different bacteria strains. The authors report that acetaldehyde concentrations were in the range of 5−15 ppm, with a decrease in levels over the 12 day fermentation period.

In this work, an analytical method similar to that of Wang et al. (*33*) was developed to monitor acetaldehyde levels in wine using headspace SPME/GC-FID with on-fiber derivatization using PFBHA. The method was then used to follow acetaldehyde levels during the micro-oxygenation of Merlot wines in a 141 L pilot-plant study and a full-scale 2400 L process over a several-month period.

MATERIALS AND METHODS

Micro-oxygenation. Pilot-plant and commercial-scale micro-oxygenation studies were carried out to test the analytical procedure. The pilot study was designed as a proof of concept experiment to test the ability of the analytical technique to monitor acetaldehyde during micro-oxygenation. Both the dimensions of the tank and the oxygen dose rate were chosen for convenience and differ considerably from the parameters used in a typical commercial micro-oxygenation process. A high oxygen dose was chosen to facilitate the generation of acetaldehyde in this study. The commercial-scale micro-oxygenation study was then carried out to test the monitoring technique under conditions typically employed within the wine industry.

In the pilot study a 141 L stainless steel tank was used. The tank is approximately 51 in. high with a diameter of 15 in., tapering to a diameter of 2 in. in the bottom 6 in. of the tank. The tank was filled with 2004 Merlot (Fresno State Winery) and was covered with a tight-fitting plastic lid. Oxygen was introduced into the wine using a micro-oxygenation unit (OENODEV SAR) at a rate of 75.9 mg/month/L of wine. The gas was added through a diffuser suspended approximately 45 in. from the top of the tank. Wine samples were periodically removed by siphoning liquid through a 0.25 in. plastic tube immersed approximately 25 in. into the tank. An initial 100 mL sample was removed and returned through a funnel at the top of the tank to flush the sampling line, and a second 60 mL sample was drawn and retained for analysis. The micro-oxygenated wine samples were analyzed on the same day as collected by GC-FID as described below. A total of 38 samples were collected during the 128 day micro-oxygenation study. A minimum of five replicate analyses were carried out for each aliquot sampled.

The commercial-scale micro-oxygenation was carried out in a stainless steel tank measuring 2.5 ft in diameter and 16 ft tall containing 2400 L of 2004 Merlot wine (Fresno State Winery). Oxygen was introduced through a diffuser at the bottom of the tank using an Oxy Genius Plus micro-oxygenation unit (Parsec) at the rate of 9.3 mg/month/L of wine. Prior to sampling, the contents of the tank were mixed ("rolled") by bubbling nitrogen through the wine for 3 min. Samples were withdrawn for analysis through a sampling valve at the bottom of the tank. Nineteen samples were collected during the 89 day micro-oxygenation process, and each was analyzed for acetaldehyde content at least five times.

Analyte Extraction and Derivatization. Sixty-five-micrometer PDMS−DVB fibers (65 μm; Supelco) were conditioned prior to their first use by heating them to 270 °C for 30 min in a GC inlet. The conditioned fibers were then coated with PFBHA by exposing them to the headspace of 17 mg/mL aqueous PFBHA hydrochloride (>98%, Aldrich) solutions. Twenty milliliters of the PFBHA solution was equilibrated at 30 °C for 30 min in 40 mL glass vials capped with septa. The fibers were exposed to the headspace of the solutions for 10 min to obtain an acceptable fiber loading. The coated fiber was then exposed to the headspace of 20 mL of the sample in a 40 mL vial held at 30 °C for 1 min. Immediately following sample loading, the adsorbed chemicals were analyzed by GC-FID or GC-MS.

Sample Analysis. Chemicals adsorbed to the SPME fibers were characterized and quantified using a Hewlett-Packard 5890/5972 GC-MS and a Hewlett-Packard 5890 GC-FID. Within the GC-FID instrument, analytes were desorbed from the fibers for 2 min in a 0.75 mm internal diameter (i.d.) SPME injector sleeve placed within the injection port at 270 °C. A 30 m, 0.25 mm i.d. SPB-5 column with a 1.0 μm film thickness (Supelco) was used with a helium carrier gas flow rate of 0.7 mL/min. The column was held at 45 °C for 1 min and was then ramped at 10 °C/min to 185 °C. The temperature was then increased to 270 °C at 30 °C/min, and the final temperature was held for 4 min, resulting in a total run time of 21.8 min. The FID gas flow rates of air and hydrogen were 462 and 61.5 mL/min, respectively, and the detector was operated at 300 °C.

The GC-MS inlet temperature and oven temperature program were identical to those of the GC-FID. However, a 30 m, 0.25 mm i.d. DB-5MS with a 0.25 μm film thickness (J&W Scientific) was used with the GC-MS, and the helium carrier gas flow rate was 1.0 mL/min. The mass spectrometer was operated using electron impact ionization (70 eV), and spectra were collected in the scan mode.

Calibration Standards. Acetaldehyde standards were generated from stock solutions of acetaldehyde (99.5%, Fisher Scientific) dissolved in either distilled water or a wine matrix (consisting of 191° proof alcohol obtained from Sunmaid Distillaries) diluted to 12% in distilled water and adjusted to pH 3.6 with tartaric acid. Stock solution concentrations were in the 1000−3000 ppm range. Aqueous and wine standards in the range of 1−100 ppm were made by diluting the stock solutions with the appropriate media. Acetaldehyde standards in commercial 2002 Chardonnay, 2003 Petite Sirah, and 2004 Merlot

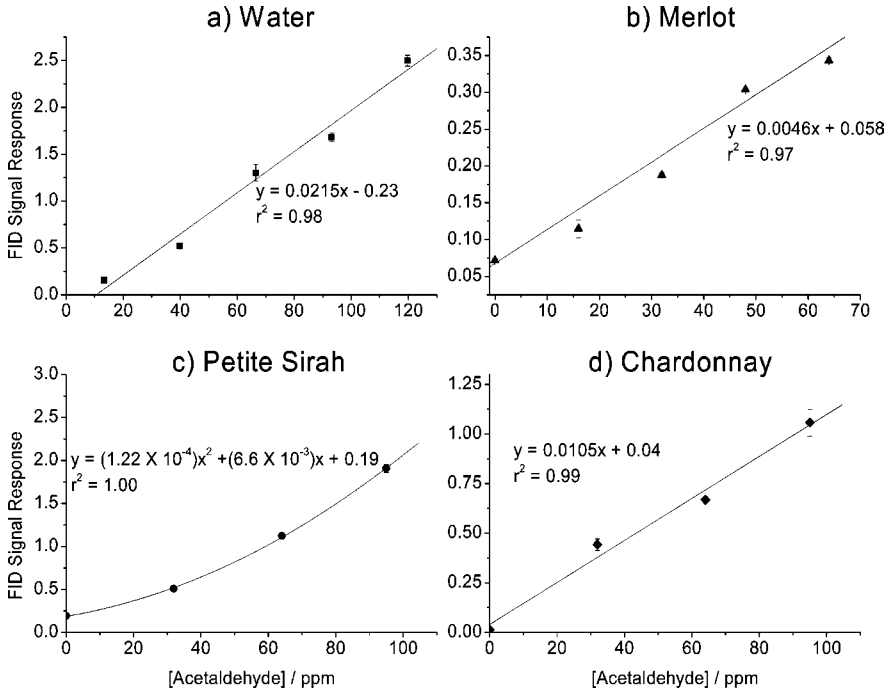

Figure 2. Calibration curve for acetaldehyde in (**a**) water, (**b**) Merlot, (**c**) Petite Sirah, and (**d**) Chardonnay. Error bars represent one standard deviation of the mean.

wines (all obtained from Fresno State Winery) were generated by spiking the appropriate volume of wine matrix stock solution into the wine on the same day that the analyses were performed. Calibration curves were generated using integrated peak areas of the underivatized PFBHA and PFBHA−acetaldehyde derivatives analyzed by GC-FID following headspace SPME extraction as described above.

Method Validation. The limit of detection and limit of quantitation were estimated by determining the concentration corresponding to a signal 3 times and 6 times higher than the average noise in three replicate blank solutions, respectively. The signal reproducibility for each aqueous and wine standard was determined from the 95% confidence limits for a minimum of three sample replicates. Linearity of the signal response was tested by comparing the least-squares fits of linear and quadratic equations to the calibration data.

RESULTS

Initially, fibers coated with the derivatization reagent were exposed to aqueous acetaldehyde solutions and then analyzed by GC-FID. On the basis of these chromatograms, peaks corresponding to PFBHA and the derivatized acetaldehyde were provisionally identified, and the GC temperature program and carrier gas flow rate were optimized to allow adequate separation of the peaks. The product of the reaction between PFBHA and acetaldehyde is an oxime, which may be formed as either the syn- or anti-conformer. The two conformers have slightly different retention times, and so two peaks are attributed to the oximes. With the optimized GC-FID parameters described above, the retention times for PFBHA and the two oximes are 15.2, 15.3, and 15.5 min, respectively. The identities of the peaks were subsequently confirmed by analysis of the mass spectra obtained by GC-MS. The mass spectrum from the peak identified as PFBHA was matched with that of the reference spectrum of this compound from the National Institute of Standards and Technology (NIST) mass spectral library database. The two peaks identified as the oximes were found to have virtually identical fragmentation patterns, indicating that the species have very similar structures. In both spectra the ion with the highest mass/charge ratio occurs at *m*/*z* 239, corresponding to the molecular weight of the compound. Additionally, the most prevalent ion in both mass spectra has a mass/charge ratio of 181, which is characteristic of PFBHA derivatives.

The work carried out in this study was performed prior to the publication of refs *33* and *36*, and so the method described above was developed independently of these studies. A number of experiments were carried out to optimize the exposure time of the SPME fibers to the derivatization reagent and the exposure time of the PFBHA-coated fibers to the samples. The protocol described above was selected as it provided a linear signal response over the desired concentration range and good reproducibility. However, under these experimental conditions, equilibrium has not been reached, and a longer exposure of the PFBHA-coated fiber to the sample results in a higher signal. Because signals from all samples are orders of magnitude higher than the limit of detection, the shorter exposure time was selected to reduce the time taken for the complete analysis.

Calibration data were obtained at an average of five different concentrations in the range of 0−100 ppm acetaldehyde. At least three separate analyses were performed at each concentration. Calibration curves were generated for acetaldehyde solutions in water, 2002 Chardonnay, 2003 Petite Sirah, and 2004 Merlot. In all samples, the sum of the integrated peak areas from the two oxime conformers was used for quantification. Due to variations in fiber coverage of PFBHA from sample to sample, the absolute peak areas of the oximes were not always consistent with the concentration of acetaldehyde in the sample. The ratio of the absolute FID peak area of the oximes to that of the underivatized PFBHA was found to be more reliable for quantifying acetaldehyde in the wine samples, and the signal response is reported here as this ratio. The calibration curves for the samples are shown in **Figure 2**. The FID signal response to changes in acetaldehyde concentration was found to be linear in all of the media except for the Petite Sirah (see below), despite the high ratio of derivatized/underivatized PFBHA at higher acetaldehyde concentrations. The parameters for the linear (or quadratic) least-squares fits of the calibration data for acetaldehyde in the different solvents are given in **Table 1**. Uncertainties in these parameters at the 95% confidence levels are <10%.

Table 1. Calibration Data for Acetaldehyde in Water and Wine Samples in the 0–100 ppm Range

solvent	calibration signal[a,b] (ppm^{-1})	[acetaldehyde] in solvent blank[b] (ppm)
water	0.0215 ± 0.002	
Chardonnay	0.0105 ± 0.0007	1.3 ± 0.2
Petite Sirah	$(6.6 \pm 1) \times 10^{-3} + (2.4 \pm 0.2) \times 10^{-4} \times$ [acetaldehyde]	19.1 ± 1.9[c]
Merlot	0.0046 ± 0.0002	15.5 ± 2.3

[a] Calibration signal is defined as peak area of oximes/peak area of PFBHA per ppm of acetaldehyde present (see text for details). [b] Uncertainties are 95% confidence limits. [c] Estimated from a linear fit to the data (see text for details).

Table 2. Method Validation Data for Repeatability and Linearity (See Text for Details)

solvent	[acetaldehyde] added (ppm)	signal variation (95% confidence limit) (%)	F value	$F_{critical}$ ($P = 0.05$)
water	13.3	9	0.81	4.41
	39.9	6		
	66.5	17		
	93.1	8		
	119.7	5		
Merlot	0	11	0.62	4.74
	16.0	34		
	32.0	5		
	48.0	2		
	64.0	3		
Petite Sirah	0	5	54.5	4.96
	32.0	7		
	64.0	7		
	95.0	8		
Chardonnay	0	16	0.31	4.96
	32.0	20		
	64.0	7		
	95.0	19		

Within the mutual uncertainties, the signal responses for the different solutions are not the same. Merlot is found to have the smallest signal response, with water giving the largest signals. The pure wine samples were found to contain acetaldehyde, and the concentrations present were determined using the appropriate signal response factors. In the case of the Petite Sirah, the quadratic fit to the data does not cross the *x*-axis, and so the reported acetaldehyde level in the wine was estimated from a linear regression. Acetaldehyde levels in the wine samples are given in **Table 1**. Levels within the Chardonnay were 1.3 ppm, whereas levels in the red wines were significantly higher (15.5 and 19.1 ppm in the Merlot and Petite Sirah, respectively). Uncertainties in these values are $\leq 15\%$ at the 95% confidence limit.

Results from the method validation analysis are summarized in **Table 2**. The limit of detection and limit of quantitation using this method are 10 and 20 ppb, respectively. Repeated analysis of the same samples gave statistically indistinguishable signals, indicating that only a small fraction of the acetaldehyde present is removed by the SPME fiber during each analysis. At the 95% confidence level, the signals obtained in all media at virtually all concentrations are reproducible to within 20%. To test the linearity of the calibration data, the *F* test was used to determine if a quadratic function fits the data better than a linear function at the 95% confidence limit. The results of the *F* test are shown in **Table 2**. An *F* value that is lower than $F_{critical}$ indicates that a quadratic equation does not fit the data better than a linear equation at the 95% confidence limit. It can be seen that for the calibrations in water, Chardonnay, and Merlot, a quadratic fit does not lead to a statistically significant improvement in the fit, and so a linear fit to the data is appropriate. However, a quadratic fit to the Petite Sirah calibration data is better than a linear fit at the 95% confidence limit, and so the quadratic equation parameters are reported.

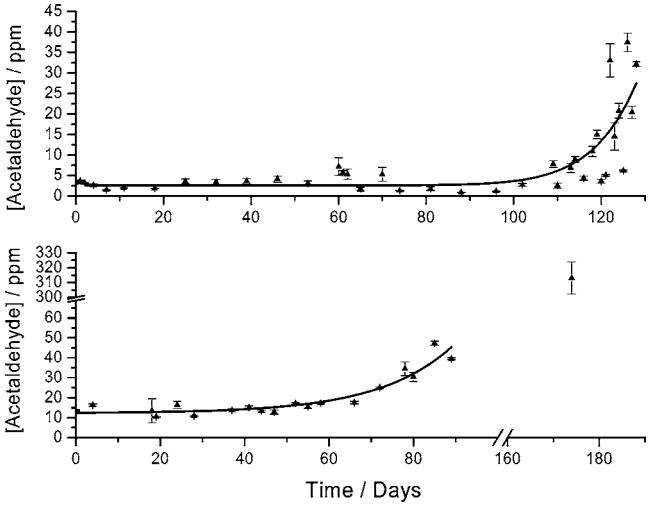

Figure 3. Acetaldehyde concentrations during the micro-oxygenation of 2004 Merlot Wine in (**a**, top) 141 L pilot plant study and (**b**, bottom) 2400 L full-scale study. Error bars represent one standard deviation of the mean from a minimum of five measurements.

Variations in the concentrations of acetaldehyde present during micro-oxygenation of the Merlot wines are shown in **Figure 3**. In the pilot-plant study, the initial acetaldehyde concentration in the wine was 3.3 ppm. Although some sample-to-sample variability was observed in the acetaldehyde concentration during the first 3 months, there was no systematic change in the acetaldehyde levels. After about 100 days, the acetaldehyde concentration began to increase, and the levels were found to double approximately every 10 days. Whereas the acetaldehyde concentrations did systematically increase in this period, large sample-to-sample variations in the measured levels were observed, as is expected with the experimental setup. The micro-oxygenation process was ended on day 128. At this point, the acetaldehyde concentration was about 30 ppm, below the literature values for the sensory threshold.

The results of the full-scale micro-oxygenation study are shown in **Figure 3**. The initial level of acetaldehyde was 13.7 ppm, and the concentration did not begin to increase until about 50 days into the micro-oxygenation process. After this point, the acetaldehyde concentration doubled approximately every 25 days. Some sample-to-sample variation was observed, but it was significantly smaller than that in the pilot study. The micro-oxygenation process was stopped on day 89 because volatile acids were detected within the wine. The acetaldehyde concentration was about 40 ppm, which is below the reported sensory threshold for this species.

DISCUSSION

It is clear from **Table 1** that the signal response for acetaldehyde is significantly different between the four different solvents. This is not unexpected given that variations in the composition of solutions may affect the loading of acetaldehyde on the PFBHA-coated SPME fibers in the headspace. Different chemical environments in solutions alter the activity of the acetaldehyde present. This will affect the equilibrium partial

vapor pressure of acetaldehyde above the solution, and therefore the amount of gas-phase acetaldehyde available for uptake by the SPME fiber will also be altered. Furthermore, wine contains other carbonyl compounds that may "compete" with acetaldehyde for reaction with the PFBHA adsorbed to the SPME fiber. Solutions with high concentrations of these compounds in the headspace are expected to produce a smaller signal response compared to solutions in which these additional carbonyls are not present. A number of additional peaks, possibly corresponding to these derivatized carbonyls, were observed in the chromatograms but were not analyzed. Although the first of these factors could potentially be overcome by "salting-out" acetaldehyde from the solutions, the interference effect from other carbonyls is more difficult to circumvent. This shows the need for acetaldehyde calibrations to be carried out in authentic samples of the wine to be studied rather than in aqueous or wine matrix standard solutions.

In several experiments, samples were spiked with acetaldehyde several days prior to analysis. For the majority of the wine samples, the signal response did not match that expected on the basis of the data reported in **Table 1**. The anomalous data reflect the fact that oxidation processes within the wine are ongoing. Uptake of ambient oxygen may increase the measured levels of acetaldehyde, whereas reaction of the added acetaldehyde with anthocyanins and flavanols and volatilization of acetaldehyde from the samples may lead to lower than expected signals. This confirms that calibrations must be carried out with freshly prepared standards to eliminate artifacts from these processes.

From **Table 1** it is clear that all three wines contained measurable levels of acetaldehyde. Uncertainties at the 95% confidence limit are below 15% in all of the wine samples on the basis of three measurements. The detection limit is 10 ppb. Given that the entire analysis can be performed in about 30 min and could be completely automated with commercially available equipment, the technique may be practical for use in the wine industry. The concentrations found in the Petite Sirah and Merlot were significantly higher than those present within the Chardonnay. The observed differences may be due to variations in phenolic content, storage, handling, and processing conditions, or differences in the amounts of sulfur dioxide added to the wines.

During the pilot-plant micro-oxygenation experiment, relative levels of acetaldehyde were found to vary much more from sample to sample than in the full-scale experiment (**Figure 3**). The key difference between the two experiments is that in the full-scale process, the wine was mixed by bubbling nitrogen gas through it prior to sampling. In the pilot-plant micro-oxygenation process, the wine was not mixed. In both experiments, the wine is not exposed to a constant stream of oxygen, but rather receives periodic doses of oxygen bubbles. Following each dose, a higher level of acetaldehyde is expected in the wine directly exposed to the oxygen. This high localized concentration would then decrease as it mixes with the rest of the wine in the tank. The sample-to-sample variation in the pilot-plant experiment likely reflects the inhomogeneous distribution of acetaldehyde within the tank and highlights the need to mix the wine prior to sampling if the average acetaldehyde concentration is to be measured.

In both micro-oxygenation experiments, the acetaldehyde levels do not begin to increase until several weeks into the process (**Figure 3**). In the reaction mechanism described above, acetaldehyde is an intermediate in the formation of ethyl-bridged oligomers from reactions involving oxygen, catechol derivatives, ethanol, anthocyanins, and flavanols (**Figure 1**). If formation of acetaldehyde from reactions R1−R4 is initially rate limiting, then the acetaldehyde concentration would effectively be independent of the levels of anthocyanins and flavanols. Thus, in the initial stages of micro-oxygenation, the acetaldehyde levels would remain almost constant despite the depletion of anthocyanins and flavanols in the condensation reactions (R5). However, when the concentrations of these species reach a critical threshold, removal of acetaldehyde in reaction R5 would become rate limiting. At this point, the steady-state acetaldehyde concentration would begin to increase as the levels of anthocyanins and flavanols available to react with the acetaldehyde decrease.

As can be seen in **Figure 3**, the change in acetaldehyde levels over the course of micro-oxygenation differs between the pilot-plant and commercial-scale experiments in several respects. The point at which acetaldehyde levels begin to rise is reached approximately 40 days earlier in the full-scale study compared to the pilot-plant study. Additionally, once the acetaldehyde levels begin to rise in the wines, the rate of increase in acetaldehyde levels is not the same in the two experiments. There are several differences between the experimental parameters in the two studies. The studies were designed with very different oxygen flow rates, but there are other factors that would affect acetaldehyde levels in the wine including the dimensions of the tanks and the storage time and conditions for the wine prior to micro-oxygenation. Because of the number of variables involved, we did not attempt to identify the reasons for these different temporal profiles.

On average, the concentrations increased with rates of the order of 1 ppm/day in both micro-oxygenation studies once the levels began to rise. Given this relatively slow increase, sample analysis once per week should be adequate to determine the onset of the increase in acetaldehyde levels. Thus, although some of the detailed features of the chemistry occurring during the micro-oxygenation process remain uncertain, monitoring acetaldehyde levels as described in this study appears to be a practical and effective method for determining the appropriate endpoint for the micro-oxygenation process.

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