Technical Brief

Nondestructive Method of Determining Acetic Acid Spoilage in an Unopened Bottle of Wine

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Nuclear magnetic resonance (NMR) spectroscopy was used to monitor acetic acid levels in intact wine bottles. An NMR probe and a spectrometer capable of examining full intact wine bottles are described and used to reliably measure the acetic acid content in wine down to 0.5 g/L, more than half the accepted limit in normal wine.

Key words: NMR, wine spoilage, acetic acid

Wine is the product of the growth and metabolism of yeasts and bacteria in grape must. It is well known that many yeasts and bacteria survive throughout the winemaking process, from mature grape through vinification to bottle corking [17]. One class of these organisms is *acetobacter*, a bacteria responsible for oxidizing ethyl alcohol into vinegar or acetic acid [4]. Although present in most wines, *acetobacter* does not generate enough acetic acid to spoil wine during bottle storage due to a lack of oxygen [10]. If the wine is stored in an anaerobic environment, conditions ensured by quality corking, then below sensory levels of acetic acid are produced and the quality of the wine is preserved [17]. Unfortunately, the sealing performance of wine corks can degrade with age, and the long-term behavior of low-quality natural corks and synthetic stoppers is not well documented. One consequence of a leaky cork is the admission of oxygen to wine, a triggering of *acetobacter* function, and the production of acetic acid—a process that leads to the spoilage of fine wines.

Current methods for identifying acetic acid in wine are extremely sensitive, detecting roughly 50 µg/L acetic acid [7], even though the accepted spoilage limit of acetic acid in wine is 1.4 g/L [13]. All current strategies for acetic acid detection require the bottle to be violated, a process that destroys the cork, seal, and label, severely devaluing both the wine and bottle [20].

The purpose of this paper is to build upon nuclear magnetic resonance (NMR) studies of wine involving small (<1 mL) samples of wine [1,3,6,8,9,11,12,15,16,18]. Given the success of NMR spectroscopy in the noninvasive and nondestructive examination of vivo problems in living and closed systems [2], it is natural to assume that full, intact bottles of wine can also be explored. Here an NMR probe and spectrometer are developed to detect less than 0.5 g/L amounts of acetic acid in wine. The method relies on the approximately 1 ppm chemical shift difference between the acetic acid methyl group hydrogen nuclei and the ethyl alcohol methyl group hydrogen nuclei [14] (the usage of parts per million, or ppm, does not correspond to a concentration, rather it refers to the energy shift of a proton NMR peak from the standard absorption of rf energy for tetramethylsilane protons at an 85.78 MHz Larmor frequency for a 2.01 T magnetic field). This approach to acetic acid quantitation does not violate the wine bottle, is harmless to the bottle contents, and can be easily extended to the exploration of other vital ingredients and or contaminants in intact wine bottles.

Materials and Methods

All titration experiments were performed on mixtures of deionized water, 200 proof ethyl alcohol obtained from Gold Shield Chemical (Hayward, CA), and 99.7% glacial acetic acid purchased from EM Science (Gibbstown, NJ). These experiments involved charging empty wine bottles with 750 mL of 12.5% (v/v) ethyl alcohol in water and having controlled concentrations of acetic acid between 0.5 and 3.2 g/L. All tested wines and red wine vinegar were either purchased from local markets or obtained as gifts from the University of California (UC) Davis Viticulture and Enology Department.

All NMR experiments were performed at either 9.1 or 2.01 T magnetic fields corresponding to ^{1}H Larmor frequencies of 399.76 MHz and 85.78 MHz, respectively. The high-magnetic-field commercial NMR spectrometer was used to check full-bottle acetic acid concentrations measured on the low-field home-built instrument by investigating 500-µL samples pre-

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pared with similar acetic acid concentrations. A schematic of the full-bottle NMR instrument and probe head is shown in Figure 1. A single-resonance NMR spectrometer delivers rf pulses to a high-power amplifier connected to the NMR probe head mounted inside a 310 mm, room temperature bore superconducting solenoid imaging magnet (Oxford Instruments, Eynsham, UK). The full, intact wine bottle is housed inside the NMR probe head, as shown by the exploded view in Figure 1. The rf coil examines the neck of the wine bottle between the base of the cork and the main body of the bottle. Although there is less sample in this region in comparison to the bottle body and base, it is much easier to establish a homogeneous static magnetic field over the small sample region and ultimately produce narrow, highly resolved NMR lines. After termination of the rf pulse, the sample emits a low μV – mV rf signal that is mixed to audio frequencies and digitized by the NMR spectrometer. Typically, block averages of 10 groups of 100 scans are collected and each group is separately Fourier-transformed to yield NMR spectra. These 10 spectra are then shifted in frequency to remove magnetic field-drift and added to yield the standard NMR spectrum. Substantial improvements in dynamic range can be made by selectively exciting and measuring just the methyl group region of the ^{1}H NMR spectrum between 1 and 2.5 ppm with a low-power 3-ms-long rf pulse and a narrow 200 Hz bandwidth audio filter, respectively [19]. Such operation removes the massive background signal from water at 4.8 ppm.

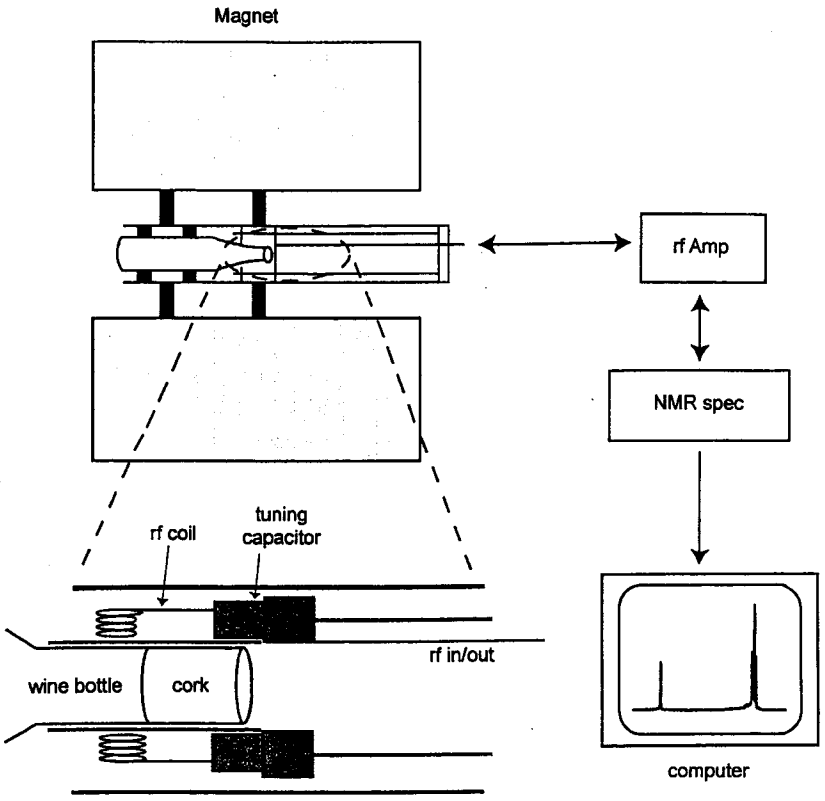

Figure 1 Experimental set-up to obtain NMR spectra of full, intact wine bottles. Exploded view shows the NMR probe head capable of housing an entire bottle of wine.

Results and Discussion

Several different nuclei, including deuterium ^{2}H, oxygen ^{17}O, carbon ^{13}C, and hydrogen ^{1}H, were initially considered as possible candidates for determining acetic acid levels in wine. Of these possibilities, the ^{1}H nucleus was chosen due to its superior sensitivity and the approximately 1 ppm chemical shift difference between the spectrum of acetic acid and the spectra of water and ethyl alcohol, the two major constituents of wine. An example of the ability of ^{1}H NMR to detect acetic acid in wine is shown in Figure 2. The ^{1}H NMR spectrum in Figure 2A obtained at 9.1 T corresponds to a 500-μL sample of the 1997 vintage UC Davis Experimental Vineyard Cabernet Sauvignon. The intense peak at 4.8 ppm is due to water, while the quartet and triplet centered at 3.6 ppm and 1.1 ppm earmark the methylene and methyl groups in ethyl alcohol, respectively. The ^{1}H NMR spectrum in Figure 2B also obtained at 9.1 T corresponds to a sample of red wine vinegar. The new peak at approximately 2 ppm clearly labels the methyl group in acetic acid, and the lack of splittings on this single line is consistent with chemical structure. The amount of acetic acid in the red wine vinegar can also be determined as 2.6% (v/v),

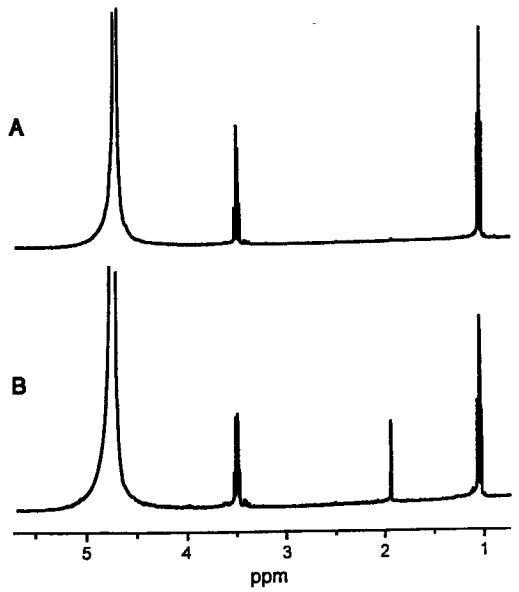

Figure 2 ^{1}H NMR spectra obtained at 9.1 T for 500-μL sample of UC Davis Cabernet Sauvignon (**A**) and homemade red wine vinegar (**B**). Spectra demonstrate the presence of acetic acid by the formation of a peak at 2.1 ppm in **B**.

or 27.6 g/L from the ratio of the methyl group peak heights in Figure 2B, and by assuming that the ethyl alcohol was 12.5% (v/v) of the full bottle volume before acetification. The ^{1}H NMR spectrum in Figure 3A was obtained for a full bottle of the UC Davis Cabernet Sauvignon using the set-up shown in Figure 1 with selective excitation of the methyl group frequencies between 0 and 3 ppm. The triplet in Figure 3A is also shown in the 500-µL sample in Figure 2A at 1.1 ppm and is due to scalar coupling with the protons in the methylene group in ethyl alcohol. The full-bottle ^{1}H NMR spectrum shown in Figure 3B corresponds to a 750-mL mixture of water, 12.5% (v/v) ethyl alcohol, and 0.5% (v/v) acetic acid. The singlet peak centered at 2.1 ppm clearly indicates the presence of acetic acid as expected from comparison to the spectrum obtained for the small volume shown in Figure 2B.

The spectrum in Figure 3B corresponds to an acetic acid concentration of 5.3 g/L, nearly 3.8 times the accepted 1.4 g/L acetic acid spoilage limit for wine. Titration data shown in Figure 4 document an NMR measurement of acetic acid concentration in prepared samples from a ratio of the integrated area of the acetic acid peak at 2.1 ppm to the integrated area of the ethyl alcohol triplet at 1.1 ppm, given the 12.5% (v/v) ethyl alcohol concentration. The triangles in this plot represent one measurement of the acetic acid concentration in a 500-µL sample at 9.1 T, whereas the circles correspond to the average of nine measurements of the acetic acid concentration from full-bottle NMR spectra at 2.01 T. This average represents the separate average of three measurements of acetic acid content in three different standard preparations. The ±1 standard deviation error bars in Figure 4 are therefore a function of both the NMR-based uncertainty and the errors in sample preparation. The dashed line of unit slope is included in Figure 4 to serve as a guide between the exact agreement of prepared and measured concentrations of acetic acid and the experimental data. Both the low-field full bottle and high-field small-sample measurements of acetic acid agree with prepared concentrations, although there is some spread in the data. In the case of the high-field small-sample results, the uncertainty between the prepared and measured concentrations is most likely due to a liquid volume measurement error in the sample preparation, as the extremely narrow ^{1}H NMR line widths (Figure 2) permit reasonable peak intensity assignment by integration. The increased line width in the full-bottle experiment (Figure 3) introduces more error into the measurement of acetic acid concentration, as shown by the error bars in Figure 4. Here it is more difficult to assign start and end points for peak integration. Consequently, errors in both liquid volume measurements during sample preparation and peak intensity determination yield concentration measurements slightly deviating from exact agreement with the dashed line in Figure 4. Better magnetic field shims yielding narrower lines will substantially increase the accuracy of the acetic acid concentration measured in Figure 4. Despite the small disagreement between prepared and measured acetic acid concentrations shown in Figure 4, upon increasing the number of measurements on a single preparation from three to nine, the error bars decrease to ±0.2 g/L, and the full-bottle method is capable of evaluating the amount of wine acetification down to at least 0.5 g/L, more than half the accepted spoilage limit of 1.4 g/L.

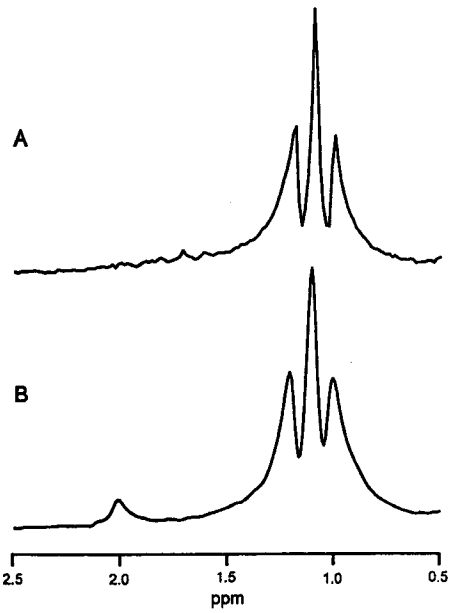

Figure 3 ^{1}H NMR spectra obtained at 2.01 T for a full, intact bottle of UC Davis Cabernet Sauvignon (**A**). Spectrum in **B** corresponds to a full bottle of 12.5% (v/v) ethyl alcohol with an added 0.5% (v/v) of acetic acid.

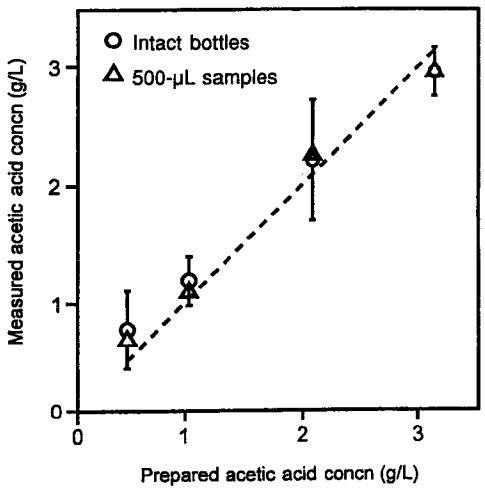

Figure 4 Titration analysis of full, intact wine bottles and 500-µL samples. The full-bottle data is representative of nine separate measurements of the acetic acid concentration. Error bars indicate standard deviation or error in the measurement.

Although most of the full-bottle NMR results reported here are appropriate for violated bottles of wine, it should be emphasized that the apparatus is capable of investigating all common wine bottle shapes, sizes, and corks. All of these factors, including the effects of lead or metallic seals, can be compensated for by carefully adjusting the home-built room tempera-

ture magnetic field shims. Additionally, the lead or metallic seals do not measurably interfere with the probe tuning or the homogeneity and intensity of the rf field across the wine bottle. Although the titration data only documents results down to 0.5 g/L acetic acid (Figure 4), levels down to 0.1 g/L have been measured with the current set-up. It is anticipated that more advanced NMR-solvent suppression techniques [5] and/or a dual-coil NMR probe head will extend this limit by another order of magnitude.

The high-static and rf magnetic fields used in the NMR experiment in no way affect the quality of wines studied here. This observation is consistent with recent findings documenting that magnetic fields do not modify wine quality (A.L. Waterhouse, personal communication, April 2002).

Conclusion

The purpose of this paper was to introduce a noninvasive, nondestructive method of determining the level of wine acetification. It is hoped that this method will become routine in the evaluation of the quality of fine wines and in the study of wine cork aging. This method of full, intact bottle analysis is not just limited to the determination of acetic acid spoilage and content in wines. Rather, it can be extended to the study of other molecular components such as aldehydes and flavonoids that develop as wines age in sealed bottles. It is this more detailed exploration that is reserved for a future publication.

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