Optimization of Headspace Solid-Phase Microextraction for Analysis of Wine Aroma Compounds

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Headspace solid-phase microextraction (SPME) is a fast and simple sampling method for analysis of volatile compounds, but quantitation can be affected by sample matrix and sampling conditions. Sampling time and temperature are particularly important in controlling analyte response, and the effects vary for different compound classes and volatilities. Tests with model solutions containing a range of typical wine volatiles show that increasing temperature and sampling time can increase sensitivity for higher boiling polar compounds but decrease sensitivity for very volatile compounds. Sample matrix elements such as ethanol concentration can also have different effects on the responses of different compounds.

KEY WORDS: aroma, solid-phase microextraction, headspace analyses

Solid-phase microextraction (SPME) is a fast, simple, solvent-free method for analysis of volatile compounds from liquid and solid samples. The technique combines extraction, concentration, and chromatographic injection into one step, dramatically reducing labor, materials, and waste disposal costs. Since its introduction a decade ago [1,21], the technique has rapidly grown in popularity for various applications. Because of the importance of volatile compounds to the aroma and flavor of wines, SPME combined with gas chromatography (GC) or GC/mass spectrometry (GC/MS) can be a significant tool for wine research and quality control analysis.

SPME was first developed as a technique to extract volatile compounds from liquid samples by immersion of a fused silica fiber coated with sorbent material in the sample, followed by desorption of the analytes in the injection port of a GC [1,21]. Further development included moving the fiber into the headspace above the sample in order to enhance mass transfer of analyte into the sorbent [20] and the recognition that the sample matrix greatly affects the extraction [2]. The technique has been used to sample aroma and flavor compounds from coffee and fruit juice [19] and from orange juice [13,16]. Steffen and Pawliszyn [16] found that salt and acid concentrations affected response for orange juice volatiles, and Jia *et al.* [13] determined that increasing the sample temperature caused faster equilibration and lower response and that the sample matrix affected response.

Several groups have studied the use of SPME-GC for analysis of wine volatiles. Reichenbacher and coworkers [5] studied the effects of temperature, time, salt concentration, ethanol concentration, and pH on the response of several terpenes using immersion sampling. In a subsequent paper [6], they compared different fibers for recovery of a variety of compound classes. Later, this same group compared headspace with direct sampling SPME for determination of terpenes and found generally higher responses using the headspace method [7]. They also found that increasing temperature decreased the response of 3-decanol (internal standard) and had a complex effect on terpene response. Vas *et al.* compared immersion and headspace SPME with solvent extraction and found headspace SPME preferable for characterization of a wide range of wine aroma compounds [17]. They also used the technique to differentiate wines from various regions [18]. Headspace SPME has also been used to analyze wine for cork taint [3,9], sulfur compounds [14], methyl isothiocyanate [11], diacetyl [12], and oak lactones [15].

The purpose of the work reported here is to further delineate the effects of sample matrix and sampling conditions on detection of wine aroma and flavor compounds of varying volatilities and functionalities.

Materials and Methods

Model solutions: Standards were obtained from Aldrich Chemical Co. (Milwaukee, WI) except for acetic acid, ethyl acetate, and sodium chloride which were procured from Fisher Scientific (Pittsburgh, PA) and 4-ethylguaiacol which came from Avocado (Ward Hill, MA). A solution containing 100 mg/L acetic acid, 50 mg/L ethyl acetate, 495 µg/L ethyl hexanoate, 196 µg/L hexyl acetate, 520 µg/L ethyl decanoate, 100 mg/L 3-methyl-1-butanol, 10 mg/L 2-phenylethanol, 10 µg/L ß-ionone, 1.0 mg/L 4-ethylphenol, and 1.0 mg/L 4-ethylguaiacol was prepared in distilled water containing 11% (v/v) ethanol and 1 m*M* tartaric acid. The solution pH was 3.1. Spiking experiments were conducted by adding small volumes of 4-ethylphenol and 4-ethylguaiacol standard solutions to samples of Pinot Noir wine which did not contain detectable concentrations of those compounds.

Sampling: A 4-mL sample was pipetted into a 16-mL sample vial; 1.0 g sodium chloride was added along

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with a small magnetic stirring bar, and the vial was capped with a septum. The sodium chloride was added to "salt out" the organic compounds, increasing their partition coefficients [1,2,5,7,16,19,21]. The vial was placed in a thermostatted aluminum block on a stirrer and an SPME fiber with a 65 μm Carbowax™ divinylbenzene coating (Supelco, Bellefonte, PA) was inserted into the headspace for a measured length of time. During the sampling time, the sample was stirred at 35% of full speed. After completion of sampling, the fiber was removed from the sample vial and inserted into the injection port of the GC for 10 minutes. Prior to use, the fiber was conditioned at 250°C for 30 minutes in the injection port.

Gas chromatography/mass spectrometry: The samples were analyzed using a 5890 GC interfaced to a 5972 MS (Hewlett Packard, Palo Alto, CA). The GC was equipped with a 30-m × 0.25-mm DB-WAX column (J & W Scientific, Folsom, CA) with a 0.25-μm coating. The carrier gas was helium at a velocity of 36 cm/sec. The injector temperature was 240°C, and the column was initially held at 40°C for 5 minutes, then programmed at 6°C/minute to 230°C. The injection mode was splitless for 5 minutes. The MS was operated in full scan mode under Autotune conditions with 70 eV electron impact ionization. Chromatographic peak areas for selected ions were calculated by the data system.

Results and Discussion

The test compounds were chosen to represent several major classes of wine aroma and flavor compounds: alcohols, acids, esters, norisoprenoids, and phenolics. An attempt was made to cover ranges of volatility as well as functionality. Terpenes were omitted from this study as they have already been the subject of much work [5,7]. The two phenolic compounds were chosen because they are markers for *Brettanomyces* sp [4]. The model solution matrix of 11% ethanol and tartaric acid at pH 3.1 was chosen to partially mimic the wine matrix without being too complex.

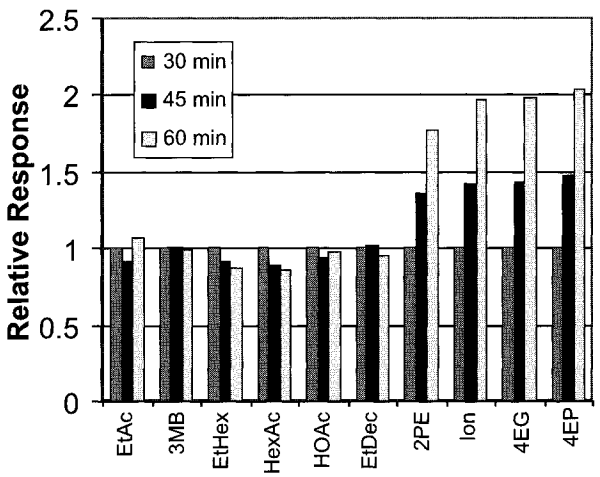

Fig. 1. Relative responses of test compounds at varying sampling times with a sampling temperature of 22°C. EtAc = ethyl acetate, 3MB = 3-methylbutanol, EtHex = ethyl hexanoate, HexAc = hexyl acetate, HOAc = acetic acid, EtDec = ethyl decanoate, 2PE = 2-phenylethanol, Ion = ß-ionone, 4EG = 4-ethyl-guaiacol, 4EP = 4-ethylphenol.

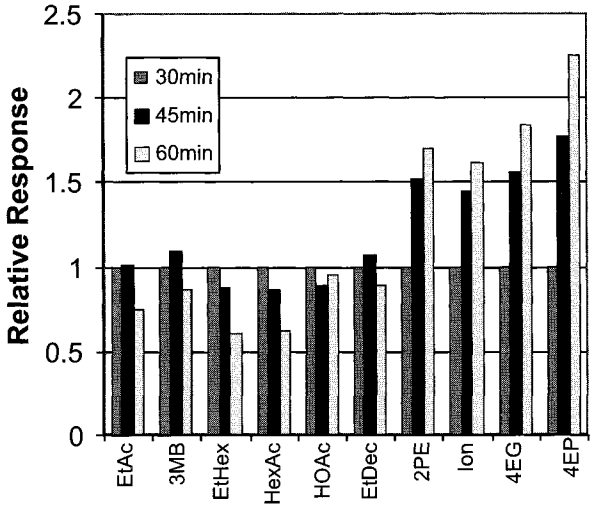

Fig. 2. Relative responses of test compounds at varying sampling times with a sampling temperature of 35°C. EtAc = ethyl acetate, 3MB = 3-methylbutanol, EtHex = ethyl hexanoate, HexAc = hexyl acetate, HOAc = acetic acid, EtDec = ethyl decanoate, 2PE = 2-phenylethanol, Ion = ß-ionone, 4EG = 4-ethyl-guaiacol, 4EP = 4-ethylphenol.

Sampling time: In headspace SPME, the amount of analyte absorbed in the fiber coating increases until equilibrium with the sample is reached. Sampling times shorter than that required to reach equilibrium can be used as long as the sampling time is carefully controlled, but sensitivity may be reduced. Sampling times of 30, 45, and 60 minutes at 22°C are compared in Figure 1. The analytes are listed in order of elution from the GC column. The responses for six of the compounds are little affected by increasing the sampling time, indicating that they have reached equilibrium within 30 minutes. For the last four compounds (2-phenylethanol, ß-ionone, 4-ethylguaiacol, and 4-ethylphenol), representing the less volatile and/or more polar components of the mixture, increasing the sampling time dramatically increases the response, indicating slower equilibration. For those compounds, an increase in sampling time beyond 30 minutes results in a large increase in sensitivity, while for the more volatile compounds there is no advantage to increasing the sampling time. The experiment was repeated at a sampling temperature of 35°C (Fig. 2). At the increased temperature, there was still a strong increase in response with increased sampling time for the last four compounds, but the difference between 45 and 60 minutes was much less, indicating faster equilibration.

Sample temperature: Headspace SPME sampling is controlled by the equilibria between the sample and the headspace and between the headspace and the fiber, and these equilibria are affected by temperature. Zhang, *et al.* [21] pointed out that an increase in sample temperature increases the headspace concentration of volatile and semivolatile compounds, favoring extraction, but that too high a temperature decreases the fiber/headspace partition coefficient, reducing absorp-

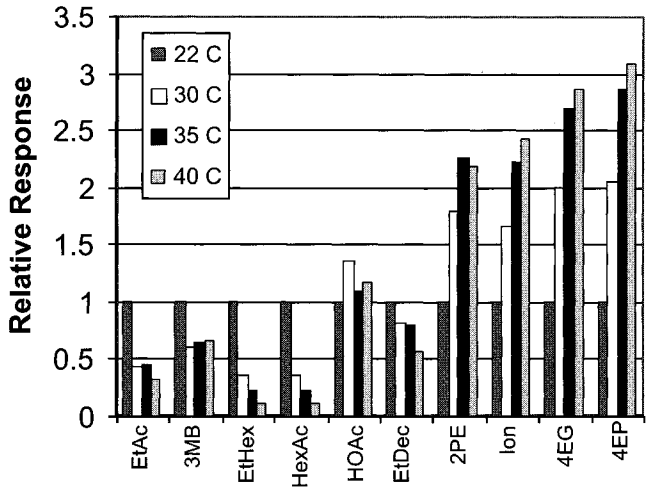

Fig. 3. Relative responses of test compounds at varying sample temperatures with 30 minute sampling. EtAc = ethyl acetate, 3MB = 3-methylbutanol, EtHex = ethyl hexanoate, HexAc = hexyl acetate, HOAc = acetic acid, EtDec = ethyl decanoate, 2PE = 2-phenylethanol, Ion = ß-ionone, 4EG = 4-ethyl-guaiacol, 4EP = 4-ethylphenol.

tion. Zhang and Pawliszyn [20] demonstrated that increasing the sample temperature is helpful in decreasing the equilibration time for higher boiling compounds, but De la Calle Garcia *et al.*De la Calle Garcia [5] found that increasing sample temperature from 25°C to 80°C significantly decreased the response for 3-decanol extracted from wine. For this study, the sample temperature was varied from 22°C to 40°C with a sampling time of 30 minutes. The results are shown in Figure 3. The increase in temperature reduced the response for the more volatile compounds, indicating a shift in fiber/headspace equilibrium. At the same time, the response for the higher boiling polar compounds increased due to increased headspace/solution partition coefficients. This demonstrates that for a complex sample, selection of an optimum sampling temperature will depend strongly on which analytes are of most interest or require the most sensitivity. Another important point made by this data is that the small temperature change from 22°C to 30°C, roughly equivalent to the change from spring to summer in a non-climate-controlled lab, can have a large effect on the results of an SPME analysis. It is, therefore, vital to carefully control the temperature of the sample, independent of room temperature.

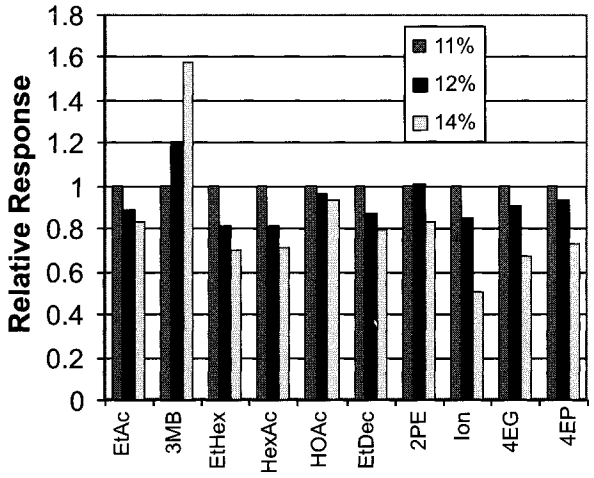

Fig. 4. Relative responses of test compounds at different ethanol concentrations, sampled at 35°C for 45 minutes. EtAc = ethyl acetate, 3MB = 3-methylbutanol, EtHex = ethyl hexanoate, HexAc = hexyl acetate, HOAc = acetic acid, EtDec = ethyl decanoate, 2PE = 2-phenylethanol, Ion = ß-ionone, 4EG = 4-ethyl-guaiacol, 4EP = 4-ethylphenol.

Sample matrix: The effects of sample matrix on immersion SPME sampling of wine have already been noted [5], and we have found that the sample matrix also has an effect on headspace SPME. Figure 4 shows the decrease in response for all of the analytes, except 3-methylbutanol, as the ethanol concentration in the model solution is increased from 11% to 14%. This is not unexpected, since most of the analytes should be more soluble in higher ethanol concentrations, thereby reducing their headspace/sample partition coefficients as demonstrated by Fischer *et al.* [10]. The cause of the anomalous behavior of 3-methylbutanol is not apparent.

We have observed that recovery of 4-ethylphenol and 4-ethylguaiacol spiked into wine is poor when calculated from calibration standards prepared in the model solution matrix. The recoveries at room temperature with 30-minute sampling were 50% (std. dev. = 2.7%, n = 8) for 4-ethylphenol and 49% (std. dev. = 2.9%, n = 6) for 4-ethylguaiacol. Increasing the sample temperature to 35°C and the sampling time to 45 minutes did not improve the recoveries appreciably. Preliminary results also indicate poor recovery of ß-ionone. It is possible that components of the wine other than ethanol and pH affect the extraction of volatiles. Dufour and Bayonove have suggested that wine polyphenols may interact with aroma compounds, reducing vapor pressure in some cases [8]. This sort of interaction would be expected to alter SPME extraction efficiency.

Conclusions

Wine is a very complex matrix containing a wide array of potential target analytes. The various analytes will respond differently to changes in SPME sampling time and temperature, and the differences may be due to both functionality and volatility. It is, therefore, important to focus on the analytes of interest when optimizing sampling conditions, and to remain aware that conditions optimum for one set of compounds will not necessarily be optimum for another set of compounds. This variation in response will also be important in the selection of internal standards for quantitative analysis, as an effective internal standard must closely mimic the behavior of the associated analytes.

In this study, we have found that sampling times of up to 60 minutes and a sample temperature of 35°C can result in increased sensitivity for less volatile analytes, especially polar compounds. For more volatile or non-polar analytes, lower temperatures and shorter sampling times may be optimal. In either case, sampling

time and temperature must be carefully controlled. Variations in temperature in the laboratory can affect SPME results.

Because of the negative relationship between ethanol concentration and most analyte responses, analysts should be aware that variation in sample parameters such as alcohol content may affect comparisons of different wines. It is also necessary for the analyst to be aware of the potential interactions of aroma compounds with other wine components, such as polyphenols, which can vary from one wine sample to another and affect quantitation.

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