Analysis of Cork Taint in Wine and Cork Material at Olfactory Subthreshold Levels by Solid Phase Microextraction

Keywords: *Wine; cork taint; 2,4,6-trichloroanisole; SPME*

INTRODUCTION

Cork from *Quercus suber* has been used as closures for wine bottles since the 17th century because of its unique physical properties, including long-lasting flexibility, hydrophobicity, and gas impermeability. Over the past 20 years the incidence of moldy and musty off-flavors in wines sealed with cork stoppers has increased significantly. 2,4,6-Trichloroanisole (2,4,6-TCA) was first reported to be the primary compound responsible for cork taint by Tanner et al. (1981). Olfactory thresholds for 2,4,6-TCA vary from 4 ng/L (Amon et al., 1989) to 10 ng/L (Tanner et al., 1981) in white wine and to 50 ng/L in red wines (Tanner et al., 1981). On the basis of estimations made by the Australian wine industry 2−5.5% of all Australian wines exhibit a cork taint (Heyes, 1995; Leske, 1995). If this figure is extrapolated worldwide, cork taint is responsible for a total loss of roughly $1 billion per year in the case of wine, not considering the other beverages and foods contaminated by 2,4,6-TCA (Maarse et al., 1988). The biosynthesis and biotransformations leading to 2,4,6-TCA are reviewed elsewhere (Maarse et al., 1988; Lee and Simpson, 1993). The analytical determination of 2,4,6-TCA so far has relied on labor-intensive procedures such as Soxhlet, liquid−liquid, or solid phase extraction and subsequent concentration (Pollnitz et al., 1996), all of which use hazardous solvents. More recent approaches have employed thermal desorption of 2,4,6-TCA from a cork sample introduced into a special injector (Hoffmann and Sponholz, 1994) or dynamic headspace sampling of cork material combined with cyrofocusing (Jäger et al., 1996), which still are time-consuming, difficult to automate, and not suitable for determination of 2,4,6-TCA in wine. Solid phase microextraction (SPME) (Arthur and Pawliszyn, 1990) not only eliminates the use of solvents but also can be automated (Arthur et al., 1992). In addition to the numerous applications of SPME for environmental analysis, it has recently been applied for analysis of monoterpenes by direct extraction from wine (Garcia et al., 1996).

The objective of the present study was to develop a rapid, solvent-free, automatable, and inexpensive SPME method for detection and quantification of 2,4,6-TCA at parts per trillion levels in wine and cork material.

EXPERIMENTAL PROCEDURES

Wine and Cork Material. To develop and optimize the analytical method, a commercial wine (1995, Riesling, Qualitätswein Pfalz, dry, 12% vol ethanol) was spiked with 10 ng/L 2,4,6-TCA (Sigma, Germany). To determine 2,4,6-TCA in authentic wines with a cork taint off-flavor, five commercial wines made of different grape varieties produced in Germany or France were selected.

Isolation of 2,4,6-TCA Using SPME. Wine samples of 5 mL were filled into a 14 mL glass vial, saturated with 2 g/L NaCl, closed with a Teflon-coated septum, and agitated at a constant velocity of 1250 rpm by a magnetic stirrer at 20 °C. Adsorption temperatures from 20 to 60 °C were generated using a temperature-controlled water bath, while adsorption temperatures of 10 °C were generated using a refrigeration chamber. SPME fibers (Supelco, Germany) coated either with 100 μm polydimethylsiloxane or 85 μm polyacrylate were utilized for a 30 min adsorption period in the headspace of the vial, followed by a 15 min desorption period in the GC injector. For quantification from the cork matrix, 100 mg of ground cork material was moistened with 5 mL of demineralized water and filled into 40 mL headspace vials, closed with Teflon-coated septums. The other conditions were kept identical to the isolation made from a wine matrix.

Table 1. Effect of Salt, Agitation, and Fiber Composition on Adsorption of 2,4,6-TCA on a SPME Fiber at 20 °C and 30 min of Equilibration Time (n = 3)

SPME condition	relative detector response, % (CV, %)
no salt	75 (4)
saturated with NaCl	100 (9)
without stirring	34 (3)
stirring [1250 rpm]	100 (5)
polyacrylate fiber	90 (5)
polydimethylsiloxane fiber	100 (5)

Quantitative Analysis. Gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring mode (SIM) utilized a Chrompack CP9000 GC and a Hewlett-Packard 5970 mass selective detector equipped with a 0.32 mm × 30 m fused silica capillary column coated with 0.5 μm cross-linked polyethylene glycol. The column was held at 60 °C for 3 min and then programmed at 5 °C/min to 240 °C, which was held for 1 min. The injector was held at 250 °C and maintained in its splitless mode for 1 min; the ion source was held at 250 °C. Helium was used as a carrier gas at 10 kPa head pressure. The mass fragments for 2,4,6-TCA, m/z 195, 197, and 210, were generated at 70 eV by electron impact. During the development of the method the peak areas of 2,4,6-TCA within one experiment were expressed as percentage of the highest peak area obtained in the specific trial, which was arbitrarily set to 100%. For the determination of 2,4,6-TCA from the headspace of wines with cork taint, 2,4,6-TCA levels were quantified by standard addition method: in a first measurement authentic 2,4,6-TCA levels were determined in the wine headspace, followed by a second measurement of the same wine with an addition of a known 2,4,6-TCA stock solution. The difference in peak area between the two measurements was related to the added amount of 2,4,6-TCA. All measurements were made in triplicate, and reproducibility is expressed as coefficient of variation (CV) or error bars in figures.

RESULTS AND DISCUSSION

Method Optimization. Following the reports of Arthur et al. (1992) the wine samples were saturated with salt to increase the yield of adsorbed analytes (Table 1). Stirring facilitated an increase of liquid surface and turbulence in the liquid and gaseous part of the system, which raised the detector signal overall by a factor of 3 (Table 1). Also, polarity of the SPME fibers was crucial; nonpolar polydimethylsiloxane coating yielded the highest absorption capacity of 2,4,6-TCA (Table 1).

Comparison of adsorption of 2,4,6-TCA to the SPME fiber in the wine matrix against adsorption of 2,4,6-TCA from the headspace above the wine matrix showed adsorption from the headspace gave a 4 times greater yield as adsorption within the wine (Figure 1). Equilibration time for adsorption from the liquid (20 min) was shorter than adsorption from the headspace (45 min)

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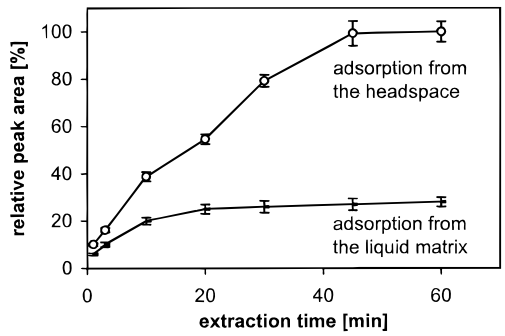

Figure 1. Recovery of 2,4,6-TCA as function of time of equilibrium on a polydimethylsiloxane SPME fiber from the headspace and from the liquid matrix ($n = 3$).

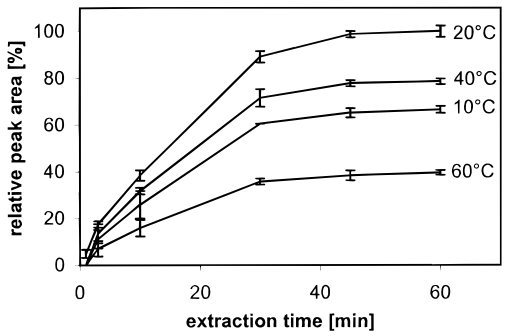

Figure 2. Recovery of 2,4,6-TCA as function of time of equilibrium and temperature on a polydimethylsiloxane SPME fiber ($n = 3$).

because adsorption of 2,4,6-TCA from a liquid follows first-order kinectics, while adsorption from the headspace is ruled by second-order kinetics, where volatilization is the rate-limiting step (Fischer et al., 1997). Besides lower sensitivity, submersion of the SPME fiber into the wine matrix contaminates the GC injector with nonvolatile wine constituents adsorbed to the fiber and decreases the lifetime of the fiber and GC column.

Although the pH in matrices can affect adsorption to SPME fibers (Pan, 1995) and terpenes were best adsorbed from wine acidified to pH 2 (Garcia et al., 1996), the potential increase in sensitivity due to acidification was omitted to keep the method as simple as possible. It has been demonstrated that increasing ethanol will generally diminish partition coefficients of aroma compounds in wine (Fischer et al., 1997) and will slightly decrease sensitivity using SPME (Garcia et al., 1996). However, no dilution of samples was done to lower ethanol content, because consequent dilution of analytes will always be more detrimental than the benefit of increased partition coefficients.

Varying the temperature from 10 to 60 °C revealed a clear optimum for the adsorption of 2,4,6-TCA at 20 °C (Figure 2) and the recovery decreased at higher temperatures, probably because of increased desorption of 2,4,6-TCA from the SPME fiber at higher temperatures. Parallel to our results, Picque et al. (1995) reported for a wide range of chemicals decreasing detector signals at increasing temperatures. To diminish the desorption of analytes from the adsorbents, Popp (1996) suggested a separate cooling of the SPME fiber only while higher temperatures were maintained in the vial to accelerate volatilization from the liquid matrix in the headspace. For the direct adsorption of monoterpenes from a wine matrix, Garcia et al. (1996) reported a linear increase of adsorptivity with increasing temperature.

The last parameter of consideration is adsorption time. Two equilibria govern the curve displayed in Figure 2: first, the partitioning between liquid and gaseous phases and, second, the partitioning between gaseous and adsorbents phases coating the SPME fiber. To reach complete equilibrium at 20 °C, an extraction period of 60 min is required (Figure 2). Since analysis time is of interest as well, adsorption time was reduced to a level at which the limit of detection (LOD), which is defined by a signal to noise ratio of 3/1, is still below the olfactory thresholds for 2,4,6-TCA, ranging from 4 to 10 ng/L in a wine matrix (Tanner et al., 1981; Amon et al., 1989). These objectives were reached at an adsorption period of 30 min, at which the LOD of 2,4,6-TCA was 2.9 ng/L in wine. After 30 min of sample adsorption, the SPME fiber is inserted for 15 min into the GC injector for thermal desorption of the sample and reconditioning. While the GC program needs a further 30 min to finish the analysis, the SPME fiber is available to adsorb the next sample within the same 30 min period. Due to equal time requirements, the determination of 2,4,6-TCA with SPME fibers could be easily automated by using a specific autoanalyzer for SPME fibers (Varian, Germany).

Authentic Wines and Their Cork Stoppers. The high sensitivity of the developed method is demonstrated by the fact that in case of the 1993 Ruländer wine (Table 2) 2,4,6-TCA was quantified, although the wine had no cork taint. However, the wine were described by a lack of fruit character. The CV for the determination of 2,4,6-TCA from the headspace of wine varied within a range of 3−8%.

In the next step, the amount of 2,4,6-TCA was investigated in the respective cork stoppers, from which the off-flavor migrates over time into the wine matrix (Diekmann, 1997). Using ground cork material as reported elsewhere (Diekmann, 1997) accelerates the migration of chlorophenols into the liquid matrix and can be considered as the worst possible case: if there is no 2,4,6-TCA in the ground cork and microbiological synthesis is ruled out, no potential for cork taint exists in this particular cork material. The CV for the determination of 2,4,6-TCA from moistened ground cork material was in the range of 2−11%. A reliable quantification of 2,4,6-TCA concentration in cork material is not feasible yet: repeated measurements of 2,4,6-TCA from the headspace over the moistened cork material in the same sample revealed very slow and long-lasting extraction kinetics, presumably due to a very low partition coefficient between the hydrophobic cork matrix and the polar water matrix. Added external or

Table 2. Concentration and CV of 2,4,6-TCA in Commercial Wines with Authentic Cork Taints and Peak Area of 2,4,6-TCA Fragments in Their Respective Cork Stoppers ($n = 3$)

investigated wines	olfactory description	concn of 2,4,6-TCA, ng/L (CV, %)	peak area of 2,4,6-TCA in cork stopper (CV, %)
1994 Riesling, Alsace, France	moldy, cork taint	18.6 (5)	
1994 Muskateller, Pfalz, Germany	moldy, cork taint	20.0 (8)	41 695 (3)
1995 Pinot noir (rosé), Pfalz, Germany	moldy, very strong cork taint	551.8 (8)	14 006 955 (4)
1995 Pinot noir (red wine), Pfalz, Germany	moldy, musty	35.5 (3)	184 441 (11)
1993 Ruländer, Pfalz, Germany	no cork taint, low fruit intensity	4.9 (3)	27 066 (2)

internal standards would be mainly dissolved in the water and not in the cork matrix and would fail to mimic the true extraction of 2,4,6-TCA from the cork material. The fact that the peak area of 2,4,6-TCA obtained from a cork stopper was up to 20 times higher than the peak area found in the respective wine reveals that measuring 2,4,6-TCA from ground cork material is by far more sensitive to the detection of 2,4,6-TCA in the system of a corked bottle than it is to the measurement of it directly in the wine. The high coefficient of determination ($r^2 = 0.999$) between the concentration of 2,4,6-TCA in commerical wines and the peak areas detected in their respective cork stoppers allows us to draw conclusions from the 2,4,6-TCA content found in cork to the potential concentration in the wine. Since the total weight of cork stoppers deviates from 3 to 6 g and storage conditions (time, temperature, agitation, etc.) differ among individual wine bottles, future research has the objective to establish a reliable prediction system for the migration of chlorophenols from the cork stoppers into the wine matrix by use of the developed SPME methodology.

CONCLUSION

The described use of SPME fibers to extract 2,4,6-TCA from the headspace over an agitated wine and moistened cork matrix is a short, inexpensive, and solvent-free method to determine 2,4,6-TCA. Due to the efficient adsorption properties of the polydimethylsiloxane phase coated SPME fibers and the high sensitivity of GC/MS, the limit of detection of 2.9 ng/L 2,4,6-TCA is low enough to detect the commercially most detrimental off-flavor in wine below its threshold range of 4−50 ng/L. Submersion of SPME fibers into the wine or cork matrix were inferior in sensitivity and would increase contamination of the injector system and shorten the lifetime of the SPME fiber and analytical GC column. The new method is suitable not only for the analysis of contaminated wines but also for the direct detection of 2,4,6-TCA in cork material. Further, it is possible to automate the procedure of extracting 2,4,6-TCA from the various matrices by SPME because sample preparation, desorption, and reconditioning of the fiber requires the same time as GC analysis. Combining all of these assets together, this method could be of great importance for the cork industry to check raw materials and commercial end products for their potential to introduce cork taints into bottled wines.

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