Screening for Volatile Fatty Acids in Agricultural Air Using Solid Phase Microextraction and Gas Chromatography – Mass Spectrometry

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Abstract. Volatile fatty acids (VFAs) are a major component of odorous gases associated with agricultural sources. Because of typically low VFA air concentrations, conventional air sampling methods including sorbent tubes and vacuum canisters are often not sensitive enough to detect them. Solid phase microextraction (SPME) was used in this research because it is very sensitive, reusable, fast, and combines sampling and sample preparation, allowing for rapid detection of low concentrations of organics in air. This research focused on 7 VFAs: acetic, propionic, isobutyric, butyric, isovaleric, valeric, and hexanoic acids. Field experiments were conducted to test this method for air sampling of beef cattle feedyard, horse manure compost, and a swine operation in Texas. Rapid SPME samples were collected by exposing DVB/Carboxen/PDMS 50/30 µm SPME fibers to air for 1 to 5 min. Sample preservation was accomplished by plugging of fiber assemblies with simple PTFE caps for transport to the laboratory for analysis on gas chromatograph-mass spectrometer (GC-MS). SPME DVB/Carboxen/PDMS 50/30 µm coating was very effective in detecting target VFAs and other gaseous components of agricultural odors. This coating was also very efficient in retaining major fraction of target VFAs.

Keywords. solid phase microextraction, SPME, volatile fatty acids, VFAs, carboxylic acids, air sampling, volatile organic compounds, VOCs, GC-MS, odor, feedyard, swine housing, compost
Introduction

Volatile fatty acids (VFAs) are a major component of odorous gases associated with agricultural sources. Many researchers have accepted VFAs as the major odor contributor among the various volatiles emitted from manure (Zhu, 1999; Wiles et al., 2000). In fact, many researchers believe that C2 to C9 VFAs are the most important odor indicators compared to all other volatile organic compounds (VOCs) found in agricultural air (Zhu, 1999; Wiles et al., 2000). Of all the compounds produced in swine manure containing only C, H, and O, VFAs are the most abundant (Zhu, 1999; Yu and Fang, 1999; Martensson et al., 1999). Development and assessment of odor abatement strategies from agricultural sources should rely on accurate air sampling and analysis methods for VFAs.

Current methods for the measurement of VFAs in air are often inadequate for very low concentrations. Individually they have been found in concentrations ranging from 0.0000015 to 6.7 mg/m$^3$ in air above hog manure (O'Neill and Phillips, 1992). The use of charcoal tubes for the analysis of VFAs can be impractical in some cases due to the long sampling time required and relatively low sensitivity of the method. Summa canisters for whole air sampling can suffer from incomplete analyte recovery. Thus, there is a need for improved methods for sampling VFAs that is fast, sensitive, and cost effective.

The primary objective was to develop an air sampling method for screening of VFAs from various agricultural sources using solid phase microextraction (SPME) and gas chromatography mass spectrometry (GC-MS). A secondary objective was to test the method with typical agricultural sources of odorous gases.

This research project focused on 7 VFAs: acetic, propionic, isobutyric, butyric, isovaleric, valeric, and hexanoic acids. A separation and detection method for GC-MS was developed for target VFAs. The selection of fiber coating sampling retention was also investigated. Field samples were collected to test the feasibility of using SPME to sample for VFAs in ambient air. Sampling sites included: horse manure compost, beef cattle feedyard, and a hog operation. Field sampling involved collecting onsite preconcentration of air samples with SPME fibers for later analysis in the laboratory with GC-MS. Concentrations of VFAs in ambient air were not determined. However, this research can be used towards the development of a SPME-based method for quantification of VFAs in air.

METHODOLOGY

SOLID PHASE MICROEXTRACTION

SPME is a solvent-free sampling and sample preparation method that utilizes a chemically active, polymeric sorbent bonded to a fused silica fiber (Fig 1.). The advantages of SPME over conventional methods are the elimination of toxic chemicals to extract analytes from samples, decreased sampling and sample preparation time, and increased sensitivity. Molecules are pre-concentrated via partitioning to the fiber coating (Fig 2.). It can be used for the analysis of many different matrices including air and water. SPME is a very sensitive sampling method that preconcentrates and introduces the entire, non-diluted sample for analysis into a GC.
Figure 1 - Schematic of SPME device. The expanded view shows the simple design of a manual SPME assembly. When the screw hub is depressed, the fiber is sampling outside the protective needle. The spring helps in withdrawing the fiber inside the needle.

Figure 2 – Schematic of SPME sampling via partitioning to the coating.

**STANDARD GAS GENERATION**

Standard gases were used in this research to calibrate the SPME and GC-MS. Standard gas concentrations of volatile organic acids were made using a Model 491B standard gas generator (Kin-Tek, LaMarque, TX). This system produced standard gas mixtures using permeation tubes.
for each VFA. Permeation tubes were made with short sections of plugged ¼” (6mm) PTFE tubing and filled with pure acid. Analyte diffused through the PTFE tubing wall at a constant rate to ultra high purity nitrogen carrier gas. Standard gas concentrations were changed by adjusting the dilution flow. The permeation rate and resulting gas concentration was determined based on periodically weighing of acid loss from each tube and measurements of actual gas flow rate.

The standard gas generator outlet was connected with ¼” stainless steel tubing to a 1L glass, flow through sampling bulb (Supelco, Bellefonte, PA). The bulb contained a Thermogreen LB-1 half-hole septum (Supelco, Bellefonte, PA) for insertion and sampling with SPME. A circulating heating/cooling bath (New Brunswick Scientific Co., Edison, NJ) controlled the temperature of the bulb at 30.5 °C. The outlet of the sampling bulb was vented to a fume hood.

**SPME Fiber Selection**

Six SPME fiber coatings were evaluated for their efficiency for collecting 7 target VFAs from a standard gas mixture (Fig. 3) using 1 min sampling time. The Carboxen/PDMS 75 µm fiber collected the most mass of acetic, propionic, isobutyric, and butyric acids normalized to their respective concentrations. The DVB/Carboxen/PDMS 50/30 µm fiber coating collected the most mass for isovaleric, and valeric acids. PDMS/DVB 65 µm coating collected the most mass for hexanoic acid only. In Fig. 3 relative standard deviations (RSDs) for triplicate samples ranged from 1 % to 17% and are represented by the error bars. The DVB/Carboxen/PDMS 50/30 µm coating that was selected for field sampling was very efficient in extracting all 7 target VFAs.

![Figure 3 – Comparison of SPME fiber coating sensitivity for 7 target VFAs based on 1 min sampling from standard gas.](image)

**Selection of Sampling Time**

Selecting the proper sampling time affects both method detection limits and quantification. Typically, sampling time selection is driven by site-specific quantification requirements and the sensitivity of detectors. Adsorptive fibers such as the DVB/Carboxen/PDMS 50/30 µm coating load VOCs very efficiently and require a much shorter sampling time for low molecular weight gases compared with high molecular weight analytes. One to five min sampling time was
selected based on the extractions of standard VFA gases with the DVB/Carboxen/PDMS 50/30 µm coating and preliminary field sampling.

**Sample Preservation**

SPME samples were collected for 1 min from the ambient air surrounding feedlots, swine facilities, and composting sites (5min). Fibers were capped with PTFE plugs and sealed in vials with PTFE lined screw top lids for transportation to the laboratory for analysis with GC-MS (Koziel and Pawliszyn, 2001) (Fig 4.).

A sample preservation experiment was conducted to determine the fraction of retained sample on SPME coating that was used for field sampling. One minute triplicates were taken with the DVB/Carboxen/PDMS 50/30 µm SPME coatings from standard gas ranging in concentration from 13.4 ppmv for acetic acid to 0.19 ppmv for hexanoic acid. The fiber assemblies were capped with PTFE plugs, placed in glass vials with PTFE lined screw cap, and stored at room temperature (average 24°C) for time intervals of 1, 2, 4, 8, 24, and 120 h (Fig. 5). At least 82 % of extracted mass remained on the fibers after as long as 5 days at room temperature. Relative standard deviations ranged from a maximum of 17 % for acetic acid and a minimum of 2 % for valeric acid. All field air samples were analyzed within 8 h after collection.

**Sample Analysis**

Samples were analyzed on a Varian CP-3800 GC coupled to a Saturn 2000 ion trap mass spectrometer (Varian, Walnut Creek, CA). The column was a CP-WAX (25 m × 0.25mm × 1.2 µm film thickness) also purchased from Varian. The initial oven temperature was 60 °C. Oven temperature was increased to 210 °C with a ramp of 10°C/min and no hold time, followed by a ramp 60 °C/min to 250 °C for a 3 min hold time to purge the column of any interferences. The injector was held in the splitless mode and isothermal at 250 °C for the entire run.

The ion trap was programmed to a mass window from 35 to 200 m/z. The emission current was 10 µamps. The trap, manifold, and transfer line temperatures were 200 °C, 120 °, and 170 °C, respectively. A scan time of 0.44 seconds/scan was used to increase the number of data points per peak and increase chromatogram resolution.
RESULTS

**Beef Cattle Feedlot**

Feedlot air was sampled using SPME with the DVB/Carboxen/PDMS 50/30 µm coating and 1 min sampling. A chromatogram of air above the feedlot surface near fresh manure is presented in Figure 6. Acetic, propionic, isobutyric acids and 5 other gases were identified (Table 1). Compounds were identified with spectral matches within the MS library (NIST, Gaithersburg, MD). VFAs were identified by matching to mass spectral libraries developed in our laboratory and GC column retention times obtained through SPME injections of small masses of pure analyte on the GC-MS. The other compounds were identified with spectral matches within the MS library.

Table 1 - Summary of identified compounds found in feedlot air with SPME.

<table>
<thead>
<tr>
<th>Retention Time (min)</th>
<th>Compound Name</th>
<th>Fit</th>
<th>Reverse Fit</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.41</td>
<td>1-Propene-1-thiol</td>
<td>883</td>
<td>538</td>
<td>488</td>
</tr>
<tr>
<td>4.23</td>
<td>3,6-Dimethoxy-1,6-dimethyl-1,4-cyclohexa</td>
<td>837</td>
<td>358</td>
<td>338</td>
</tr>
<tr>
<td>6.43</td>
<td>Thiazole, 2-(phenylthio)-</td>
<td>822</td>
<td>188</td>
<td>167</td>
</tr>
<tr>
<td>6.55</td>
<td>Acetic acid</td>
<td>993</td>
<td>564</td>
<td>564</td>
</tr>
<tr>
<td>6.89</td>
<td>2-Butanone, 3-methoxy-3-methyl-</td>
<td>848</td>
<td>423</td>
<td>413</td>
</tr>
<tr>
<td>7.51</td>
<td>Propionic acid</td>
<td>972</td>
<td>567</td>
<td>562</td>
</tr>
<tr>
<td>7.84</td>
<td>Isobutyric acid</td>
<td>942</td>
<td>739</td>
<td>711</td>
</tr>
<tr>
<td>8.10</td>
<td>1-Acetyl-3-(4-pyridyl)-pyrazoline</td>
<td>857</td>
<td>116</td>
<td>113</td>
</tr>
</tbody>
</table>
1. Acetic
2. Propanoic
3. Isobutyric

Figure 6 - Chromatogram from an air sample collected with the DVB/Carboxen/PDMS 50/30 µm fiber 0.1 m above a beef cattle feedlot surface in an alley between pens.

**Hog Farms**

Chromatogram of 1 min air sample collected near exhaust fan with DVB/Carboxen/PDMS 50/30 µm SPME fiber coating is presented in Figure 7. Isobutyric, butyric, and valeric acids and 6 other gases were identified (Table 2).

Figure 7 - Chromatogram of hog house air collected approximately 0.1 m from exhaust fan using 1 min sampling time with DVB/Carboxen/PDMS 50/30 µm SPME coating. (M Counts = millions of area counts. Unnumbered peaks represent unidentified compounds.)
Table 2 - Summary of identified compounds in air from a hog house exhaust fan.

<table>
<thead>
<tr>
<th>Peak Order</th>
<th>RT (min)</th>
<th>Peak Name</th>
<th>Fit</th>
<th>Rev. Fit</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.53</td>
<td>Hexane-2,2,3,4,5,5-hexamethyl-(meso)-</td>
<td>786</td>
<td>479</td>
<td>380</td>
</tr>
<tr>
<td>2</td>
<td>3.50</td>
<td>1-Cyclohexen-1-ol acetate</td>
<td>921</td>
<td>225</td>
<td>225</td>
</tr>
<tr>
<td>3</td>
<td>3.76</td>
<td>3,3-Dimethyl-5-(2,2-dimethylpropyl)terti</td>
<td>857</td>
<td>379</td>
<td>370</td>
</tr>
<tr>
<td>4</td>
<td>4.07</td>
<td>3-Hydroxy-4-methylbenzaldehyde</td>
<td>817</td>
<td>449</td>
<td>413</td>
</tr>
<tr>
<td>5</td>
<td>4.91</td>
<td>Benzene 1,2,4-trimethyl</td>
<td>923</td>
<td>946</td>
<td>902</td>
</tr>
<tr>
<td>6</td>
<td>6.65</td>
<td>1-Decene 4-methyl-</td>
<td>846</td>
<td>756</td>
<td>742</td>
</tr>
<tr>
<td>7</td>
<td>7.42</td>
<td>Isobutyric acid</td>
<td>408</td>
<td>288</td>
<td>139</td>
</tr>
<tr>
<td>8</td>
<td>8.37</td>
<td>Butyric acid</td>
<td>649</td>
<td>253</td>
<td>187</td>
</tr>
<tr>
<td>9</td>
<td>9.52</td>
<td>Valeric acid</td>
<td>238</td>
<td>169</td>
<td>158</td>
</tr>
</tbody>
</table>

Finding of butyric and valeric acids is in agreement with the findings of Zahn et al. (1997) from an analysis of airborne VOCs collected with Tenax TA/Carbon 569 desorption tubes from air downwind of a swine slurry storage basin.

**Horse Compost**

Air above horse manure compost piles differing in age were sampled with DVB/Carboxen PDMS 50/30 µm SPME fiber coating for 5 min. Chromatogram of a relatively fresh manure pile is presented in Figure 8. Acetic, propionic, butyric, isobutyric, valeric, and hexanoic acids were identified. VFA intensities were higher in new compost than in the old compost. Preliminary data suggests that the concentration of VFAs in compost air decreases with the age of the manure. Suggesting that the screening of VFAs could possibly be used to test the maturity of compost. Identified compounds in horse compost air are listed in Table 3.

![Figure 8 - Typical chromatogram of horse manure compost gases collected with SPME DVB/Carboxen/PDMS 50/30 µm fiber coating for 5 min.](image)
Table 3 - Summary of identified compounds found in horse manure compost air with SPME-GC-MS.

<table>
<thead>
<tr>
<th>Peak Order</th>
<th>Retention time (min)</th>
<th>Peak Area</th>
<th>Peak Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.54</td>
<td>1,080,574</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>2</td>
<td>7.52</td>
<td>43,292</td>
<td>Propionic acid</td>
</tr>
<tr>
<td>3</td>
<td>7.81</td>
<td>150,823</td>
<td>Isobutyric acid</td>
</tr>
<tr>
<td>4</td>
<td>8.54</td>
<td>499,419</td>
<td>Butyric acid</td>
</tr>
<tr>
<td>5</td>
<td>9.15</td>
<td>228,957</td>
<td>Valeric acid</td>
</tr>
<tr>
<td>6</td>
<td>9.79</td>
<td>848,099</td>
<td>Hexanoic acid</td>
</tr>
</tbody>
</table>

**Conclusion**

Solid phase microextraction DVB/Carboxen/PDMS 50/30 µm fiber coating and GC-MS was used to screen and analyze volatile fatty acids in ambient air from cattle feedyard, swine farm, and horse manure compost. The target VFAs included acetic, propionic, isobutyric, butyric, isovaleric, and hexanoic acids. Short sampling times ranging from 1 to 5 min were sufficient to collect enough mass on SPME coating and subsequent detection on GC-MS. Sample preservation was accomplished by plugging of fiber assemblies with PTFE caps for transport to the laboratory for analysis on GC-MS. Gases identified in feedyard air and swine houses are consistent with those listed in the literature. Adsorptive SPME coating including the DVB/Carboxen/PDMS 50/30 µm, PDMS/DVB 65 µm, and Carboxen/PDMS µm were generally very efficient in extracting VFAs from air. The DVB/Carboxen/PDMS 50/30 µm fiber coating was also very efficient in retaining target VFAs. At least 82% of extracted mass was retained on fiber after 5 days of preservation at room temperature. SPME could provide a viable alternative to conventional air sampling methods. This research could be used to develop a quantification method for VFAs in air based on SPME.

**REFERENCES**


Yu, Q. and Fang, P. 1999. Production of Volatile Fatty Acids and Alcohols from Dairy Wastewater Under Thermophilic Conditions, Center for Environmental Engineering Research, Department of Civil Engineering, The University of Hong Kong, Hong Kong.


**Safety Emphasis**

All work with liquid and gas VFAs were done under a fume hood. Excess standard VFA gases were vented through a fume hood.