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**Characterization and Quantification of Livestock
Odorants using Sorbent Tube Sampling and Thermal
Desorption coupled with Multidimensional Gas
Chromatography–Mass Spectrometry–Olfactometry
(TD-MDGC-MS-O)**

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Abstract. *Characterization and quantification of livestock odorants is one of the most challenging analytical tasks because odor-causing gases are very reactive, polar and often present at very low*

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concentrations in a complex matrix of less important or irrelevant gases. The objectives of this research is to develop a novel analytical method for characterization of the livestock odorants including their odor character, odor intensity, and hedonic tone and further quantitative analysis of the key odorants responsible for livestock odor emissions. Sorbent tubes packed with Tenax TA were employed for sampling. The automated one-step thermal desorption coupled with multidimensional gas chromatography-mass spectrometry-olfactometry system was developed for simultaneous chemical and odor analysis. Fifteen odorants identified from different livestock species operations are quantified. In addition, odor character, odor intensity and hedonic tone associated with each of the target compounds are also analyzed. The method developed in this research is being used on a multistate, multispecies project focused on quantifying odor and chemical analysis of odor.

Keywords. Odor, VOCs, air sampling, livestock, emissions, thermal desorption, GC-O, mass spectrometry

Introduction

Odor emissions from livestock facilities affect air quality in surrounding communities. Many volatile organic compounds (VOCs) have been identified, including acids, alcohols, aldehydes, amines, volatile fatty acids (VFAs), hydrocarbons, ketones, indoles, phenols, nitrogen-containing compounds, sulfur-containing compounds, and others (Lo et al, 2008; Schiffman et al, 2001). Compounds contributing to the livestock odor have been identified, such as VFAs, p-cresol, phenol, 4-ethylphenol, indole, skatole, and sulfur-containing compounds (Koziel et al, 2006; Bulliner et al, 2006; Cai et al, 2006; Keener et al, 2002; Oehrl et al, 2001).

Livestock odor can be measured using dynamic forced-choice olfactometry, which relies on air sample collection in bags for subsequent evaluation with panelists. This method allows for quantification of the overall odor. However, it does not allow for identification of individual odorous compounds that might be significant to the overall odor controlling.

Gas chromatography (GC)-mass spectrometry (MS)-olfactometry offers the advantages of combining sensory assessment with the identification and quantification of compounds. Some researchers have reported using this method for identification of odorous compounds from swine facilities (Koziel et al, 2006; Bulliner et al, 2006; Cai et al, 2006; Keener et al, 2002). Rabaud et al (2002) used thermal desorption-GC-olfactometry/MS to identify and quantify odor compounds from a dairy. However, relatively few references exist on the relationship between livestock VOC concentrations and the odor character (Zahn et al, 2001a and 2001b; Greenman et al, 2005).

The focus of this research is to develop an odor characterization method for specific livestock odorants including their odor character, odor intensity, and hedonic tone and develop quantitative analysis method for the key odorous compounds responsible for livestock odor emissions using TD-MDGC-MS-O system.

Experimental

Thermal Desorption- Multidimensional GC-MS-Olfactometry (TD-MDGC-MS-O) system

Simultaneous chemical and sensory analyses of livestock odorants were completed using the thermal desorption- multidimensional GC-MS-olfactometry (TD-MDGC-MS-O) system. The thermal desorption (TD) system is using a Model 3200 automated thermal desorption inlet for Agilent 6890 GC developed by Microanalytics based on a PAL® autosampler. The unique design of the Model 3200 system allows it to utilize a single-step desorption and sample introduction method that eliminates cryotrapping. This design allows the Model 3200 to desorb samples directly into the column interface, eliminating many of the problems associated with dual or two-step desorption such as those associated with the presence of trapped water in sorbent tubes.

Multidimensional GC-MS-O (from Microanalytics, Round Rock, TX, USA) was used for all air samples analyses. The system integrates GC-O with conventional GC-MS (Agilent 6890N GC/5973 MS from Agilent, Wilmington, DE, USA) as the base platform with the addition of an olfactory port and flame ionization detector (FID). The system was equipped with two columns in series connected by a Dean's switch. The non-polar pre-column was 12m, 0.53mm i.d.; film thickness, 1 µm with 5% phenyl methylpolysiloxane stationary phase (SGE BP5) and operated with constant pressure mode at 8.5 psi. The polar analytical column was a 25m×0.53 mm fused silica capillary column coated with poly (ethylene glycol) (WAX; SGE BP20) at a film thickness

of 1 μm . The column pressure was constant at 5.8 psi. Both columns were connected in series. System automation and data acquisition software were MultiTraxTM V. 6.00 and AromaTraxTM V. 7.02 (Microanalytics, Round Rock, TX, USA) and ChemStationTM (Agilent, Santa Clara, CA, USA). The general run parameters used were as follows: injector, 260 °C; FID, 280 °C, column, 40 °C initial, 3 min hold, 7 °C min^{-1} , 220 °C final, 10 min hold; carrier gas, GC-grade helium. The GC was operated in a constant pressure mode where the mid-point pressure, i.e., pressure between pre-column and column, was always at 5.8 psi and the heart-cut sweep pressure was 5.0 psi. The MS scan range was 33-280 amu. Spectra were collected at 6 scans s^{-1} using scan and selected ion monitoring (SIM) simultaneously. Electron multiplier voltage was set to 1000 V. MS tuning was performed using the default autotune setting using perfluorotributylamine (PFTBA) weekly. The TD-MDGC-MS-O system is shown in Figure 1.

Human panelists were used to sniff separated compounds simultaneously with chemical analyses. Odor caused by separated VOCs was evaluated with a 64-descriptor panel, intensity scale, and hedonic tone scale in Aromatrx software.

Table 1 Typical odorous compounds quantified in this work

	Compounds	CAS #	MW	Retention time (min)	MS Ion ⁽¹⁾	Correlation equation ⁽²⁾	R ²
1	Acetic Acid	64-19-7	60.05	12.78	45, 60 , 15	$y = 16.938\ln(x) - 61.507$	0.9453
2	Propanoic Acid	79-09-4	74.08	14.40	74 , 28, 48	$y = 16.248\ln(x) - 57.867$	0.9673
3	Isobutanoic Acid	79-31-2	88.11	14.91	43 , 27, 73	$y = 20.864\ln(x) - 64.9$	0.9563
4	Butanoic Acid	107-92-6	88.11	16.00	60 , 27, 73	$y = 18.168\ln(x) - 36.671$	0.8158
5	Isopentanoic Acid	503-74-2	102.13	16.73	60 , 43, 87	$y = 10.253\ln(x) + 23.501$	0.7794
6	Pentanoic Acid	109-52-4	102.13	17.88	60 , 73, 27	$y = 20.802\ln(x) - 63.623$	0.9507
7	Hexanoic Acid	142-62-1	116.16	19.68	60 , 73, 27	$y = 28.718\ln(x) - 106.47$	0.9592
8	Guaiacol	90-05-1	124.14	20.06	109 , 124, 81	$y = 12.949\ln(x) + 0.1546$	0.9041
9	Heptanoic Acid	111-14-8	130.19	21.38	60 , 73, 41	$y = 32.645\ln(x) - 135.18$	0.9577
10	Phenol	108-95-2	94.11	22.13	94 , 66, 39	$y = 7.705\ln(x) - 24.844$	0.7413
11	p-cresol	106-44-5	108.14	23.28	107 , 77, 90	$y = 10.253\ln(x) + 6.5324$	0.7794
12	4-Ethylphenol	123-07-9	122.17	24.61	107 , 122, 77	$y = 12.8\ln(x) - 25.964$	0.7622
13	2-Aminoacetophenone	551-93-9	135.16	25.41	120 , 135, 92	$y = 12.949\ln(x) - 2.192$	0.9041
14	Indole	120-72-9	117.15	28.23	117 , 90, 63	$y = 20.864\ln(x) - 52.773$	0.9563
15	Skatole	83-34-1	131.18	28.88	130 , 77, 103	$y = 13.011\ln(x) - 9.634$	0.9128

Note: (1) The ions shown in black italic type were used for quantification.
(2) Correlation equation shows the correlation of odor intensities to odorants mass in one tube.

Sampling

Sampling sorbent tubes were constructed of 304 stainless steel and then double passivated with a proprietary process. They were packed with 65 mg Tenax TA. Silanized glass wool plugs and stainless steel screens were placed in the two ends of the tubes to hold the sorbent.

Before the first use, sorbent tubes were conditioned by thermal cleaning (260 °C for 5 hrs) under a flow rate of nitrogen of 100 mL min⁻¹. For subsequent uses, pre-conditioning at 260 °C for 30 min was applied.

Field air samples were taken using a SKC pump with the set flow rate of 70 mL min⁻¹ for 1hr, were stored at 4 °C, and were analyzed within 7 days. The sampling flow rates were detected on-line using a Bios DryCal digital flow meter.

Standards and Calibration

Fifteen compounds were selected as the target compounds for this work. The selection was based on the previous studies relative to typical odorous volatile organic compounds emitted from livestock facilities (shown in Table 1) (Lo et al, 2008; Koziel et al, 2006; Bulliner et al, 2006; Cai et al, 2006; Keener et al, 2002; Oehrl et al, 2001). Sulfur VOCs were not quantified due to the limitations of Tenax TA sorbent. Standard solutions were prepared by diluting stock standard solutions in methanol and were stored at 4 °C in dark. Stock standard solutions of VFAs and phenolics were prepared by adding certain weights of neat chemicals in a 40 mL pre-cleaned vial, and then filled the vial with a certain weight of methanol. Response factors for odorants were determined by direct injection of 1.0 µL of standard solution onto the GC column and measuring recovery of each odorant.

For sorbent tube analysis, 5 µL or 10 µL of the standard solution was spiked into a sorbent tube using an ATIS™ Adsorbent Tube Injector System (Supelco). A nitrogen flow of 50 mL min⁻¹ for 5 min with the block heater temperature of 75 °C was needed to transfer the target odorants onto the sorbent tubes.

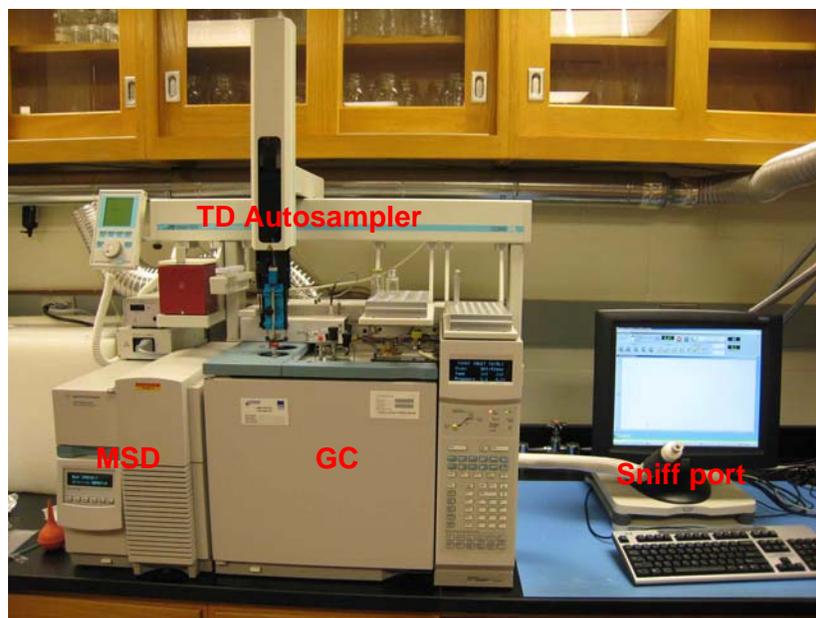


Figure 1 Thermal desorption - multidimensional gas chromatography - mass spectrometry - olfactometry (TD-MDGC-MS-O) system for simultaneous chemical and olfactometry analyses

Data Analysis

Identification and quantification of compounds were performed by analyzing the MS data using the HP ChemStation and the BenchTop software. Compounds were initially characterized using the MS library search software and then confirmed using true standards. Confirmation included matching retention times, spectra and selected ions. Quantification was performed by external standard. The selected ion for quantification of each compound was listed in Table 1. The integrated area of a selected ion was obtained for each compound for data analysis.

The odor event of that particular compound was examined and compared with the odor characters from LRI and Flavornet (Acree 2004). It is important to note that the human response to odors varies and therefore some variations related to odor character and intensity are natural between panelists.

Results and Discussion

Correlation of odor intensities to odorants concentration

Quantification of odorants concentration and odor intensity was performed simultaneously using TD-MDGC-MS-O system,. Target compounds were separated in GC column and isolated compounds were split into mass detector and sniff port with the split ratio of 1:2. The concentration of the compounds was quantified with the mass detector, and the odor character, intensity, duration time, and hedonic tone was identified and quantified via the sniff port by the panelist. Figure 2 shows the chromatogram and aromagram of a standard sample with 15 typical odorous VOCs. Figures 2 and 3 also illustrate that the start times of some odor events were different from that of the corresponding chromatogram peak. With the increase of the chromatographic retention time, the start time of odor event in aromagram was delayed increasingly longer, up to 2.85 min. The odor event duration time also increased with the increase of retention time. As a result, some odor events were difficult to separate at first, especially for the compound with retention time longer than 18 min. In order to quantify the odor event accurately, it is important to separate each odor event correctly.

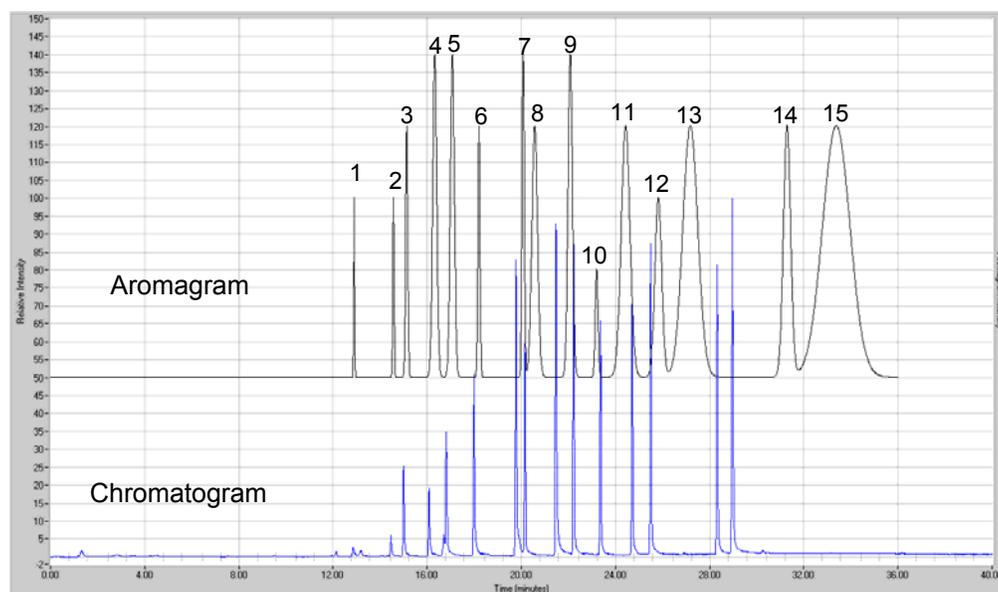


Figure 2 Chromatogram and aromagram of 15 VOCs standards

- (1) Acetic Acid; (2) Propanoic Acid; (3) Isobutanoic Acid; (4) Butanoic Acid; (5) Isopentanoic Acid; (6) Pentanoic Acid; (7) Hexanoic Acid; (8) Guaiacol; (9) Heptanoic Acid; (10) Phenol; (11) p-cresol; (12) 4-Ethylphenol; (13) 2-Aminoacetophenone; (14) Indole; (15) Skatole.

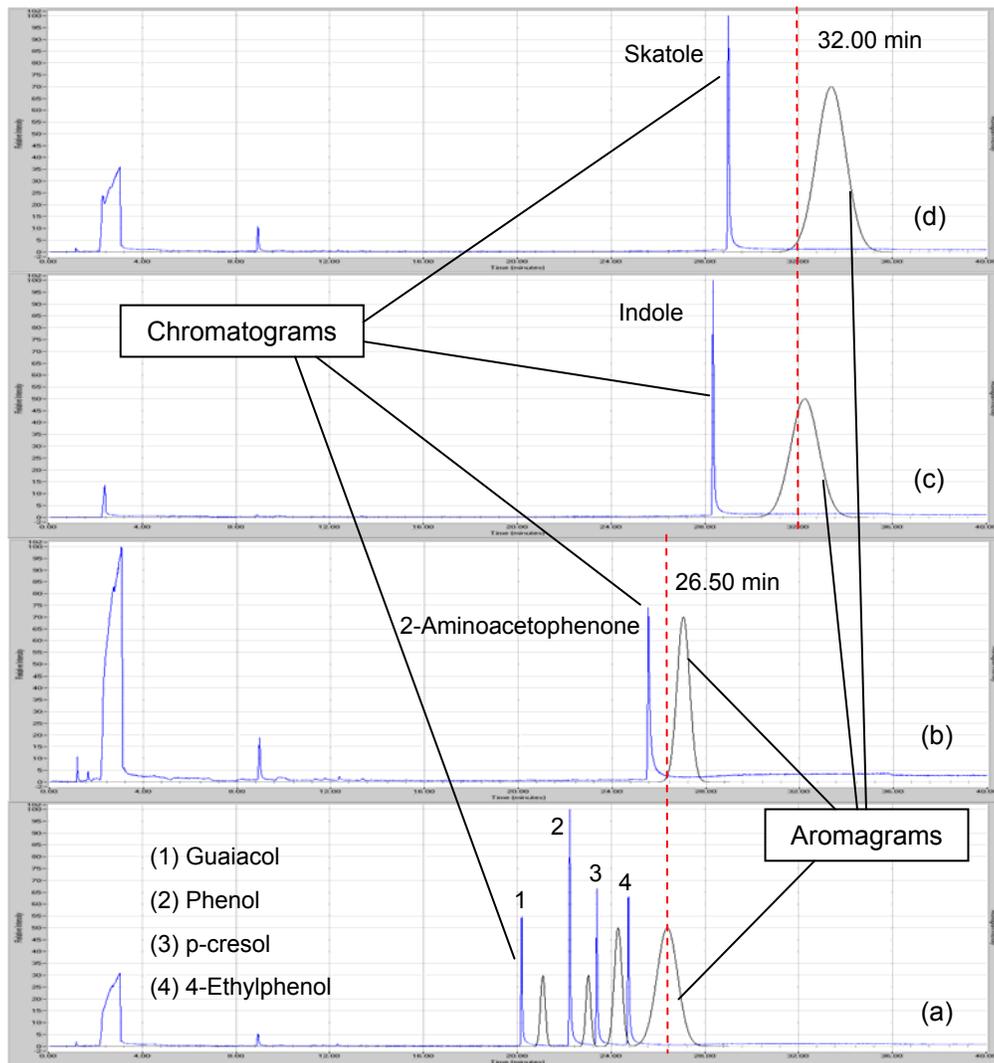


Figure 3 Chromatograms and aromagrams of VOCs with late eluting times

For compounds with longer retention time, especially 2-aminoacetophenone, indole and skatole, the GC-MS-O analysis of single compound was first performed to determine the effects of the odor event delay. From Figure 3, it could be found that the odor events for indole and skatole could not be separated. The same was true for 4-ethylphenol and 2-aminoacetophenone. The odor events for other compounds were separated. In order to allow comparison of odor in different experiments, the odor event for indole was artificially stopped and the odor event for skatole was started at 32 min. The same was true for 4-ethylphenol and 2-aminoacetophenone, where the odor event for 4-ethylphenol was artificially stopped and the odor event for 2-aminoacetophenone was started at 26.50 min. Figure 4 shows the correlation of odor intensities to odorants mass in one tube. For the TD-MDGC-MS-O system used in this work, the make-up air flow rate is constant, so the correlation of odor intensities to odorants mass should be similar with that of odor intensities to odorants concentration. For many odorants used in the food and

fragrance industry, there is a linear relationship between log olfactory intensity reported by the individual and the air concentration of the odorant present in air (Turk and Hyman, 1991). Zahn et al. (2001) also reported the total concentrations of VOCs emitted from swine manure correlate well with the log stimulus intensity. This relationship between perceived olfactory stimuli and intensity of sensation is referred to as the fundamental psychophysical law (Stevens, 1957). From Figure 4 and Table 1, it was found that the mass of each VOCs correlate well with the log stimulus intensity according to Steven's law. All of the correlation coefficients (R^2) are greater than 0.74, and 10 correlation coefficients are greater than 0.90.

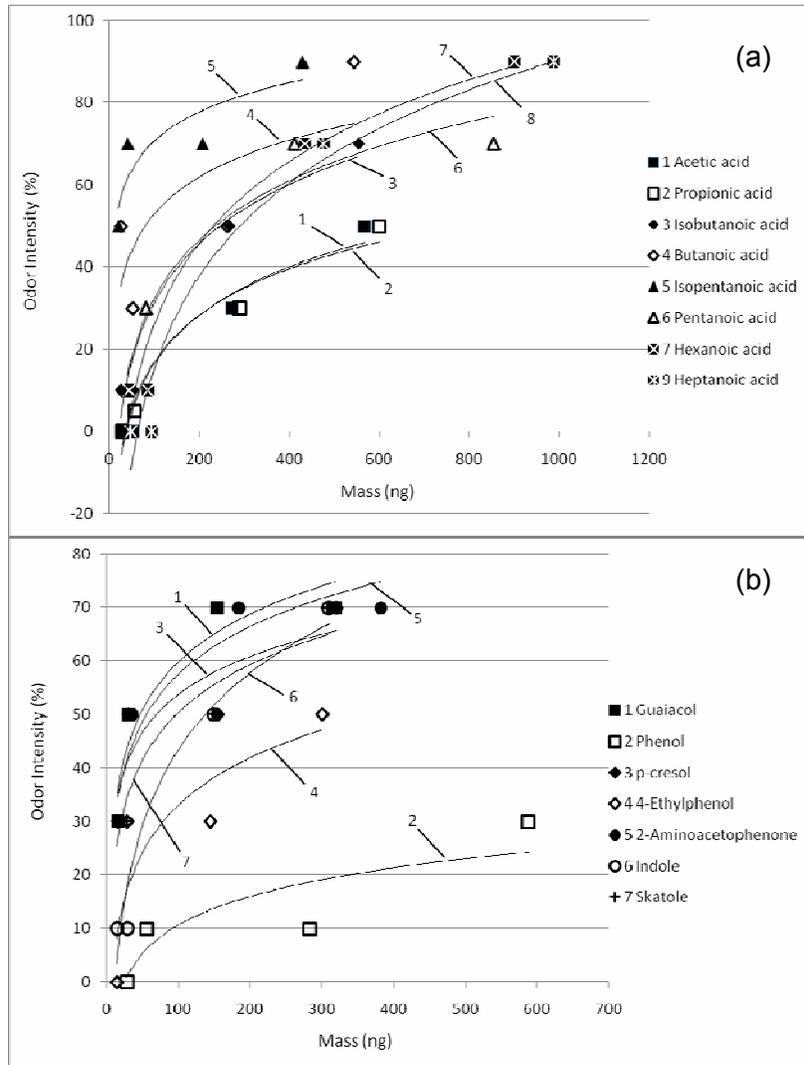


Figure 4 Correlation between the odor intensity and the mass of 15 typical VOCs in one tube

Application to field air analysis

Ten air samples were taken from two swine barns and one dairy barn using sorbent tubes. A typical TD-MDGC-MS-O result for a sample taken from a swine barn was shown in Figure 5 and Table 2.

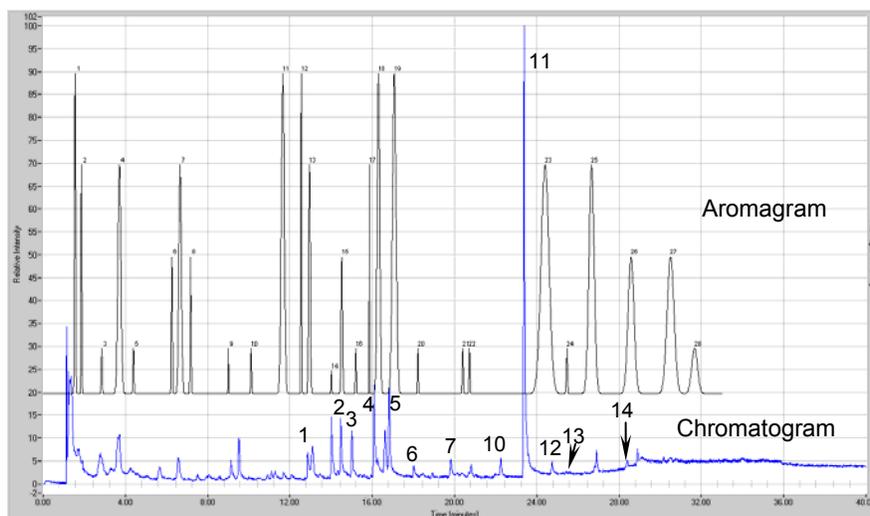


Figure 5 Chromatogram and aromagram of a typical air sample from swine barn

(1) Acetic Acid; (2) Propanoic Acid; (3) Isobutanoic Acid; (4) Butanoic Acid; (5) Isopentanoic Acid; (6) Pentanoic Acid; (7) Hexanoic Acid; (10) Phenol; (11) p-cresol; (12) 4-Ethylphenol; (13) 2-Aminoacetophenone; (14) Indole.

Table 2 Chemical and sensory quantification of target compounds for a typical sample from a swine barn

No.	Compound	Concentration (ppb)	Odor Character	Odor I ⁽¹⁾ (%)	Odor Area (I×D×100)	Hedonic Tone
1	Acetic Acid	27.2	Acidic	50	1,098	-2
2	Propanoic Acid	34.4	Body odor, Fatty acid	30	598	-1
3	Isobutanoic Acid	4.73	Body odor	10	119	-1
4	Butanoic Acid	13.0	Body odor, Fatty acid	70	2,445	-2
5	Isopentanoic Acid	2.93	Body odor, Fatty acid, Sweet	70	3,424	-3
6	Pentanoic Acid	0.99	Acidic, Fatty acid	10	119	-1
7	Hexanoic Acid	0.29	Acidic, Fatty acid	10	139	-1
8	Guaiacol	N/D	N/D	N/D	N/D	N/D
9	Heptanoic Acid	N/D	N/D	N/D	N/D	N/D
10	Phenol	0.55	N/D	N/D	N/D	N/D
11	p-cresol	8.79	Burnt, Phenolic, Medicinal	50	5,391	-2
12	4-Ethylphenol	0.20	Burnt, Phenolic	10	159	-1
13	2-Aminoacetophenone	0.03	Burnt, Smoky, Phenolic, Medicinal	50	3,494	-2
14	Indole	0.03	Burnt, Barnyard, Taco Shell	30	2,845	-2
15	Skatole	N/D	N/D	N/D	N/D	N/D

Note: (1) I is the Odor intensity; D is the odor duration time.
(2) N/D = "not detected".

Gas concentrations, odor character, odor intensity, odor area, and hedonic tone were obtained simultaneously. Correlations between odor intensity and the odorant concentrations were found for all 10 samples. Results are shown in Figure 6. Correlation coefficients for most of VFAs were bigger than 0.75. Figure 6 shows the correlations between the odor intensity and the mass of isopentanoic acid and p-cresol, two important odorous compounds defining the character of livestock odor.

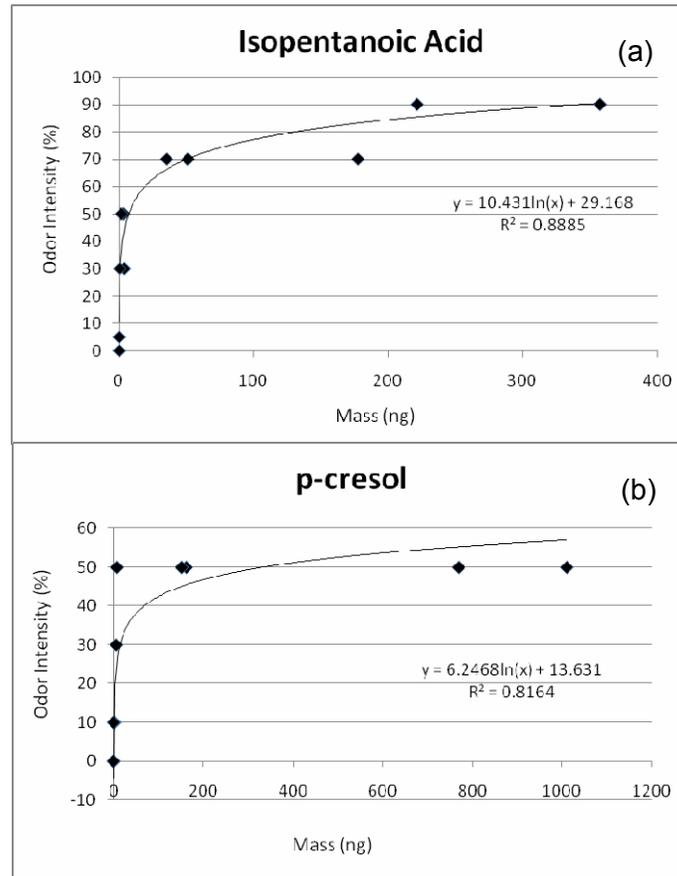


Figure 6 Correlations between the odor intensity and the mass of isopentanoic acid and p-cresol collected on sorbent tubes during field air sampling

Conclusion

Several preliminary conclusions can be made:

- (1) The TD-MDGC-MS-O system could be used to estimate concentrations of VFAs and phenolic compounds associated with CAFOs.
- (2) Odor character, odor intensity, and odor hedonic tone can be assessed for separated target compounds simultaneously with chemical analyses.
- (3) Concentrations of odorous compounds correlated well with the log stimulus intensity.

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