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Method for sampling and analysis of volatile biomarkers in process gas from aerobic digestion of poultry carcasses using time-weighted average SPME and GC–MS

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Abstract
A passive sampling method, using retracted solid-phase microextraction (SPME) – gas chromatography–mass spectrometry and time-weighted averaging, was developed and validated for tracking marker volatile organic compounds (VOCs) emitted during aerobic digestion of biohazardous animal tissue. The retracted SPME configuration protects the fragile fiber from buffeting by the process gas stream, and it requires less equipment and is potentially more biosecure than conventional active sampling methods. VOC concentrations predicted via a model based on Fick's first law of diffusion were within 6.6–12.3% of experimentally controlled values after accounting for VOC adsorption to the SPME fiber housing. Method detection limits for five marker VOCs ranged from 0.70 to 8.44 ppbv and were statistically equivalent ($p > 0.05$) to those for active sorbent-tube-based sampling. The sampling time of 30 min and fiber retraction of 5 mm were found to be optimal for the tissue digestion process.

Keywords
Process monitoring, Gas, Biomarkers, Aerobic digestion, Animal mortalities, Volatile organic compounds, Solid-phase microextraction, Gas chromatography–mass spectrometry

Disciplines
Agriculture | Bioresource and Agricultural Engineering | Food Biotechnology | Poultry or Avian Science | Toxicology

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Method for sampling and analysis of volatile biomarkers in process gas from aerobic digestion of poultry carcass using time-weighted average SPME and GC-MS

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ABSTRACT:

A passive sampling method, using retracted solid-phase microextraction (SPME) – gas chromatography-mass spectrometry and time-weighted averaging, was developed and validated for tracking marker volatile organic compounds (VOCs) emitted during aerobic digestion of biohazardous animal tissue. The retracted SPME configuration protects the fragile fiber from buffeting by the process gas stream, and it requires less equipment and is potentially more biosecure than conventional active sampling methods. VOC concentrations predicted via a model based on Fick's first law of diffusion were within 6.6 to 12.3% of experimentally controlled values after accounting for VOC adsorption to the SPME fiber housing. Method detection limits for five marker VOCs ranged from 0.70 to 8.44 ppbv and were statistically equivalent ($p>0.05$) to those for active sorbent-tube-based sampling. The sampling time of 30 min and fiber retraction of 5 mm were found to be optimal for the tissue digestion process.

Chemical compounds studied in this article

Dimethyl disulfide (PubChem CID: 12232); Dimethyl trisulfide (PubChem CID: 19310); Pyrimidine (PubChem CID: 9260); Phenol (PubChem CID: 996); *p*-Cresol (PubChem CID: 2879)

KEYWORDS: process monitoring, gas, biomarkers, aerobic digestion, animal mortalities, volatile organic compounds, solid-phase microextraction, gas chromatography – mass spectrometry.

1. Introduction

Catastrophic natural disasters or large-scale disease outbreaks can result in massive loss of poultry and livestock (Nutsch & Kastner, 2010). During emergency situations, timely and biosecure disposal of animal carcasses is necessary to prevent the spread of disease, as well as soil, air, and water pollution (Nutsch et al., 2010). Public concern regarding contamination of valuable ground water resources by mass burial has led to an interest in alternative treatment and disposal options. Aerobic digestion (AeD) has been shown to accelerate decomposition of sheep and poultry carcasses and associated water and air pollutants (Williams, Edwards-Jones, & Jones, 2009; Gwyther, Jones, Golyshin, Edwards-Jones, & Williams, 2012; Koziel, Ahn, Glanville, Frana, van Leeuwen, & Nguyen, 2017).

When animal losses are the result of highly contagious diseases such as Foot-and-Mouth disease or highly-pathogenic avian influenza, it is preferable to be able to monitor completion of tissue decomposition without collecting and transporting potentially biohazardous samples. Akdeniz et al. proved that monitoring process gases for specific marker volatile organic compounds (VOCs) is a promising method to track the progress of swine mortality composting (Akdeniz, Koziel, Ahn, Glanville, Crawford & Raman, 2009; Akdeniz, Koziel, Ahn, Glanville, Crawford & Raman, 2010a; Akdeniz, Koziel, Ahn, Glanville, & Crawford, 2010b; Akdeniz, Koziel, Glanville, Ahn & Crawford, 2011). It is less subjective and labor-intensive than visual assessment of decomposition, and also reduces biosecurity risks associated with excavating the compost to determine if carcass decomposition is complete. Pyrimidine, dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) were found to be three reliable biomarker VOCs of the completion of swine carcass degradation (Akdeniz et al., 2011). Recent reports confirmed that DMDS and DMTS are generated during early stages of decomposition of pig carcasses (Dekeirsschieter, Stefanuto, Brasseur, Haubruge, & Focant, 2012; Stadler, Stefanuto, Brokl,

Forbes, & Focant, 2013), and cow and chicken tissues (Cablk, Szelagowski, & Sagebiel, 2012). Phenol and *p*-cresol also have been shown to be highly odorous compounds released during the middle and late stages of domestic pig decomposition (Dekeirsschieter et al., 2009; Dekeirsschieter et al., 2012; Stadler, Stefanuto, Brokl, Forbes, & Focant et al., 2013). Odor released during animal tissue decomposition is an important concern as it adversely influences public acceptance of disposal operations (Glanville, Ahn, Akdeniz, Crawford, & Koziel, 2016).

Akdeniz et al. (2009; 2011), used sampling ports, tubing, pumps, and glass bulbs to collect gases from decomposing swine mortalities. The sampling bulbs were subsequently transported to the lab to be resampled via solid-phase microextraction (SPME) followed by analysis of VOCs. These methods are accompanied by (i) biosecurity risks during gas sample transfer into and out of the gas collection bulbs or sorbent tubes; (ii) sorption of VOCS on interior surfaces of tubing or bulbs; (iii) the need for pumps and additional equipment; and (iv) the need for additional procedural steps during sampling, sample preparation, and equipment clean-up.

Biosecurity risks are reduced by using passive gas sampling via retracted SPME. Only the SPME fiber is transported to the lab. SPME assemblies can be isolated and stored for maximum sample recovery. Several studies have reported on passive sampling via retracted SPME and time-weighted averaging (TWA) for quantification of VOCs from indoor air (Koziel, Jia, Khaled, Noah, & Pawliszyn, 1999; Martos, & Pawliszyn, 1999; Koziel, Noah, & Pawliszyn, 2001; Chen & Pawliszyn, 2003), and from processes such as: hot syngas and stream gas from a biomass gasification and pyrolysis (Woolcock, Koziel, Cai, Johnston, & Brown, 2013; Woolcock, Koziel, Johnston, Brown, & Broer, 2015); and vehicle exhaust (Baimatova, Koziel, & Kenessov, 2015). Passive TWA-SPME has advantages over ‘conventional’ SPME (i.e., exposed

87 outside of needle assembly). These are: (1) retracted SPME shields the fiber from breakage; (2)
88 effects of turbulence on the SPME boundary layer and quantification are minimized.
89 Analytes reach the retracted SPME fiber primarily via diffusion and the TWA approach to
90 quantification depends on Fick's first law of diffusion. Developing a new TWA-SPME method
91 requires (1) careful study and optimization gas sampling parameters for the quantification model
92 and (2) quantifying target VOC adsorption onto SPME fiber housing (metallic parts of needle
93 assembly) and determining its effects on concentration predictions.
94 The effluent gas from AeD of animal tissues is a complex mixture of chemicals with
95 potentially useful information about the process status. However, many potentially useful
96 biomarkers are present at sub-ppmv levels among many less-relevant gases and bioaerosols. To
97 date, very few studies have compared TWA-SPME with conventional sampling and
98 quantification methods (Woolcock et al., 2015), and no TWA-SPME method exists for sampling
99 biomarker VOCs from the potentially infectious process for the purpose of tracking completion.
100 The goal was to develop a passive TWA-SPME method for collection, identification, and
101 quantification of biomarker VOCs released during emergency disposal of biohazardous animal
102 carcasses via AeD. Our working hypotheses were that (i) VOC adsorption onto the SPME fiber
103 housing (metallic parts of needle assembly) can be reproducible and quantified, (ii) retracted
104 SPME fiber behaves as a zero sink for target VOCs, and (iii) the developed quantification
105 method is in agreement with Fick's first law of diffusion model. Specific objectives were to
106 determine the effects of (1) adsorption onto SPME fiber housing; (2) sampling time; (3) VOC
107 concentration; (4) diffusion path length; and to establish (5) detection limits; and (6) validate
108 TWA-SPME sampling method using volatile biomarkers in the process gas.
109

2. Materials and Methods

2.1 Targeted biomarker chemicals

Based on previously discussed studies of marker VOCs produced during animal tissue digestion, five standards for DMDS, DMTS, pyrimidine, phenol, and *p*-cresol were purchased from Sigma-Aldrich (St. Louis, MO, US). Ethanol (200 proof) was from the Chemistry Stores at Iowa State University. Ultrahigh pure 99.995% helium (He) was purchased from Praxair (Des Moines, IA, US).

VOC standard stock solution in ethanol was prepared daily by adding 10 mg of each compound in a total volume of 10 mL. The final concentration of the standard mixture solution was approximately 1 mg mL^{-1} for each compound. The stock solution was stored at 4 °C and diluted with ethanol (volume $volume^{-1}$) prior to use. Standard gases were generated by injecting a known volume of VOC diluted standard mixture (1 to 10 µL) into 250 mL glass sampling bulb equipped with Thermogreen half hole type septa using a 10 µL gastight syringe (Hamilton, Reno, NV, US).

The theoretical concentration for each analyte, expressed in ppm, was calculated from the equation below:

$$C = [22.4 \times 10^6 \times (T/273) \times (1/P) \times M] / (V \times MW) \qquad (1)$$

where C is the gas contaminant concentration (ppmv), T is the absolute temperature of the system (K), P is the system pressure (atm), M is the mass of the contaminant added (g), V is the volume of the system (L), and MW is the molecular weight of the contaminant (g mol^{-1}). The concentration in ppm can be converted into mass per unit volume by the expression:

$$C_{M/V} = (C_{ppm} \times MW \times P) / (R \times T) \qquad (2)$$

where $C_{M/V}$ is the gas contaminant concentration (mg m^{-3}), C_{ppm} is the gas concentration (ppmv), R is the molar gas constant (L atm K^{-1} mol^{-1}).

Glass sampling bulbs (250 mL), Thermogreen half-hole type septa, and glass vials were from Supelco (Bellefonte, PA, US). All glassware was thoroughly washed, rinsed, and baked overnight at 125 °C before use (Bulliner, 2006; Cai, Koziel, Lo, & Hoff, 2006). All work related to chemicals was performed under fume hoods of the Atmospheric Air Quality Laboratory at Iowa State University.

2.2 SPME and GC-MS conditions

The 85 µm Carboxen (CAR)/PDMS (Supelco, Bellefonte, PA, US) fiber was used for a sampling of the five target VOCs in the gas phase using the TWA-SPME method. Passive sampling was facilitated using a commercial SPME fiber holder (Supelco, Bellefonte, PA, US) that was modified for the TWA-SPME sampling method by adding additional notches for different desired diffusion path lengths. A detail of the modified SPME fiber holder was discussed elsewhere (Koziel & Novak, 2002).

All TWA-SPME gas samples were analyzed using a gas chromatography - mass spectrometry (GC-MS) system consisting of 6890N GC and 5973 MS (Agilent Technologies, Santa Clara, CA, US). Ultrahigh pure He was used as the carrier gas at constant pressure (5.1 psi). GC conditions were as follows: injection temperature = 250 °C, transfer line temperature = 240 °C, column He flow rate = 7.5 mL min^{-1}, polar capillary column SGE-BP20-054440 (SGE Inc., Austin, TX, US), and dimension (length = 30 m, diameter = 530 µm, thickness of the coating = 0.50 µm). The GC program started at 40 °C for 3 min, the temperature ramping rate of 10 °C min^{-1} to 240 °C and held there for 3 min.

Temperatures of MS source and MS quad were 230 °C and 150 °C, respectively. Single ion monitoring (SIM) mode was chosen for detection of the target VOCs. Mass-to-charge ratios (m z^{-1}) were 94, 126, 80, 94, and 107 for DMDS, DMTS, pyrimidine, phenol, and *p*-cresol, respectively. Scanning frequency was 8.33 cycles min^{-1}, the electron multiplier voltage was 2,329 V. Chromatography data acquisition software consisting of MS detector (ChemStation, Agilent Technologies, Santa Clara, CA, US) was used to analyze data. Separated compounds were identified using mass spectral matches with ChemStation's National Institute of Standard and Technology – Mass Spectrometry (NIST MS) Library. SIM chromatograms were integrated for peak area counts (PACs). PACs were converted into mass using MS detector response factors (RFs). These RFs were developed by direct injection of 1 µL standard target VOCs (n= 3 replicates of n=3 of concentrations) into GC-MS using the same conditions of MS and program. Quantified masses of target VOCs were then used for comparisons with TWA-SPME model (i.e., experimental vs. theoretical) and to estimate measured gas concentrations.

2.3 Fick's first law-based model for TWA-SPME sampling with retracted fiber

The principle of SPME-TWA sampling technique is based on the Fick's first law of diffusion which states that the amount of analyte collected on a SPME fiber is proportional to their molecular diffusion rates in gas phase (D_g) and the SPME needle cross-sectional area (A), and is inversely proportional to the diffusion path length (Z, i.e., distance between needle opening and the retracted fiber tip). As long as the fiber is not saturated, and the fiber coating behaves as a zero sink (i.e., the rate of sorption is not affected by sorption capacity), the mass extracted (n) is proportional to the integral of the concentration over a sampling time (t) (Koziel et al., 1999; Martos et al., 1999).

$$n(t) = \frac{C_g A D_g t}{Z} \qquad (3)$$

For a defined exposure time, the gas-phase analyte concentration can be calculated from:

$$C_g = \frac{n(t) Z}{A D_g t} \qquad (4)$$

In equations (3) and (4), A, Z, and t are known and controlled values. $n(t)$ can be determined using analytical equipment such as GC-MS system (Woolcock et al., 2013).

Series of experiments were performed to determine the effects of t, C, and Z on mass extracted by SPME using standard gases of target VOCs. Standard gas samples were collected by inserting the SPME needle with a retracted 85 μm CAR/PDMS fiber into the glass sampling bulb that contained the VOC standard mixture at T = 23 ± 0.5 °C and P =1 atm (Fig. 1A). During the TWA-SPME sampling, the analytes partition into the SPME coating (Koziel et al., 1999), albeit this extraction process is much slower compared with conventional sampling with exposed SPME. The TWA SPME advantage is that the extraction is not affected by turbulence outside of the SPME needle, while the SPME process and its calibration are controlled by diffusion (Fig. 1B) (Martos et al., 1999).

<Figure 1>

Fig. 1. Schematic of (A) diffusion-based gas sampling for target VOCs in a process gas flow using the TWA-SPME method with 250 mL gas sampling bulb and (B) retracted SPME fiber in the TWA sampling mode.

2.4 Semi-empirical models for gas-phase diffusion coefficient (D_g)

To estimate D_g for the five target VOCs the Fuller-Schettler and Giddings (FSG) model was used (Fuller, Ensley, & Giddings, 1969)

$$D_{AB} = \frac{0.00143T^{1.75}\left(1/M_A + 1/M_B\right)^{1/2}}{p\left[\left(\sum v\right)_A^{1/3} + \left(\sum v\right)_B^{1/3}\right]^2} \quad (5)$$

where D_{AB} is the diffusion gas coefficient of solute gas A in carrier gas B; T is the absolute temperature (K); M_A and M_B are the molecular weights (g mol^{-1}) of solute A and carrier gas B, respectively; P is the total pressure (atm); and $\sum v$ is the sum of the atomic diffusion contributions.

2.5 Effects of adsorption onto SPME needle housing (metallic parts of needle assembly) on TWA-SPME method

Prior to calculating experimental mass extracted on the SPME fiber, the amount of analytes adsorbed on the SPME fiber needle housing must be accounted for. To do this, three set of experiments (herein called trial #1, 2, and 3) were undertaken to determine the extent of adsorption contributed by the fiber needle housing. A broken fiber which has no CAR/PDMS coating was used in these trials. All experiments were carried out in triplicates at T = 23 ± 0.5 °C and at P = 1 atm. In trial #1, the amount of target VOCs adsorbed on the fiber needle housing was examined at four different sampling times (t = 10, 20, 30 and 60 min). The standard gaseous concentrations of DMDS, DMTS, pyrimidine, phenol and *p*-cresol were 1.44, 1.54, 1.52, 1.21, and 1.42 ng mL^{-1} (0.37, 0.30, 0.46, 0.31 and 0.32 ppmv), respectively and Z = 0.5 cm. In trial #2, the amount of target VOCs absorbed on the fiber needle housing was observed at C_g ranging from 0.04 to 3.11 ng mL^{-1}, for the 5 target VOCs, at Z = 5 mm and t = 30 min, respectively. In trial #3, the amount of target VOCs absorbed on the fiber needle housing was measured at three

different Z (5, 15 and 30 mm), with t = 30 min, and the gas concentrations of DMDS, DMTS, pyrimidine, phenol, and *p*-cresol set at 0.19, 0.15, 0.26, 0.19, and 0.20 ppmv, respectively.

2.6 Effect of sampling time on TWA-SPME method

Sampling time, C_g, Z, T, and pressure were kept the same as in trial #1. The experimentally extracted mass was calculated by subtracting the mass extracted on the needle housing from the mass extracted on CAR/PDMS fiber.

2.7 Effect of VOC concentration on TWA-SPME method

The accurate and consistent response of the TWA-SPME method was determined by sampling at different concentrations of analytes in the gas phase in triplicate. Five different standard VOC mixtures (concentrations ranging from 0.04 to 3.11 ng mL^{-1}) were used for the TWA-SPME method with 85 µm CAR/PDMS fiber. Sampling time, C_g, Z, T, and pressure were kept the same as in trial #2.

2.8 Effect of diffusion path length on TWA-SPME method

According to the Fick's first law of diffusion, the amount of analytes extracted on the SPME fiber is inversely proportional to Z (Eq. 3). The effect of Z was validated by the use of one standard gas mixture (concentrations ranged from 0.29 to 1.59 ng mL^{-1}). SPME fiber was retracted at three different Zs (5, 15, and 30 mm), sampled continuously at t = 30 min, T = 23 ± 0.5 °C and P = 1 atm.

2.9 Method detection limits

243 Method detection limits (MDLs) were estimated as a product of (1) the students' t value
244 appropriate for a 99% confidence level and a standard deviation estimate with (n-1) degrees of
245 freedom and (2) the standard deviation of the replicate measurements (USEPA, 1970). Six
246 replicates of a standard VOC mixture gas sample were used. The concentration of each VOC
247 ranged from 0.07 to 0.4 ng mL^{-1}. The SPME fiber was retracted at 5 mm for t = 30 min, at T= 23
248 ± 0.5 °C, and P = 1 atm. Estimates of D_g were obtained using the FSG model (Eq. 5).
249
250 2.10 Validation of TWA-SPME sampling method using volatile biomarkers in process gas
251 The reliability and feasibility of the TWA-SPME method were validated by comparing its
252 results with those obtained using a sorbent tube-based method. Both were applied to process
253 gases generated by a laboratory scale AeD system for poultry carcasses. Gas samples were
254 collected from 4 identical AeD reactors simultaneously using glass sampling bulbs (for TWA-
255 SPME method) and sorbent tubes on the 11^{th} day (Test #1) and 42^{nd} day (Test #2) of the poultry
256 carcass subjected to aerobic decomposition. The schematic of the gas sampling system of 5
257 target VOCs emitted from AeD reactor of poultry carcass is shown in Fig. 2. The temperature of
258 process gas was 28 ± 0.5 °C, and relative humidity was 100%. Laboratory-scale AeD system was
259 designed, constructed and validated for this research with similar features to (Williams et al.,
260 2009; Gwyther et al., 2012).
261 <Figure 2>
262 **Fig. 2.** Schematic of the process gas sampling system of 5 target VOCs emitted from the aerobic
263 reactor of poultry carcass using TWA-SPME and sorbent tube-based methods.
264

For the TWA-SPME method: gas samples were collected from AeD reactors using 250 mL gas sampling bulbs (Supelco, Bellefonte, PA, US) with a flow rate at 3 L min^{-1} (air mass flow controller model GFC 17, Aalborg, Orangeburg, NY, US). After 2 min (8 hydraulic residence times), stopcocks of glass sampling bulbs were closed, and gas samples were captured inside the bulbs. Then glass sampling bulbs containing gas samples were taken to Atmospheric Air Quality Laboratory where they were sampled with Z = 5 mm retracted fiber (85 µm CAR/PDMS) and t = 30 min (Fig. 1). Headspace TWA-SPME gas samples were analyzed using the same GC-MS system used for analyses of standard VOC gas samples.

For sorbent tube-based method: The method of building, and cleaning sampling sorbent tubes was reported elsewhere (Zhang et al., 2010). In this research, the same type of sorbent tubes (65 mg Tenax TA 60/80, Supelco, Bellefonte, PA, US) was used for gas sampling. Gas samples from AeD reactor were taken using an SKC pocket sampling pump model 210-1002 (SKC Inc., Eight Four, PA, US) with a set flow rate of 50 mL/min for 15 min (Zhang et al., 2010). The sorbent tubes #1 and #2 (Fig. 2) were used as gas sampling tubes, and breakthrough tubes, respectively. The sampling flow rates were checked with an NIST-traceable DryCal digital flow meter, model Defender 520 (Bios International, Butler, NJ, US).

All gas samples were analyzed immediately after sampling from AeD reactors using thermal desorption - GC-MS (TDGC-MS) system. The thermal desorption system is using a Model 3200 automated thermal desorption inlet for Agilent 6890 GC (Agilent, Wilmington, DE, US) developed by Microanalytics (Round Rock, TX, US) based on a PAL® autosampler (Alexandria, VA, US). All volumes and concentrations of gaseous samples were corrected to EPA's standard sampling conditions (T_{std} = 25 °C or 298 K, and P_{std} = 760 mm Hg) (USEPA, 1999).

3. Results and Discussion

Specific objectives (1-6) were addressed for a development of the TWA-SPME method. These objectives also test the working hypotheses (i, section 3.1) and (ii, sections 3.2-3.4) and basic prerequisites for passive air sampling (Martos et al., 1999).

3.1 Effects of adsorption onto SPME needle housing (metallic parts of needle assembly) on TWA-SPME method

There were three trials to validate the effects of needle housing on TWA-SPME method. In these trials, the surface of stainless steel needle housing was considered as a plane surface. Therefore, it has limited surface area for VOC gas adsorption. Experiments showed that the amount extracted on SPME needle housing is relatively small, reproducible and can be accounted for, and thus easy to calibrate. In trial #1, the effect of needle housing was evaluated against the extraction time (Fig. 3; Part A). It can be seen that adsorbed masses of 5 target VOCs on needle housing remains within the linear adsorption for short extraction times (t = 10, 20 and 30 min). However, at longer t = 60 min, the maximum masses adsorbed on needle housing for DMDS, DMTS, pyrimidine, phenol and *p*-cresol were 0.02, 0.02, 0.04, 0.01 and 0.03 ng, respectively (relative standard deviations, RSDs ranged from 1.0 to 11.5%). An examination of the data shows that the adsorption linearly increases with the short extraction times, but at longer extraction time it appears to approach saturation with the surface of the needle assembly. The results show that the total amount VOC adsorbed is within the capacity limits of the adsorption surface of the needle housing. In principle, this is in agreement with previous findings by Baimatova et al. (2015) and the Langmuir theory (Langmuir, 1918).

In trial #2, the effect of adsorption onto SPME needle housing was evaluated against the different VOC concentrations (Fig. 3; Part B). The amount of adsorption on the needle housing ranged from 3.91 to 6.20 % of that observed on the SPME fiber at the same experimental conditions. Results show that the amounts of adsorbed gases were proportional to the C_g. It was found that the maximum amounts of adsorbed substances are primary determined by the surface area on which the adsorption occurs (Langmuir, 1918).

In trial #3, the effect of needle housing was evaluated against different *Z*s (Fig. 3; Part C). Equation (3) gives the desired relation between *n* and *Z*, in which, *n* is inversely proportional to *Z*. However, statistical analysis showed that *n* changes at different *Z*s were not significantly different at any tested level. Results indicate that the mass of VOCs adsorbed on the needle housing were not dependent on *Z* (Fig. 3; Part C). Fick's first law was therefore not applicable to the stainless steel needle housing under the experimental conditions.

<Figure 3>

Fig. 3. Contribution of VOC adsorption onto the SPME fiber housing (metallic parts of needle assembly); Part A: effect of sampling times (*t*) (trial #1), Part B: effect of concentrations (C_g) (trial #2), and Part C: effect of diffusion path lengths (*Z*) (trial #3).

3.2 Effect of sampling time on TWA-SPME method

The results from this study are shown in Fig. 4 (Part A). The RSDs of the means ranged from 1.0 to 6.4%. It is reported that the zero sink assumption is satisfied if the adsorption of analytes is linear with *t* (Ouyang & Pawliszyn, 2008). According to (Eq. 3), *n(t)* was indeed directly proportional to *t*. The linear correlation of the R^2 values ranged from 0.988 to 0.999. The

theoretical mass estimated by TWA-SPME model deviates from the experimental *n(t)* by an average of 11.0% (ranging from 0.5 to 29.4%) for all target VOCs and all *t*. For individual VOCs, the TWA-SPME model differs from the experimental mass extracted between 0.5% and 15.8%. The TWA-SPME method is thus validated due to acceptable variation between the theoretical model and experimental results. The use of t=30 min resulted in the lowest average difference of 8.8 % (min = 1.1% and max = 17.7%) between the TWA-SPME model and the experimental *n(t)*. Thus, t = 30 min was chosen as the proper sampling time for the TWA-SPME method to quantify the target VOCs. These results verified that the 85 µm CAR/PMDS fiber behaves as a zero sink for all target VOCs, because of the strong affinity and large capacity of this type of fiber (Kataoka, Lord, & Pawliszyn, 2000).

3.3 Effect of VOC concentration on TWA-SPME method

Three repetitions of each standard VOC mixture resulted in R^2 values > 99 % which indicate that there is a very good linear relationship between the theoretical model and the experimental results (Fig. 4; Part B). Mean RSDs were 4.4% (ranging from 1.4 to 7.8%), which indicates a high degree of precision of this TWA-SPME method. In accordance with equation (3), *n(t)* was indeed directly proportional to the analyte concentrations in the gas phase, C_g. This is one of the useful, significant features of the TWA-SPME sampling method, i.e., relatively low RSDs for extractions at long extraction times. Analytes displacement and competitive adsorption are common for t = 30 min with conventional sampling with exposed adsorptive SPME fiber. Results of this test verify the second requirement of the TWA-SPME sampling method. The TWA-SPME model deviated from the experimental mass extracted on SPME fiber by average 6.58% (ranging from 0.28 to 16.39) for all target VOCs, thus validating the TWA-SPME

356 method. Additionally, the TWA-SPME model has the most accurate prediction at the highest
357 VOC concentrations (mean percentage difference = 3.93%, ranging from 0.50 to 6.54%).
358
359 3.4 Effect of diffusion path length on the TWA-SPME method
360 Results of this test are shown in Fig. 4 (Part C). All data points represent (n=3) replications.
361 Mean RSDs were 3.16% and ranged from 1.0 to 6.5%. Results show that the mass extracted on
362 SPME fiber vs. mass predicted by the Fick's first law model deviates by average 16.1% (ranging
363 from 7.9 to 32.4%) for all target VOCs and all data, with *p*-cresol having the best fit to the
364 model. The mass extracted predictions deviated by 12.3%, 15.4%, and 20.6% for Z = 5, 15, and
365 30 mm, respectively. The use of Z = 5 mm resulted in the lowest average difference (12.3%, min
366 = 7.9% to max=18.7%) between predicted and the measured mass extracted. The linear
367 correlations demonstrate that there is a very good relationship between $n(t)$, and the reciprocal of
368 Z. The current results agree with the previous research (Chen et al., 2003; Woolcock et al.,
369 2013). It indicates that VOC uptake by an SPME fiber is well described by Fick's first law of
370 diffusion (Koziel et al., 1999; Martos et al., 1999; Woolcock et al., 2013) when the amount of
371 analytes extracted by metal SPME needle assembly is subtracted from the total mass.
372
373 <Figure 4>
374 **Fig. 4.** Validation of the TWA-SPME model for five target VOCs – Part A: effect of sampling
375 times (t), Part B: effect of concentrations (C_g), and Part C: effect of diffusion path lengths (Z),
376 i.e., mass extracted on SPME fiber vs. mass predicted by the Fick's first law model.
377
378 For a typical passive sampler, a large surface area requires a large face velocity (ranging
379 from 4.6 to 15 m min^{-1}) to ensure a large amount of analyte is sampled. With a very small

passive sampler such as the retracted SPME fiber, therefore, it requires a very small face velocity. Previous work with standard gas from *n*-pentane to *n*-nonane has shown that there was no significant difference between the face and bulk concentrations determined with static standard gas and those obtained with a face velocity as low as 0.6 cm min^{-1} (Chen et al., 2003). Other research proved that the secondary diffusion boundary layer does not exist outside the tip of the needle of SPME fiber with a minimum gas flow velocity of ~ 10 cm s^{-1} for sampling standard mixture of benzene, toluene, ethylbenzene, and *p*-xylene (Koziel, Jia, & Pawliszyn, 2000). In this study, the cross-sectional area of the needle housing of 85 µm CAR/PDMS SPME fiber is extremely small (7.5×10^{-4} cm^2). Therefore, TWA passive sampling with SPME can be reasonably expected to sample accurately. Considering these present results and comparing them with current literature and, it can be concluded that the third requirement of the TWA-SPME method, in which C_{Bulk} equals C_{Face} is met.

3.5 Method detection limits

The method detection limits (Table S1) for the five target biomarker VOCs in the gas phase were reported as 1.50, 0.7, 1.50, 8.44, and 5.76 ppbv for DMDS, DMTS, pyrimidine, phenol, and *p*-cresol, respectively. This new method was significantly more sensitive than the standard methods (NIOSH 2542 for DMDS, and DTMS, MDLs = 200 to 10,000 ppbv; NIOSH 2546 for phenol and *p*-cresol, MDLs = 250 to 15,000 ppbv) (Table S2). No comparison can be made for pyrimidine because there is no standard method.

3.6 Validation of TWA-SPME sampling method using volatile biomarkers in process gas

Side-by-side measured C_g using the TWA-SPME and sorbent tube-based methods were compared. The selective ion monitoring (SIM) chromatograms in Fig. 5 illustrate the target VOCs found in the process gas of the AeD system.

<Figure 5>

Fig. 5. Example of selective ion monitoring (SIM) chromatograms of gas samples collected from the aerobic reactor of poultry carcass using TWA-SPME with 250 mL glass sampling bulb and sorbent tubes. All TWA-SPME gas samples were analyzed by the GC-MS system. All sorbent tube gas samples were analyzed by the TDMDGC-MS-O system. Identified peaks: (1) DMDS, (2) pyrimidine, (3) DMTS, (4) phenol, (5) *p*-cresol.

For the TWA-SPME method, RSDs were 2.1 to 8.4% and 9.8 to 13.2% for tests I and II, respectively. For sorbent tube-based method, RSDs of test I were 4.1 to 7.9 % and those of test II were 5.8 to 13.1%. These RSDs indicate a high degree of precision of both measurement methods. In general, there was a good agreement between the TWA concentrations obtained from the AeD reactor using the 85 μm CAR/PMDS fiber and sorbent tube-based method (Table 1). Differences of measured VOC concentrations between the two methods in the test I were 13.2, -3.6, 8.3, 10.9, and 14.5% for DMDS, DMTS, pyrimidine, phenol, and *p*-cresol, respectively. These were reported in test II as -1.6, -7.0, 12.7, -11.6, and -1.1 %. No statistically significant difference was observed (one-way ANOVA test with p-values > 0.05) between two sampling methods.

Table 1. Comparison of measured VOC concentrations of 5 target gaseous VOCs released from poultry carcass aerobic decomposition using TWA-SPME and sorbent tube-based sampling methods. Gas samples from (n=4) aerobic reactors were collected on Day 11 and 42 of aerobic digestion trial. Diffusion coefficients were calculated using the FSG model. All concentrations were adjusted to standard temperature and pressure.

VOCs	Measured gas concentration using TWA-SPME (µg/L)	RSD (%)	Measured gas concentration using sorbent tubes (µg/L)	RSD (%)	Diff. (%)	*p*-values
Test #1 (11th day of aerobic digestion trial)						
DMDS	$5.23\times10^{-1} \pm 5.14\times10^{-2}$	2.1	$4.58\times10^{-1} \pm 2.28\times10^{-2}$	5.0	13.2	0.083
DMTS	$1.23\times10^{-1} \pm 1.21\times10^{-2}$	2.3	$1.28\times10^{-1} \pm 6.51\times10^{-3}$	5.1	-3.6	0.539
Pyrimidine	$1.95\times10^{-3} \pm 2.30\times10^{-4}$	8.4	$1.79\times10^{-3} \pm 7.36\times10^{-5}$	4.1	8.3	0.268
Phenol	$1.57\times10^{-1} \pm 2.07\times10^{-2}$	7.1	$1.40\times10^{-1} \pm 1.12\times10^{-2}$	7.9	10.9	0.226
p-Cresol	$6.32\times10^{-2} \pm 8.01\times10^{-3}$	3.7	$5.47\times10^{-2} \pm 3.31\times10^{-3}$	6.1	14.5	0.121
Test #2 (42nd day of aerobic digestion trial)						
DMDS	$8.00\times10^{-2} \pm 1.70\times10^{-3}$	9.8	$8.13\times10^{-2} \pm 1.07\times10^{-2}$	13.1	-1.6	0.822
DMTS	$1.56\times10^{-3} \pm 3.61\times10^{-5}$	9.8	$1.67\times10^{-3} \pm 9.75\times10^{-5}$	5.8	-7.0	0.093
Pyrimidine	$1.40\times10^{-3} \pm 1.18\times10^{-4}$	11.8	$1.24\times10^{-3} \pm 9.98\times10^{-5}$	8.1	12.7	0.072
Phenol	$1.23\times10^{-2} \pm 8.68\times10^{-4}$	13.2	$1.38\times10^{-2} \pm 8.40\times10^{-4}$	9.1	-11.6	0.066
p-Cresol	$2.50\times10^{-3} \pm 9.37\times10^{-5}$	12.7	$2.53\times10^{-3} \pm 1.21\times10^{-4}$	7.8	-1.1	0.739

4. Conclusions

A new method based on the TWA-SPME concept was developed and validated for sampling and quantification of five biomarker VOCs in a complex matrix of gases and potentially infectious aerosols associated with an aerobic digestion (AeD) process. The TWA SPME approach demonstrated a relatively simple sampling and sample preparation method that can be used for process gas monitoring without the need of more sophisticated and dedicated hardware

and instrumentation, making it attractive for scientists and engineers working with low budgets and limited laboratory equipment. Results from this study indicate that:

i. The amount of VOC adsorption on the metallic SPME needle housing was reproducible and ranged from 3.91 to 6.20 % of that measured on the SPME fiber alone. As its sorptive limit was approached, the linear extraction (i.e., the increase of sorbed mass with time) no longer applied to the needle housing. However, the amount adsorbed onto metallic needle housing can be easily determined and subtracted from the total mass extracted by SPME, making the Fick's first law of diffusion model applicable for routine calibrations and process gas sampling/analysis.

ii. VOC mass uptake by retracted SPME fiber was in agreement with Fick's first law of diffusion. The retracted SPME fiber responded proportionally to sampling time and changing concentration of analytes. Retracted SPME behaved as a zero sink for the target analytes, i.e., the mass loading rate of additional analytes was not affected by the amount of analytes already sorbed on the fiber during sampling for optimized sampling conditions.

iii. There was a very good relationship between extracted mass $n(t)$ and the reciprocal of retraction distance Z. The use of Z = 5 mm and t = 30 min sampling time resulted in the lowest average difference (8.8% and 12.3%, respectively) between the predicted and the measured mass extracted.

iv. The method detection limits were 1.50, 0.70, 1.50, 8.44, and 5.76 ppbv for DMDS, DMTS, pyrimidine, phenol, and *p*-cresol, respectively. This new method was significantly more sensitive (based on MDLs) than the standard methods (NIOSH 2542 for DMDS, and

458 DTMS, MDLs = 200 to 10,000 ppbv; NIOSH 2546 for phenol and *p*-cresol, MDLs = 250
459 to 15,000 ppbv; There is no standard method for pyrimidine.
460 v. The new method is accurate and repeatable (i.e., no statistically significant difference was
461 observed differences of measured concentrations between TWA-SPME and the sorbent
462 tube-based method).
463 Additionally, this study demonstrated that the TWA-SPME technique could be used to
464 quantify the target VOCs found in complex process gas samples emitted from AeD of poultry
465 carcasses in emergency situations. This method could be easily adapted for qualitative and
466 quantitative analysis of VOCs emitted from food and beverage processing industries, health,
467 environment and forensic applications where practical, minimally-invasive monitoring and high
468 sensitivity is needed.
469
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