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Characterization of Volatile Organic Compounds from Human Analogue Decomposition Using Thermal Desorption Coupled to Comprehensive Two-Dimensional Gas Chromatography−Time-of-Flight Mass Spectrometry

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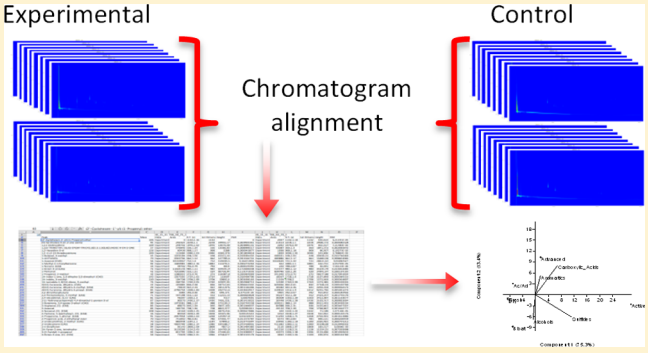
Supporting Information

ABSTRACT: Complex processes of decomposition produce a variety of chemicals as soft tissues, and their component parts are broken down. Among others, these decomposition byproducts include volatile organic compounds (VOCs) responsible for the odor of decomposition. Human remains detection (HRD) canines utilize this odor signature to locate human remains during police investigations and recovery missions in the event of a mass disaster. Currently, it is unknown what compounds or combinations of compounds are recognized by the HRD canines. Furthermore, a comprehensive decomposition VOC profile remains elusive. This is likely due to difficulties associated with the nontarget analysis of complex samples. In this study, cadaveric VOCs were collected from the decomposition headspace of pig carcasses and were further analyzed using thermal desorption coupled to comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (TD-GC × GC−TOFMS). Along with an advanced data handling methodology, this approach allowed for enhanced characterization of these complex samples. The additional peak capacity of GC × GC, the spectral deconvolution algorithms applied to unskewed mass spectral data, and the use of a robust data mining strategy generated a characteristic profile of decomposition VOCs across the various stages of soft-tissue decomposition. The profile was comprised of numerous chemical families, particularly alcohols, carboxylic acids, aromatics, and sulfides. Characteristic compounds identified in this study, e.g., 1-butanol, 1-octen-3-ol, 2-and 3-methyl butanoic acid, hexanoic acid, octanal, indole, phenol, benzaldehyde, dimethyl disulfide, and trisulfide, are potential target compounds of decomposition odor. This approach will facilitate the comparison of complex odor profiles and produce a comprehensive VOC profile for decomposition.



Decomposition chemistry is a multidisciplinary field within forensic science that investigates chemical processes occurring post-mortem and their impact on the surrounding environment. The process of decomposition begins immediately after death and is visible as a series of gross events.[1] Although these events and the various stages of decomposition have been documented and are relatively predictable, the underlying chemistry of decomposition is not as well understood. The two principal processes of decomposition are autolysis and putrefaction. Autolysis is the enzymatic self-digestion of cells. Digestive enzymes from within cells degrade outer membranes releasing the intracellular components: proteins, carbohydrates, and lipids.[2] These macromolecules are then broken down by bacteria into their constitutive building blocks through a process known as putrefaction.[2] Putrefaction produces several of the visible features of decomposition, most notably color changes, gases, and bloating. Additionally, the putrefactive breakdown products may produce distinctive VOCs that can be utilized for the detection of human remains.[3−7]

The recovery of remains is important in the event of a mass disaster as well as during forensic investigations. Processes of decomposition are highly dependent on the environment, but the deposition of remains itself will also influence the

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surrounding environment by altering its visible appearance, vegetation, soil characteristics, and odors.[8−11] The variety of methods available for the detection of remains relies on this alteration of the environment and includes aerial photography, thermal imagery, geology, botany, geophysical methods such as ground penetrating radar, and human remains detection (HRD) canines.[9,11] HRD canines are specifically trained to recognize the scent of human decomposition and utilize their air scenting abilities to indicate its origin.[12,13] Although HRD canines are commonly used by search and rescue teams and police forces, little is known about how they differentiate and recognize the general odor of human decomposition.[14,15] Volatile organic compounds (VOCs) are known to be odorous molecules;[16] however, VOCs produced by the decomposition of remains has only recently begun to be investigated,[14,17−25] and a consistent list of compounds that accurately describes the decomposition scent is yet to be elucidated.[3]

Discrepancies in profiles reported in the literature are likely the result of the variation in methods used for collection and analysis, the tissues utilized, and the biotic and abiotic factors within the decomposition environments.[3,19] Current published studies have been conducted in a variety of specific environments; e.g., burial in the southern United States,[24,25] discovered remains on the shores of Greece,[22,23] and terrestrial environments in Belgium.[17,19,20] Decomposition is a dynamic process highly influenced by the environment in which it takes place.[6,26−28] Many factors such as temperature, access to oxygen, and microbial community will affect the process and therefore the production of VOCs.[3,19,27] In the hope of characterizing the compounds used by canines for HRD, it is necessary to identify the complete decomposition VOC profile, while taking into account the natural VOC profile produced within that environment. Utilizing advanced analytical methods aids in identifying compounds that are particular to decomposition and can be applied to the development of canine training strategies.

Decomposition VOC studies have primarily used chromatographic techniques such as conventional one-dimensional (single-column) gas chromatography (1DGC) and mass spectrometry.[11,17,19−25] Conventional ^{1}D GC is the method of choice for a variety of applications due to the versatility of available capillary column phases.[29] However, applying this chromatographic method for nontarget analysis of complex samples such as decomposition headspace is difficult as adequate separation of each individual constituent may not be achieved. An alternative tool for the analysis of these samples is comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC−TOFMS). Detailed descriptions of this method are available in the literature.[29−32] In practice, two GC columns coated with different stationary phases are coupled in series. A modulator is used to continuously sample narrow bands of all the species eluting from the first column (first dimension, ^{1}D) and to reinject them into the second column (second dimension, ^{2}D).[33] The high sampling rate (modulation period, P_M) of the modulator ensures that the separation achieved in ^{1}D is maintained through the entire separation process. High speed ^{2}D chromatograms of typically 2−12 s of length are thus generated and recorded at the detector. The combination of these ^{2}D chromatograms allows a two-dimensional visualization (first dimension, $^{1}t_R$, and second dimension, $^{2}t_R$, retention times) of the separation process that illustrates the increased peak capacity. Because peak widths in ^{2}D are in the range of 50−400 ms, a fast acquisition detector is required to properly reconstruct elution currents. TOFMS is capable of high (up to 1 000 Hz) acquisition rates and is the detector of choice.[29] Additionally, compared to scanning MS instruments that scan from high to low masses while the chromatographic peak is eluting, nonscanning TOFMS acquire full mass spectra at once and the data collected are therefore not subject to mass skewing.[34] This allows spectral data to be used in deconvolution algorithms capable to separate unique mass spectra to solve chromatographic coelution issues in the mass spectral domain and improve compound identifications. The combination of both retention times obtained from GC × GC and the deconvoluted spectral data makes such a system powerful in terms of accurate identifications of compounds in a complex mixture.[29,30,35,36] Recent research has shown the improved separation and identification of decomposition VOCs using GC × GC, compared to conventional ^{1}D GC.[17,19]

In addition to the GC−MS instrument employed, the method of sample collection is also important for ensuring the complete VOC profile is trapped and analyzed. The most common sample collection techniques for the analysis of decomposition VOCs have been solid-phase microextraction (SPME),[14,21,33] thermal desorption (TD),[11,22−25] and solvent desorption.[17,19,20] Although solvent desorption provides a liquid sample that can be stored, the resulting large solvent peak can mask the more volatile compounds.[17,19,34]

Few studies have combined thermal desorption (TD) and GC × GC−TOFMS,[35,36] and to date no published studies have utilized this methodology for decomposition headspace analysis. However air sampling using TD tubes provides several advantages compared to other approaches. TD tubes are available in single or multiple sorbents formats, they are made of robust stainless material, they can easily be loaded using simple pumping devices, they present high field portability, and TD devices allow splitting of the trapped VOCs between the injector and a secondary tube that can be used for duplicate analysis or archiving.[37,38] In contrast, SPME offers less possibilities in terms of sorbent phase combinations and generally requires an equilibrium status to be reached between the sample matrix, the headspace, and the fiber, which is not always feasible in a field setting.[34,39] Both methods can be used for qualitative and quantitative analysis; however, the introduction of an internal standard is more challenging with SPME analyses. For those reasons and because of the novelty of combining TD with GC × GC−TOFMS for decomposition odor analysis,[11,19] TD tubes were used for trapping cadaveric VOC in this study.

GC × GC−TOFMS analyses produce a wealth of complex information that requires a robust data processing approach. Current software packages available for chromatogram alignment prior to the cross comparison of samples exhibit various levels of success. On the basis of an earlier report on the use of the Fisher ratio analysis procedure for third-order separation data,[40] a statistical comparison tool has been made available as a commercial data processing software.[41] It was reported by Koh et al.[42] that this statistical comparison tool was more efficient for the alignment of chromatograms than other open source software. The application of this tool to large data sets decreases the time required for data processing and increases the statistical robustness of the results. The aim of this study was to apply an optimized GC × GC−TOFMS method coupled with TD to the analysis of decomposition VOCs and utilize the statistical comparison tool to distinguish composition

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differences between decomposition and control samples in order to characterize the volatile breakdown products of soft-tissue decomposition.

EXPERIMENTAL SECTION

Human Analogues. Because of ethical restrictions associated with the use of human remains, pig carcasses (*Sus scrofa domesticus*) were used as human analogues because of their similarities to an adult human torso.[8,26,43−45] In forensic taphonomy studies, pig carcasses are considered similar to humans remains as they share a comparable internal anatomy and fat distribution across the torso, they both lack heavy fur, they are omnivores, and they are likely to share a similar internal gut flora.[43,45] Additionally, pig carcasses were utilized in this study to identify similarities/differences in the decomposition scent when compared to literature published for human decomposition scent. This information was required by local police canine handlers to determine whether porcine remains were suitable alternative training aids for HRD dogs in Ontario, Canada.

Experimental Field Setup. The decomposition trial was conducted in an enclosed grassy area in Oshawa Ontario, Canada (43.947° N, 78.898° W) during late summer (July and August 2011). Two adult pig carcasses (23 kg) were killed by captive head bolt and transported in accordance with guidelines set out by the Government of Ontario's Ministry of Agriculture, Food and Rural Affairs. Both carcasses were placed directly on the soil surface, on top of light grassy vegetation, within an hour of death. Wire cages were placed over the carcasses between sampling periods to prevent avian and mammalian scavenging while allowing full access to the local entomological fauna. Samples were collected (as outlined below) every second day throughout all stages of soft-tissue decomposition and once when the remains were determined to be in the dry remains stage. Two corresponding control sites without carcasses were established adjacent to the experimental site and sampled following the same procedure and schedule as the experimental carcasses. A weather station containing a HOBO data logger was placed at the facility and recorded the temperature hourly. The accumulated degree days (ADD) for each sampling day was calculated by summing the average daily temperature (°C) recorded by the data logger. The precipitation level was measured with a field rain gauge (mm), and accumulations were recorded during field observations. The decomposition stage and general observations were also documented at each site visit.

Sample Collection. The headspace above the carcasses was accumulated for 30 min prior to sampling by covering the remains with a stainless steel hood measuring 100 cm × 70 cm × 40 cm (280 L). The hood was fixed with a stainless steel bulk head connector which fastened the sampling tube to the hood creating a continuous path from the inside of the hood to sample the VOCs. During sampling, one end of the sampling tube was fixed to the bulk-head union and the other was connected to a LaMotte (Chestertown, MD) model BD constant flow air sampling pump. Approximately 1 L of headspace was collected onto a multisorbent thermal desorption tube containing Tenax GR (2,6-diphenylene-oxide and 30% graphite) and Carbopack B (poly(ethylene glycol) and nonsilicone phase coating) (Markes International Ltd., Llantrisant, U.K.) at a rate of 0.2 L/min. Following sample collection, tubes were capped with brass long-term storage caps fitted with PTFE ferrules and placed in a sealed mason jar for storage. Prior to desorption and analysis, 1 μL of a 60 ng/μL bromobenzene/methanol internal standard (IS) was added to the sampling tube using a standard GC injection syringe. Bromobenzene was used as the IS in accordance with previous decomposition VOC literature.[24,25] Chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany).

Thermal Desorption Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (TD-GC × GC−TOFMS). Thermal desorption and injection of samples was carried out using a Markes International Ltd. (Llantrisant, U.K). Unity 2 series thermal desorber. The sample tube was desorbed at a temperature of 300 °C for 5 min. Samples were recollected on a cold trap with a Tenax sorbent bed at a temperature of −10 °C. Desorption of the cold trap occurred at 300 °C for 3 min. The split flow during desorption and injection was adjusted according to decomposition in order to avoid saturation of the MS ion source. The GC × GC−TOFMS instrument was the unit-mass resolution Pegasus 4D (LECO, Corp., St. Joseph, MI). The modulator was mounted in an Agilent 7890 gas chromatograph equipped with a secondary oven and a quad-jet dual stage modulator. Liquid nitrogen was used to create the cold jets. Details regarding the system have been reported elsewhere.[30,46] The GC oven temperature was initially set at 35 °C and held for 5 min before being increased at 5 °C/min to 240 °C where it was held for another 5 min. The temperature offset for the secondary oven was 5 °C and operated in the iso-ramping mode. The temperature of the modulator had an offset of 10 °C compared to the temperature of the primary GC oven. Modulation was carried out on the very beginning of the ^{2}D column. The P_M was 4 s with a hot pulse duration set at 700 ms and a cooling time between stages of 1300 ms. Carrier gas was helium and a constant flow of 0.8 mL/min was used. The GC column set used was made from the combination of a 30 m × 0.25 mm i.d. Rxi-5Sil, 5% phenyl 95% dimethylpolysiloxane (Restek Corp., Bellefonte, PA) with a film thickness of 0.25 μm as ^{1}D and a more polar 1.2 m × 0.10 mm i.d. mid polar BPX-50, 50% phenyl polysilphenylene-siloxane (SGE, Austin, TX) with a film thickness of 0.10 μm as ^{2}D. This column set was already successfully used in previous VOC mixtures analysis from pig decomposition.[17,19,52] Deactivated universal presstight connectors (Restek Corp.) were used for connecting the capillary columns. The transfer line connecting the secondary column and the MS source was operated at a temperature of 250 °C. The source temperature was 250 °C, operated in electron ionization mode with a filament bias voltage of −70 eV. The data acquisition rate was set at 100 spectra/s for a mass range from 29 to 450 m/z. The detector voltage was 1500 V. Daily mass calibration and tuning were performed using perfluorotributylamine (PFTBA).

Data Processing. GC × GC−TOFMS data were first acquired and processed with the ChromaTOF (4.42) software (LECO Corp.).[41] This software was used for peak apex finding, mass spectral deconvolution, library searching, and integration. The combination of slices corresponding to a compound was performed by comparing the mass spectra under pre-established match criteria. Wiley (2008) and National Institute of Standards and Technology (NIST, 2008) databases were utilized for spectral identifications with a match factors threshold >700. A detailed description of the raw data processing procedure can be found in previous reports.[19,47] Following alignment of data processing tables, statistical information from various peak calculations was compared

from each class and between classes. Fisher ratios were calculated from the compound table for each analyte in order to identify compounds showing the highest variance. The IS was used to calculate peak area ratio in order to normalize peak areas prior to statistical analysis.[48] Results were finally exported as .csv files and applied to external principal components analysis (PCA) software. PCAs were conducted using PAST 2.14 statistical software.

RESULTS AND DISCUSSION

GC × GC−TOFMS Optimization. The analysis of decomposition VOCs requires a powerful separation technique such as GC × GC−TOFMS, which provides distinct advantages in terms of resolution and separation over conventional 1D GC. To obtain the maximum capacity, the following separation parameters were optimized: temperature range, temperature ramp, and modulation period. In order to optimize these parameters, replicate injections were required. However, because of the limitations in terms of sampling and repeat injections of thermal desorption, samples collected previously from porcine decomposition and desorbed by diethyl ether elution were utilized.[19] These samples were collected during active decomposition and were representative of the sample type to be analyzed with TD-GC × GC−TOFMS.

The first optimized parameter was the temperature range. The initial temperature was fixed at 35 °C to correlate with the boiling point of diethyl ether and to ensure the more volatile compounds of interest would be separated efficiently. For the final temperature, 280 °C was initially selected based on the previous literature[25] and was held for 5 min. However, no compounds were being detected in the last minutes of the run and therefore the final temperature was progressively decreased down to 240 °C, which was found to be the optimal temperature to elute all volatile compounds.

The second step in the optimization was to determine the optimal oven temperature ramp. In classical ^{1}D GC, a slow temperature ramp may result in undesirable peak broadening and consequently poor chromatographic resolution. In GC × GC, because of the modulation step, limited peak broadening is less critical as enough ^{1}D peak width is required to ensure proper modulation is performed and satisfies the conservation rule. A compromise temperature ramp was thus optimized to ensure proper modulation without sacrificing ^{1}D resolution. A range of different temperature ramps was tested, from 1.5 °C/min to 10 °C/min. To determine the impact of this parameter, the peak dispersion in the ^{2}D space was also taken into account to optimize the use of the chromatographic plane for the separation of the sample components. A faster ramp (10 °C/min) concentrated all the peaks along the ^{1}D axis, with a poor utilization of chromatographic space. Decreasing the ramp speed to 1.5 °C/min increased the peak dispersion on the ^{2}D axis. However the peaks still clustered in a sigmoidal shape across the chromatogram. A compromise between the two extremes was reached, and the optimal temperature ramp was 5 °C/min. The optimization of P_M was simultaneously performed to minimize wrap-around and the loss of ^{1}D separation by producing good slicing of ^{1}D peaks.[29,30,49] A P_M of 4 s was selected as the optimal parameter for this analysis. These conditions were used for the analysis of the decomposition headspace.

Data Reduction. In the analysis of complex mixtures, the data analysis is the most time-consuming part. This is particularly true for nontarget analyses where the cross-sample alignment and comparison is a critical step.[42,48,50] During alignment, the data processing software utilizes algorithmic comparisons of peak features (^1t_R, ^2t_R, and mass spectra) to correct for variations during cross-sample comparison.[42,50,51] GC × GC−TOFMS allows for complete characterization of complex VOC mixtures; however, the data obtained across all samples represents a considerable amount of information to be treated. In order to identify meaningful compounds during nontarget investigations, both chromatographic and spectral information within the data sets are required for subsequent statistical analysis.[48]

Following initial processing using ChromaTOF software, including spectral deconvolution and MS library searching,[19] the statistical comparison feature of the software was used to compare the VOC profile of pig and control samples for each sampling day. It relies on a mass spectral match criterion of 60% to align multiple chromatograms and integrate peak area.[42] Fisher ratios were calculated to highlight unknown chemical differences among known classes of samples. The aligned data sets were exported to Microsoft Excel spreadsheets for further data handling. Within Excel, relative peak area ratios were calculated against the IS and compounds were grouped into 1 of the 11 following chemical classes: alcohols, aldehydes, aromatics, carboxylic acids, esters, halogens, hydrocarbons, ketones, nitrogens, sulfides, and others. In practice, the initial data processing identified approximately 10 000 hits over the entire set of experimental chromatograms. These hit tables included VOCs issued from both the environment and the pig decomposition as well as instrument related signals such as column bleeds and multiple artifact hits resulting from various levels of peak tailing. Following data reduction and prestatistical treatment, the data matrix contained above 300 peaks that were found to be specific to decomposition and present on at least one experimental day. This matrix was used to perform PCA. A list of the major decomposition VOCs present in each chemical family and the time they were detected across the soft-tissue decomposition process are presented in Table S-1 in the Supporting Information. The number, types, and distribution of compounds during soft tissue decomposition are similar to those found by previous researchers.[19] However, the use of the statistical comparison tool allows increased automation and is less time-consuming. In particular, this tool utilizes raw peak tables generated by the initial ChromaTOF processing, therefore eliminating manual screening and handling of the data. A GC × GC apex plot recorded on experimental day 6 (ADD 150.2) is shown in Figure 1 and demonstrates the variety of compounds identified using this data processing methodology.

Decomposition Process. The average daily temperature throughout the study was 21.1 °C. The average daily high was 30.3 °C with an absolute maximum of 41.9 °C. The average daily low was 16.1 °C with an absolute minimum of 8.4 °C (Figure S-1 in the Supporting Information). The total rainfall for the period of study was approximately 160 mm (Figure S-1 in the Supporting Information). The carcasses exhibited both autolytic and putrefactive changes as soft tissue decomposition progressed through the five stages of decomposition. On the day of deposition (day 0, ADD 0) the carcasses were in fresh stage, and by day 2 (ADD 54.9) the carcasses were in the bloat stage and exhibited the characteristic distension of the torso due to the accumulation of gases. Differences in the duration of the active decay stage between the two carcasses were observed,

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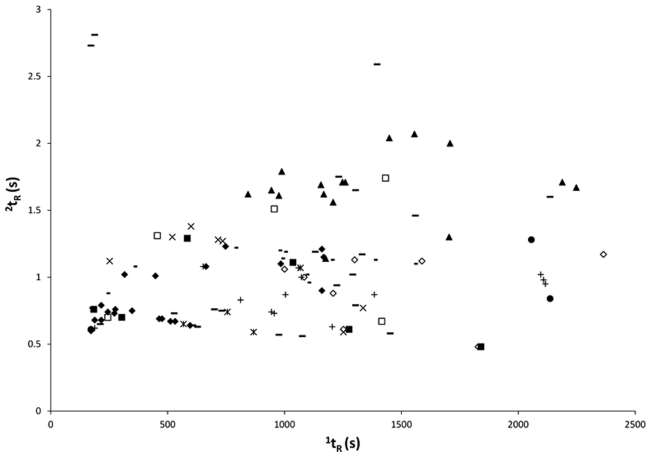

Figure 1. GC × GC apex plot of decomposition VOCs identified using Statistical Compare on experimental day 6 (ADD 150.2): (◆) alcohols, (■) aldehydes, (▲) aromatics (×) carboxylic acids, (∗) esters, (●) halogens, (+) HCs, (−) ketones, (—) nitrogens, (◊) others, (□) sulfides.

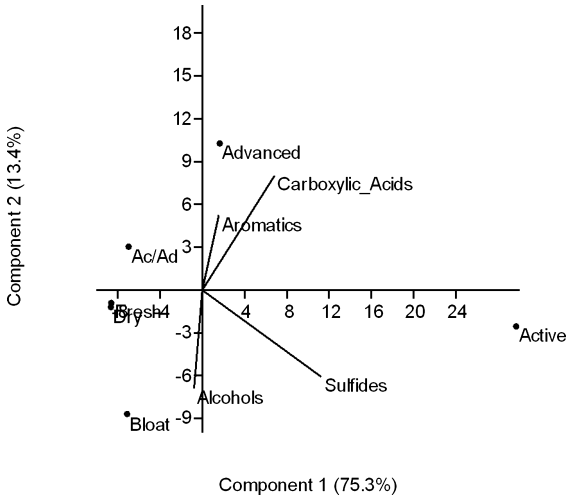

Figure 2. Principal component analysis scatter plot of the calculated PCA scores. The peak area ratios for each compound were summed to produce the total peak area ratio for a compound class. These values were then averaged across each decomposition stage and input for PCA analysis.

from day 4 (ADD 107.2) to day 6 (ADD 150.2) and from day 4 to day 8 (ADD 191.7), respectively. This stage was characterized by large amounts of insect activity, the rapid loss of soft tissue, and the purging of fluids. Following active decay, the advanced decay stage progressed through day 11 (ADD 260.9) and was last observed on day 14 (ADD 326.5), at which point there was minimal soft tissue remaining. On the final sampling day (day 40, ADD 845.7), the carcasses were characterized as being in the dry remains stage, consisting mainly of bones and desiccated skin.

Decomposition Chemistry and VOCs. In order to characterize the relationship of the VOC classes between decomposition stages, PCA was conducted. PCA is an Eigen analysis that projects multivariate data onto a new principal components axis which account for as much of the variance of the original data set as possible. Each principal component (PC) extracts a portion of the variance in the original data, with the first PC extracting the greatest amount of variance. A scatter plot of the calculated principal component scores is shown in Figure 2 and is representative of both carcasses.

Each stage is separated across the four quadrants of the plot except for the fresh and dry remains stage. Although decomposition is a continuous sequence of events, this separation of the stages illustrates that a distinct combination of processes may characterize each stage. The clustering of the fresh and dry remains stage is likely due to the decrease in VOC production following soft-tissue decomposition. Human remains detection becomes more difficult after extended post-mortem intervals when no soft tissue remains, indicating a need for further investigation into the decomposition VOCs during such time intervals.

Vectors of Figure 2 represent variables (chemical class) that explain the distribution of the points. The bloat stage was characterized by higher levels of alcohols and sulfides; active decay demonstrated high levels of alcohols, sulfides, carboxylic acids, and aromatics; whereas advanced decay exhibited higher levels of carboxylic acids and aromatic compounds. Although the levels of the various compounds were variable between the two carcasses, the same relative trends were seen in both. Overall, the greatest number and diversity of compounds was found during active decay and the onset of advanced decay. This is consistent with the decomposition observed in the field as it is during these stages that there was a marked loss of soft-tissue, and a distinctive odor was associated with the remains. In addition to the chemical classes identified by the PCA, other compounds were identified including aldehydes, ketones, hydrocarbons (HC), and nitrogen containing compounds (Table S-1 in the Supporting Information).

Human soft tissue comprises approximately 20% protein, 10% fat, and 1% carbohydrates.[52] Through decomposition, these tissues are progressively broken down into the basic building blocks such as amino acids, fatty acids, and sugars, respectively. The four major classes of compounds identified by PCA, alcohols, sulfides, carboxylic acids, and aromatics, are a result of putrefactive breakdown of these molecules and include potential target compounds of soft tissue decomposition.

The predominant alcohols identified across decomposition were short chain alcohols ranging from three to eight carbons in length. These alcohols could have originated from a variety of sources including the bacterial degradation and fermentation of amino acids, fatty acids, and carbohydrates.[3,53] As a class, alcohols demonstrated a trend with three maxima occurring at ADD 55.0, 150.2, and 260.9 (Figure 3a). The early stages of putrefaction exhibited high levels of 1-propanol and 1-butanol whereas later putrefactive decay included longer chains such as 1-octen-3-ol. This shift in the predominant alcohols found in the decomposition headspace could be explained by the change in the availability of oxygen within the remains and the resultant bacterial metabolism.[52] The metabolism of threonine by *Clostridium sp.*, an anaerobic organism, produces 1-propanol[53] whereas the oxidation of fatty acids such as linoleic acid by fungi can produce 1-octen-3-ol.[3,54] Although not a dominant group as identified by the PCA, aldehydes and ketones had higher levels in the later stages of decomposition (peaks at 191.7 and 260.9 ADD, respectively), as shown in Figure 3a. Dominant compounds of these classes included butanal,3-methyl, octanal, 3-octanone, and 1-octene-3-one. These classes of compounds are also believed to be a result of aerobic degradation of fatty acids.[3,26,52] Oxygen containing compounds reported here have also been identified as

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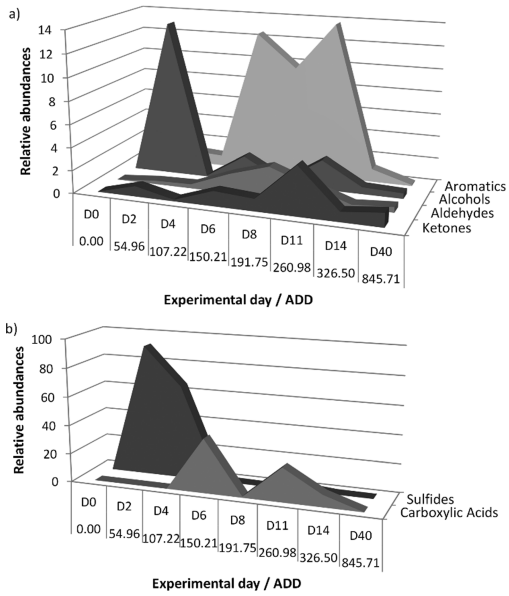

Figure 3. Relative abundances for compounds within the dominant chemical classes for (a) ketones, aldehydes, alcohols, and aromatic compounds and (b) carboxylic acids and sulfide compounds.

components of the decomposition VOCs profile within other studies.[11,19,21,22,33,55]

The sulfide group was heavily comprised of the poly sulfide compounds, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS) and dimethyl tetrasulfide (DMQS), and was dominant during early processes of decomposition, particularly the bloat stage (Figure 3b). The presence of these polysulfides was anticipated as they are one of the most reported compounds within decomposition headspace.[11,17,19−25,33] These compounds result from the desulfhydralation of sulfur containing amino acids such as cysteine and methionine and are commonly found in association with decomposing remains.[2,20−25] The metabolic products of amino acids, such as tyrosine, tryptophan, and phenylalanine yield the main compounds within the aromatic group, particularly phenol, 1H-indole, 1H-indole,3-methyl (also known as skatole), as well as several benzoic compounds such as benzonitrile and benzaldehyde.[3,4] Aromatics such as benzaldehyde[11,17,19,21,25] and phenol[11,17,19,20,22] are commonly found in decomposition headspace. However, compounds such as indole and skatole that are generally expected to be associated with decomposition[5,26] have been reported in fewer studies.[17,19,21] Swann et al.[56,57] identified selected biogenic amines and amino acids within purged decomposition fluid over the active and early advanced stages of decay. The aromatic compounds identified in the current study were predominant across the same stages (ADD 107.2−260.9), as shown in Figure 3a. It is likely that these compounds are released from the carcass within the fluid matrix and, upon release into the environment, volatilize into components of the decomposition headspace.

Predominant carboxylic acids identified during this study were two to six carbons in length and are also known as volatile fatty acids (VFAs). In particular, acetic acid, butanoic acid, 3-methyl and 2-methyl, and hexanoic acid were major components of the carboxylic acid profile. Decomposition research has identified VFAs, including those listed above, within decomposition fluid-soil solutions[4,57−59] and within decomposition headspace.[11,19−21,33] Swann et al.[58] identified the temporal trends of VFAs within decomposition fluid from pig carcasses during the summer months in Southern Ontario, Canada. Authors identified a cyclic trend with two maxima, the first and biggest occurring at ADD 126.0 and the second upward trend occurring at ADD 310.0.[58] A similar temporal trend was also observed during the current study with a maximum at ADD 150.2 and a second peak at ADD 260.9 (Figure 3b). The formation of VFAs from amino acids and/or carbohydrate degradations is an anaerobic process, facilitated by the carcass's intrinsic bacteria.[3,53,59] However, the second peak of VFAs occurred around the same time as some aerobic compounds (e.g., 1-octen-3-ol and 3-octanone), illustrating that the shift from a predominately anaerobic system to an aerobic one is not discrete but a gradual transition.

A recent paper by Dekeirsschieter et al.[19] investigated VOCs from pig carcass surface decomposition and, despite the decomposition occurring in different environments, they reported a similar VOC profile to this study. In both, the predominant compound classes included alcohols, carboxylic acids, aromatics, and sulfides along with aldehydes and ketones as major contributors to the overall profile. Additionally, the major compounds from within these classes were common to both studies, e.g., 1-butanol, 2-and 3-methyl butanoic acid, trimethylamine, 2-octanone, 1H-indole, DMDS, DMTS. Notably, Dekeirsschieter et al. conducted their study within a forested environment in Belgium during the spring season (March−May).[19] However, the current study took place in an open field in Southern Ontario, Canada, during the summer months (July−August). Despite differences in location (Walloon Brabant, Belgium vs Southern Ontario, Canada), decomposition environment (forest vs open field), and average temperature during the study (13.1 °C vs 21.1 °C), the analysis of decomposition headspace via GC × GC−TOFMS has produced a consistent VOC profile. This suggests that although the decomposition environment clearly impacts the process of decomposition and subsequently the VOC profile,[3,20,27] a consistent profile of decomposition VOCs can be identified across different geographical locations when utilizing the advanced capabilities of GC × GC−TOFMS.

This and other studies[11,17,19,20] have used pig carcasses as human body analogues in order to study volatiles from decomposition. While others have published results obtained from human remains,[21−25,33] to date there has been no direct comparison of the VOC profile between pig carcasses and human remains decomposed in the same environment. Pig carcasses are commonly used as human body analogues within decomposition chemistry and entomology research.[11,26,43−45] However, their use as human decomposition odor analogues has been questioned.[14] The primary argument against utilizing pig carcasses as human decomposition odor mimics is that their profiles show few similarities to those produced by human remains.[14] Nevertheless, the variability of the decomposition VOC profiles found in the literature is high[3] and few compounds have been consistently identified in all studies, with dimethyl disulfide being the most reported. A recent study[14] compared volatiles from animal tissues and indicated that the volatile profile of pig tissue was the "least similar" to that of the human profile. However, a number of the compounds reported by Cablk et al.[14] as missing from the pig profile have been recorded in this and other studies that analyzed the decomposition headspace of pig carcasses.[11,19,20] Some of these same compounds were additionally reported as "unique compounds not shared by any animal species",[14]

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including propanoic acid, pentanoic acid, hexanoic acid, and cyclohexanone. Although the current variability in VOC profiles makes comparisons between individuals and between species difficult, further investigation with high powered instrumentation such as TD-GC × GC−TOFMS will allow for more detailed comparisons and facilitate the characterization of the fundamental decomposition odor profile.

CONCLUSIONS

The aim of this study was to apply a novel method within decomposition VOC research, TD-GC × GC−TOFMS, to the analysis of decomposition VOCs and to utilize the statistical comparison tool of the software in order to characterize the volatile breakdown products of soft-tissue decomposition. The power of GC × GC−TOFMS coupled with thermal desorption was apparent in the number of peaks identified after initial processing. Handling the raw data using the statistical comparison option of the software allowed for a quick and efficient reduction of the identified compounds to those relevant to this study. The generated profile was able to characterize decomposition and identify potential key compounds of decomposition odor, exhibiting the usefulness of this method for the nontarget analysis and comparison of complex data sets. The key chemical families, alcohols, sulfides, aromatics, and carboxylic acids characterized the continuous process of decomposition and the dominant compounds, e.g., 1-butanol, 2-and 3-methyl butanoic acid, DMDS, DMTS, phenol, and indole, are potential target odorants of decomposition. Additionally this work demonstrated that the VOC profile of pig carcasses contains some similarities with human remains, particularly compounds that had previously been reported as being exclusive to the VOC profiles of human decomposition. Importantly, the utilization of TD-GC × GC−TOFMS has generated a decomposition VOC profile that was shown to be similar to other reported studies in distinct geographical locations. It is recommended that this methodology be applied to chemically profiling decomposition scent in alternative geographical locations and decomposition environments. A better understanding of the complete VOC profile will assist with the improvement of HRD canine training aids for the detection of remains within forensic investigations and mass disaster victim recovery.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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