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CLUSTER ISSUE

Reading Cadaveric Decomposition Chemistry with a New Pair of Glasses

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The chemical processes of human cadaver decomposition are complex and not well understood. The study of decomposition chemistry aims to elucidate the postmortem processes, particularly relating to the production of volatile organic compounds (VOCs) throughout the various decomposition stages. The use of thermal desorption coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (TD-GC×GC-TOFMS) has allowed for the VOC profile of decomposition odor above pig carcasses (human analogues) to be determined. An enhanced data-processing approach combining Fisher ratio calculations with principal component analysis assisted in the identification of the major classes of compounds that contribute to the VOC profile and their variation across decomposition stages. Detection and profiling of these VOCs is valuable for understanding the mechanisms by which human-remains detection (HRD) dogs locate victims in mass disasters and forensic investigations.

The study of human remains (HR) decomposition chemistry is motivated by the need for developing specific tools to locate dead or injured bodies during mass disaster victim-recovery cases, forensic investigations, and the search for clandestine graves. Nowadays, several approaches are available to locate HR; they include the use of ground-penetrating radar and thermal imagery, chemical analyses of soils, visual searches for modified environments, and the use of HR detection (HRD) canines.[1–4] To be efficient, HRD dogs require continuous training in the recognition of decomposition odor. However, it is unknown what compounds or combinations of compounds are recognized by HRD dogs and there is little consensus within the literature on the odor signature of human decomposition.[5] The current limited understanding of the decomposition process encourages the exhaustive chemical study of cadaveric decomposition mixtures.

Human cadaveric decomposition is a complex chemical process that is still not well characterized. It takes place soon after death and produces a large variety of organic molecules of which the nature and relative proportions are a function of the post mortem interval (PMI). Some of these molecules are volatile organic compounds (VOCs) and are, at least partly, responsible for the scent of decomposition. Temporally, several stages of decomposition can be defined,[6,7] mainly based on visual criteria. On a chemical basis, it starts with autolysis, the enzymatic self-digestion of cells that release intracellular components such as proteins, nucleic acids, lipids, and carbohydrates.[7] These components are further broken down by microorganisms such as bacteria and fungi. This catabolism of tissues starts the putrefaction process and results in the production of gases, skin color changes, and bloating of the corpse. The resulting excess amount of internal pressure leads to the purge of the fluids and starts the active decay stage of the decomposition. This is the stage where most of the decomposition molecules are released. Skatole (3-methylindole), indole, dimethyl disulfide, and other volatile fatty acids are known to be produced at this stage. Next to these, many additional molecules are, however, present in a broad range of concentrations. The production of VOCs slows down at the advanced decay stage at which the corpse dries out and tissues breakdown until the only remains are bones and hair.[8]

For physiological, biochemical, ethical, and legal reasons, domestic pigs (*Sus scrofa domesticus*) are conventionally used as surrogates for the study of human decomposition.[9] Typically, gas chromatography coupled with mass spectrometry (GC–MS) is used to separate and identify molecules trapped in the headspace of pig carcasses.[10] The most commonly used methods for trapping cadaveric VOCs are solvent desorption (SD) of solid sorbents,[6] solid-phase microextraction (SPME),[11] and direct thermal desorption (TD).[12] Because of the complexity of cadaveric VOC mixtures, classical single-dimensional GC (1DGC) suffers from peak-capacity issues and, despite the use of time-of-flight MS (TOFMS) deconvolution algorithms, cannot fully succeed in separating all possible compounds of interest.

Comprehensive two-dimensional GC (GC×GC) coupled with TOFMS[13] is a powerful tool that combines several advantages such as enhanced peak capacity, enhanced sensitivity, structuration of the chromatogram, and deconvolution of unskewed mass spectral data.[14] Both SD[6] and TD[15] approaches have recently been combined with GC×GC-TOFMS to investigate VOC

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samples produced during various stages of pig carcass (human analogues) decomposition. These independent studies illustrated the power of the approach in terms of isolation and identification of large numbers of cadaveric VOCs. Furthermore, this approach generated a decomposition profile after classical multivariate statistical treatment using principal component analysis (PCA).

Following these first successful attempts, and based on some of the issues that appeared in terms of comprehensive chemical comparisons across replicate samples of various stages of decomposition and control samples, we further developed the data-processing approach for the discovery of significant chemical differences between samples. It is based on supervised multiclass analysis by means of Fisher ratio (FR) calculation[16] for each analyte present across multiple samples. Compared to the direct PCA approach, this statistical comparison relies on the creation of different classes of samples (e.g., pigs versus controls, various stages of decomposition, etc.) prior to data processing. As the FR is the class-to-class variations divided by the sum of the within-class variations, this high-variance-based numerical value is used to rapidly identify analytes that are the most different between classes of samples. This FR calculation is of particular interest for classes of samples made of limited numbers of biological replicates, as is the case for cadaveric decomposition studies where using large numbers of cadavers is not possible.

A set of three duplicate cadaveric VOC samples produced during the active decay stage and their matching controls were analyzed by TD-GC×GC-TOFMS and processed using a pixel-based software package.[17] All GC×GC chromatograms were aligned based on a set of reliable peaks found across all chromatograms. A single composite image combining all these constituents was created and used as the processing template, after some background chromatographic noise was manually removed. Pixel regions were defined around each of the peaks and a template matrix containing retention times from both dimensions (^1t_R and ^2t_R), as well as the pre-deconvoluted mass spectral information was created. A total of 221 regions were delineated inside the composite image (Figure 1).

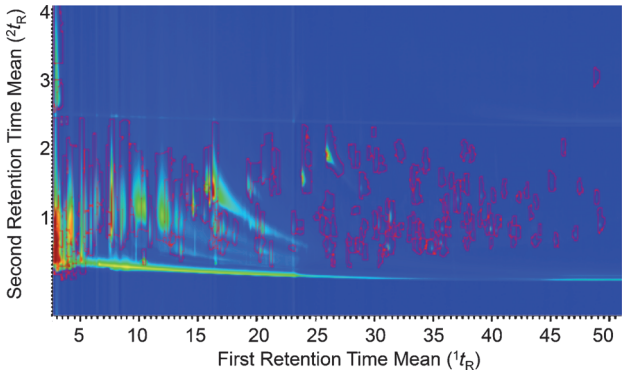

Figure 1. Composite image created from the combination of TD-GC×GC-TOFMS chromatograms generated by replicate analyses of cadaveric VOCs sampled during the active decay stage of pig carcass decomposition.

Inside each class, a mean volume value was calculated for all analytes (regions) and normalized to the sum of all volumes of the class. Within-class and between-classes variance was calculated and the percent-response FR values were obtained. FR values ranged between 6×10^{-3} and 1215. Because of the relatively limited number of regions, it was not necessary to apply any FR threshold in view of data matrix simplification. Figure 2

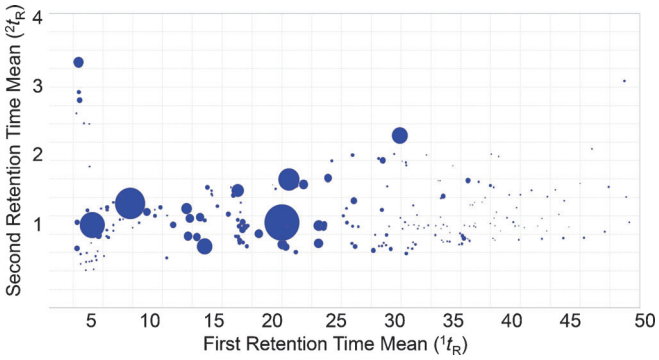

Figure 2. Two-dimensional Fisher ratio plots showing the biggest class-to-class differences between three days of active decay for the 221 regions defined inside the composite image.

illustrates the distribution of the FR values over the chromatographic retention plan. The distribution of the peaks (regions) of interest over the two-dimensional plot highlights the importance of the second-dimension separation. As previously reported, it further indicates that several different families of molecules are to be considered when performing chemical comparisons across replicate samples of various stages of decomposition. Based on the chemical logic responsible for the structured elution of these families, one could further imagine the development of a predictive model to facilitate the creation of a standardized active decay visual profile that could be used to screen VOC samples for PMI estimation. As for the portrayed snapshot of the decomposition process, carboxylic acids, aromatics, sulfurs, alcohols, nitro compounds, as well as aldehydes and ketones appeared to be the major contributors to the VOC signature. The imaging strategy could further be supported by implementing specific scripts to assist the classification of chromatographic peaks based on recognizable features in fragmentation patterns (presence of specific masses, specific ratio, specific losses, and so on).[18–20]

The high variance data provided by the FR calculations were exported to external software[21] for additional statistical treatment by means of PCA. This supervised multivariate analysis allowed supplemental data reduction and visual differentiation of the progression of active decay (Figure 3).

In summary, the use of TD-GC×GC-TOFMS for the characterization of VOCs present within the headspace of pig carcasses during decomposition appears to be efficient when coupled to specific data-mining strategies. The combined use of FR and PCA highlights the chemical variations during the most active stage of the decomposition process. Such an exhaustive approach will possibly contribute to a better understanding of

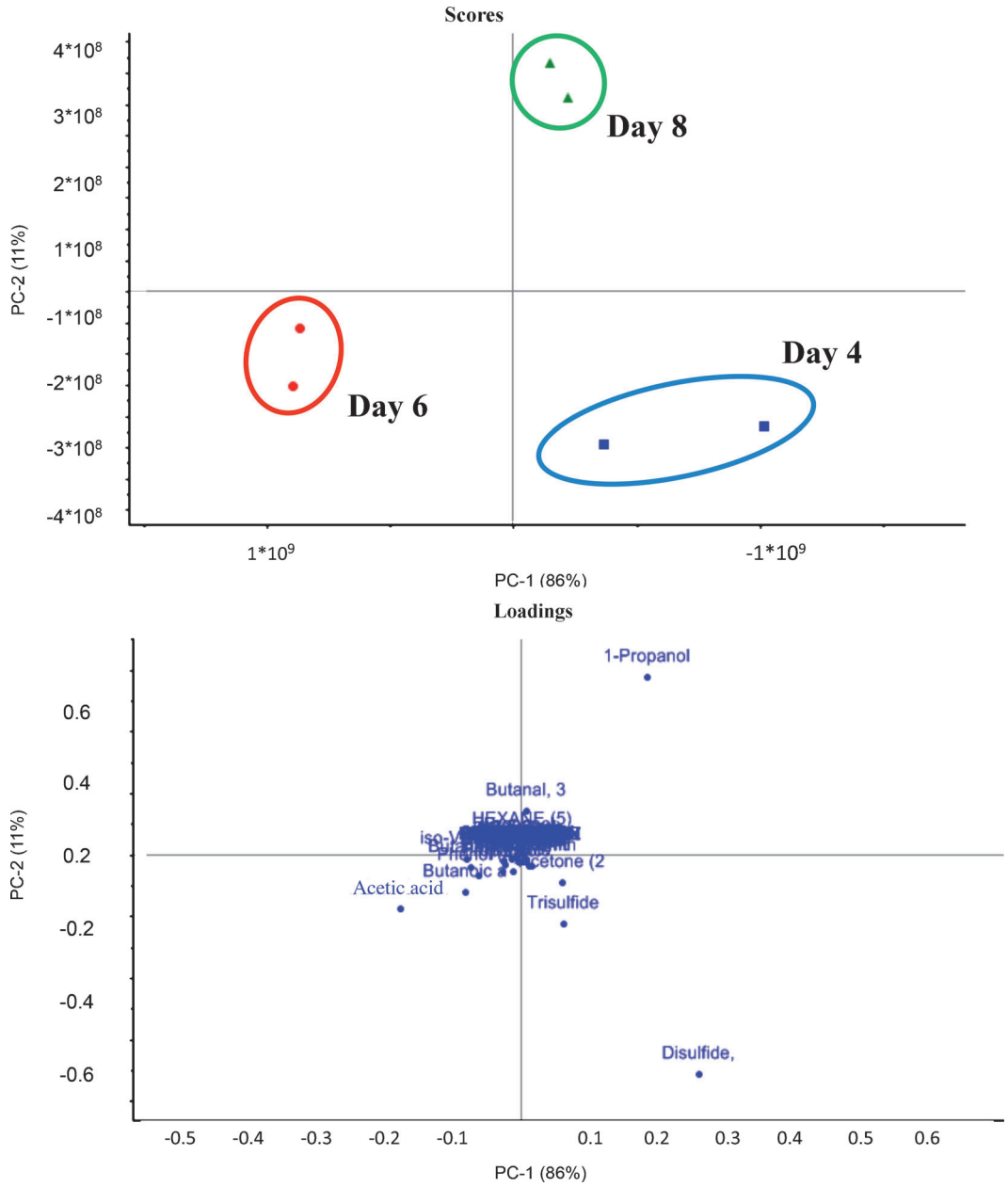

Figure 3. Principal component analysis score (top) and loadings (bottom) plots for the three days of active decay for the 221 regions defined inside the composite image.

the volatile characteristic profile recognized by HR dogs and further lead to the production of improved canine training aids.

Experimental Section

Samples and VOC collection

Samples were issued from an outdoor project. A field trial using pigs as human analogues was carried out at the University of Ontario Institute of Technology (UOIT) Geoforensic Research Facility (GRF) in 2012. A control site that contained no decomposition material was delineated within the area of the deposition site at a distance of 10 m. VOC collections were carried out by placing a sampling hood over the remains or control site for a period of 30 min and then connecting an adsorbent tube (Tenax GR/Carbopack B, which consisted of 2,6-diphenylene oxide and 30% graphite) to the sampling port of the hood. A volume of 1 L of headspace was sampled at a constant flow rate of 0.2 L min^{-1}. Tubes were capped, placed in air-tight containers, and stored at room temperature until analyses were performed.

Analyses

VOCs were thermally desorbed from the sampling tubes by using a Markes International Ltd. Unity 2 series thermal desorber (JSB, Belgium).[15] Following introduction by means of thermal desorption, analyses were performed using GC×GC-TOFMS. A Pegasus 4D

(LECO Corp., St Joseph, MI, USA) TOF mass spectrometer was used. This system is based on a nonmoving quad-jet dual-stage modulator made of two cold-nitrogen jets and two pulsed hot-air jets responsible for trapping and refocusing compounds eluting from 1D. This liquid-nitrogen modulator was mounted in an Agilent 7890 GC oven (Palo Alto, CA, USA). Further details on the modulation process are available elsewhere.[22] The carrier gas was helium at a constant flow. The GC column set used was made of the combination of an Rxi-5Sil MS fused-silica column (30 m × 0.25 mm inside diameter (i.d.) × 0.25 μm film thickness (d_f); Restek Corp., Bellefonte, PA, USA) as the first dimension and a Rxi-17Sil MS fused silica column (1.1 m × 0.1 mm i.d. × 0.1 μm d_f) as the second dimension. The GC × GC temperature program started at 35 °C, was held for 5 min, and increased to 240 °C at a rate of 5 °C min^{-1} where it was held for 5 min. The 2D oven temperature offset was 5 °C. The modulation period (PM) was 4 s with a hot pulse duration of 700 ms and a modulator temperature offset of 10 °C. The transfer-line temperature was set at 250 °C. The TOFMS ion-source temperature was 250 °C, operated in electron ionization (EI) mode at 70 eV, with a mass range of 29–450 amu, an acquisition frequency of 100 Hz, and a detector voltage of 1500 V.

Data processing and statistical analyses

ChromaTOF[23] and GC Image[17] software packages were used for the acquisition and processing of the data, respectively. The Image Investigator, part of the GC Image software package, was used to analyze multiple chromatograms and examine statistical characteristics and trends. Library searching was performed using the NIST/EPA/NIH Mass Spectral Library[24] and the Wiley Registry of Mass Spectral Data[25] with a match-factor threshold greater than 700. Details on the basic data-processing procedures are available elsewhere.[6,15,26] The matrix of data containing all of the calculated peak volumes was submitted for PCA with mean-centering pretreatment to elucidate clustering tendencies. A singular value decomposition (SVD) algorithm of the chemometrics package in The Unscrambler X was used.[21]

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Keywords: decomposition · gas chromatography · mass spectrometry · thermal desorption · volatile organic compounds

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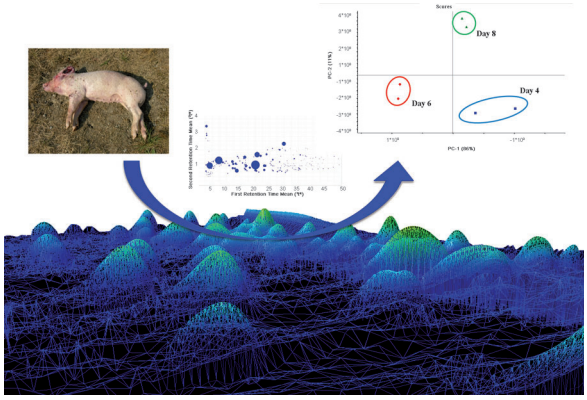

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Reading Cadaveric Decomposition Chemistry with a New Pair of Glasses

Smell it out! Thermal desorption has been coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry for the characterization of a cadaveric volatile organic compound mixture produced during the active decay stage (see figure). The combined use of the Fisher ratio and principal component analysis provided further characterization of the chemical profile of decomposition.