

Preliminary Investigation of the Influence of Fire Modification on the Odour of Decomposition using GC×GC-TOFMS

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The ability of cadaver-detection dogs to locate human remains relies on their recognition of decomposition odour, which is comprised of a complex mixture of volatile organic compounds. Numerous studies have focused on characterising the decomposition odour profile; however, little is known about the odour produced from remains that have been burnt prior to decomposition. To better understand the influence of fire modification on the odour of decomposition, this study investigated the decomposition odour profiles produced from burnt pig remains using comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS), an advanced analytical technique that is particularly valuable for the separation of complex mixtures.

Introduction

Cadaver-detection dogs are a specialised type of scent-detection canine commonly employed to assist in locating human remains. Over time, human remains will begin to decay releasing a complex mixture of volatile organic compounds (VOCs) as by- or end-products known as the 'smell of death' or decomposition odour. The ability of cadaver-detection dogs to locate human remains relies on their recognition of this odour [1]. Over the past two decades, several studies have focused on chemically characterising the decomposition odour profile, with many of these studies recently reviewed by Verheggen et al. [2] and Iqbal et al. [3].

Although research has been conducted to better understand the effect of burning on the pattern and rate of decomposition [4], very little is known about the odour of burnt human remains, and the ability of cadaver-detection dogs to locate such remains. Severely burnt human remains may arise as the result of natural disasters (i.e., typically in the absence of an ignitable liquid) such as bushfires or as a result of arson-related crimes (i.e., often in the presence of an ignitable liquid). While many of the odour compounds produced by burnt

human remains are expected to change based on the uncontrolled variables of combustion, it is hypothesised that several key decomposition compounds will be consistently present after burning and could be used to enhance cadaver-detection dog recovery of burnt remains.

Traditionally, the analysis of both decomposition odour and fire debris has been performed using one-dimensional (1D) gas chromatography – quadrupole mass spectrometry (GC-qMS). However, this presents an analytical challenge – each profile, in and of itself, is complex and dynamic in nature, as well as unpredictable. Unfortunately, traditional 1D GC-qMS methods often suffer from several limitations when analysing such complex mixtures including insufficient peak capacity, inability to manage dynamic range, and restricted selectivity in one dimension.

Advanced analytical instrumentation, such as comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS), on the other hand, offers: 1) increased peak capacity resulting in improved resolution and the separation of matrix interferences; 2) enhanced sensitivity and lower limits of detection resulting in increased peak detectability and an improved

characterisation of dynamic range; 3) additional selectivity with two individual GC columns separating compounds based on different retention mechanisms; 4) ordered chromatograms that produce distinct patterns of structurally-related compounds; and 5) automated deconvolution of co-eluting peaks (provided by TOFMS). The advantages of GC×GC (and GC×GC-TOFMS) have been discussed at length in the literature for both decomposition odour profiling [2,3,5-12] and the forensic analysis of fire debris [12-17], and GC×GC-TOFMS has been shown to provide a more comprehensive overview of the VOC profile.

The objective of this preliminary study was to investigate the influence of burning on the odour of decomposition, both in the absence and presence of ignitable liquids (i.e., gasoline), using pig carcasses as human odour analogues. After burning, the pig carcasses were left to decompose naturally on the soil surface. The chemical odour profiles were sampled periodically from the headspace above the decomposing pig remains using sorbent tubes, and were analysed using thermal desorption (TD) coupled to GC×GC-TOFMS. This investigation also served to identify key points in study design to be considered when performing future studies.

Experimental

Remains

In this study, pig carcasses were selected as a substitute for human remains due to the legal and ethical restrictions surrounding the burning of human cadavers. In the past, pigs have typically been chosen as analogues to mimic human decomposition due to the many anatomical and physiological similarities they share with humans [18]. Because the pig carcasses used herein were purchased postmortem and were not killed specifically for the purposes of this research, animal ethics approval was not required following the guidelines of the Australian Code for the Care and Use of Animals for Scientific Purposes (8th ed. 2013) (<http://www.nhmrc.gov.au/guidelines-publications/ea28>).

Two domestic pig carcasses (*Sus scrofa domestica* L.), weighing approximately 50–60 kg each, were purchased postmortem at the end of the day on April 27, 2016 (i.e., Day 0). The pigs were transported to the University of Technology Sydney (UTS) field site within 1 h of death. Once at the UTS field site (an open Eucalypt woodland located in Western Sydney, NSW, Australia), each pig carcass was clothed in a white 100% cotton t-shirt and black polyester briefs (Kmart, Sydney, NSW, Australia), to mimic a clothed human cadaver, before being placed directly on the soil surface approximately 2 m apart. Anti-scavenging cages containing small mesh wire were positioned over the pig carcasses to prevent vertebrate scavenging while still allowing invertebrate colonisation. A control site, in the absence of remains, was established approximately 20 m from the pig carcasses in order to measure the natural VOC profile produced by the surrounding environment.

Experimental design

The experimental burns took place less than 24 h after death. On the morning of April 28, 2016 (i.e., Day 1), the two clothed pig carcasses were transported to the Fire & Rescue NSW (FRNSW) Fire Investigation Research Unit (FIRU) in Londonderry, NSW, Australia, where the burns were carried out outdoors with the assistance of trained fire and rescue personnel. Two different burn scenarios were organised: 1) bushfire scenario – i.e., in the absence of an ignitable liquid; and 2) arson scenario – i.e., in the presence of an ignitable liquid (i.e., gasoline).

The two burns were performed consecutively beginning with the bushfire scenario in order to prevent cross-

contamination from the gasoline used in the arson scenario burn. For each burn, the pig carcass was placed on its side on top of a pile of leaf litter and brush situated on a stainless steel tray. Additional brush was piled on top of the pig carcass in the arson scenario to imitate a situation in which an arsonist might try to use fire to dispose of a body, destroy forensic evidence, or conceal identification. A DT85 data-logger and five 20 m long, 3 mm diameter Type N thermocouples (TC Measurement & Control, Melbourne, VIC, Australia) were used to track the temperature of each burn. The thermocouples were placed underneath the hind legs, torso, and head of each pig carcass, as well as above the upper and lower torso regions.

The bushfire burn was started with a propane gas torch. For the arson scenario burn, approximately 750 mL of unleaded 91 octane gasoline was purchased locally. A 30 mL portion of the gasoline was retained as a reference sample, while the remaining 720 mL was poured onto the pig carcass and the surrounding brush. A handheld butane gas lighter was used to ignite the gasoline and begin the fire. Each fire lasted a total of 20 min before being extinguished with the use of water. The fires were extinguished while soft tissue was still visually present on the pig carcasses to allow for subsequent decomposition of the remains following the burns.

After burning, the carcasses were transported back to the UTS field site, where the carcasses were once again placed on the soil surface (in an orientation identical to which they were burned), covered with anti-scavenging cages, and left to decompose naturally. Photographs and visual postmortem observations (including the stage of decomposition: *fresh*, *bloat*, *active decay*, *advanced decay*, and *dry/remains* – adapted from Payne [19]) were collected on: Day 0 following death; Day 1 before, during, and after the burns; and each subsequent sample collection day. Ambient temperature (°C) and rainfall (mm) were recorded hourly using a Hobo Weather Station (OneTemp, Marlestone, NSW, Australia).

VOC sample collection

VOC samples were collected from the headspace above the control site and each pig carcass on Day 1 before and after burning, as well as once per week after burning (i.e., Days 9, 16, 23, 30, 37, and 44) until the decomposition process reached the *dry/remains* stage. The VOC sample collection technique was adopted from a

method previously developed for odour profiling in the air surrounding vertebrate carrion – see reference [20] for method details. VOC samples were collected on stainless steel sorbent tubes containing Tenax TA (35/60 mesh; Markes International Ltd, Llantrisant, RCT, UK).

TD-GC×GC-TOFMS analysis

Thermal desorption of the sorbent tubes was achieved using a Unity 2 Thermal Desorber and Series 2 ULTRA multi-tube autosampler (Markes International Ltd) followed by sample analysis using a Pegasus® 4D GC×GC-TOFMS system (Leco, Castle Hill, NSW, Australia).

Following sample collection, each sorbent tube was thermally desorbed with a split flow of 20 mL/min helium at a temperature of 320°C for 15 min and collected onto a general purpose cold trap (i.e., Tenax TA/Carbograph 1TD) maintained at -10°C. Secondary desorption was performed at 320°C for 3 min with a split flow of 50 mL/min helium (total split = tube split + trap split = 36.4:1). The reference gasoline sample (i.e., 0.2 µL gasoline injected directly onto sorbent tube) was analysed using a thermal desorption method with identical parameters, with the exception of a higher split ratio to reduce sample overload: tube desorption split flow of 100 mL/min helium and a trap desorption split flow of 100 mL/min helium (total split = 153:1).

GC×GC-TOFMS experimental conditions were previously optimised to achieve a near-theoretical maximum in peak capacity gain for the forensic analysis of ignitable liquids and can be found in reference [16].

Data processing

Chromatographic visualisation and data processing were carried out using ChromaTOF® (version 4.51.6.0; Leco). The baseline was automatically smoothed using an 80% offset. The signal-to-noise cut-off for peak searching was set at 150 for base peaks and 20 for sub-peaks using an expected peak width of 8 s in the ¹D and 0.1 s in the ²D. Using a minimum similarity match of 800 or greater, the 2011 National Institute of Standards and Technology (NIST) mass spectral library database was used for the tentative identification of peaks.

Chromatographic alignment was achieved using the Statistical Compare software feature in ChromaTOF®. Samples were separated into three classes: control ($n = 7$), pig burnt *without* gasoline ($n = 7$), and

BEFORE BURNING

DAY 1



AFTER BURNING

*Without gasoline**With gasoline*

DAY 1



DAY 9



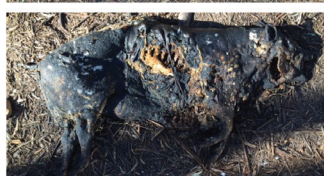
DAY 16



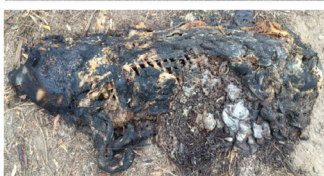
DAY 23



DAY 30



DAY 37



DAY 44



Figure 1: Photographs of the pig carcasses on Day 1 before and after burning, and on each subsequent sampling day after burning (left = pig carcass burnt without gasoline; right = pig carcass burnt with gasoline).

pig burnt with gasoline ($n = 8$). During chromatographic alignment, peak re-searching was performed using a signal-to-noise cut-off of 20. A minimum similarity match of 600 or greater was required for peak alignment. Analytes that did not meet this threshold and that were not detected in at least two samples across the three classes were removed from the final

compound list. Maximum retention time deviations permitted between samples during alignment were restricted to 2 s in the 1D and 0.03 s in the 2D . Using the analyte peak areas (calculated using unique mass), the ratio of between-class variance to within-class variance (i.e., the Fisher ratio) was calculated for each analyte. Analytes with Fisher ratios above a critical value

(F_{crit}) were considered class-distinguishing analytes. The F_{crit} threshold was computed in Microsoft Excel using the F -distribution based on the number of classes in the analysis, the degrees of freedom for each class and the significance level chosen ($\alpha = 0.05$). Analytes with a Fisher ratio greater than a $F_{crit} = 3.52$ were exported as a *.csv file and imported into Microsoft Excel for the manual removal of chromatographic artefacts and further processing.

Principal component analysis (PCA) was carried out using The Unscrambler[®] X (version 10.3.31813.89; CAMO Software, Oslo, Norway). Data pre-processing steps performed prior to PCA included mean centering, variance scaling and unit vector normalisation. The dataset was evaluated and confirmed to contain no outlying samples using the Hotelling's T^2 95% confidence limit.

Results and discussion

Experimental burn conditions and observations

The fire without gasoline recorded temperatures ranging from 91.8 – 832.2 °C. After burning, varying degrees of charring were observed on both sides of the pig carcass with the topside presenting the most consistent degree of burning (Figure 1). The extent of burning in the head, neck, and torso regions was classified as level 2 on the Crow-Glassman Scale (CGS) [21], while the limbs, which remained intact, were categorised as CGS level 1 burning.

The fire with gasoline recorded lower overall temperatures ranging from 250.0 – 669.2 °C. After burning, charring was observed to be limited to the topside of the pig carcass (Figure 1), with the exception of the limbs and head, which showed extensive charring on all sides. The extent of burning in the pig carcass burnt with gasoline was classified as CGS level 2 for the head and limbs as well as the neck, torso, and posterior regions specific to the topside of the pig carcass. The neck, torso, and posterior regions on the underside of the pig were categorised as CGS level 1 burning. All four limbs were found to be very delicate, with the front limbs in cadaveric spasm and small portions of the hind legs unrecoverable after the fire. Following the fire, a large tear was discovered in the lower abdominal region resulting in intestinal herniation (i.e., exposure of the visceral organs – Figure 1). Fly activity was observed near the exposed organs shortly after the fire was extinguished.

Weather conditions

This study was conducted during the Australian autumn from 27 April - 10 June, 2016. The mean temperature during the trial was 14.6°C with maximum and minimum trial temperatures of 29.5°C (Day 20) and -0.2°C (Day 33), respectively. A total of 151.2 mm of rainfall was recorded throughout the trial, occurring on Day 12 (0.8 mm), Day 38 (27.6 mm), and Day 39 (122.8 mm).

Decomposition observations

Photographs of the pig carcasses throughout the trial are displayed in Figure 1. On Day 0, approximately 1 h after death, the pig carcasses displayed no visible signs of decomposition and were characterised as being in the *fresh* stage of decomposition. Before burning on Day 1, approximately 20 h after death, both pig carcasses exhibited algor mortis and livor mortis, along with rigor mortis in all four limbs. On Day 9, the pig carcass burnt *without* gasoline was characterised as being in the *bloat* stage of decomposition, while the pig carcass burnt *with* gasoline exhibited a flattened/deflated torso and was characterised as already being in the *active decay* stage of decomposition. The *bloat* stage of decomposition was not observed for the pig burnt *with* gasoline. This stage may have transpired between Day 1 and Day 9, and therefore was not observed, or it may not have occurred as a result of the large tear/intestinal herniation noted in the abdominal region. Bloating began to give way to *active decay* in the pig carcass burnt *without* gasoline by Day 16, reaching *advanced decay* by Day 30 and *dry/remains* by Day 44. The pig carcass burnt *with* gasoline reached *advanced decay* much earlier, by Day 16, and remained in this stage until Day 44 when the carcass reached the *dry/remains* stage.

The difference in the initial rate of decomposition observed between the two pig carcasses is hypothesised to have occurred as a result of the differences observed in invertebrate scavenging. The exposed visceral organs in the pig carcass burnt *with* gasoline attracted flies as early as Day 1, potentially accelerating the initial rate of decomposition observed when compared to the pig carcass burnt *without* gasoline. Furthermore, flies were observed to lay eggs predominantly in the head region of the pig carcass burnt *without* gasoline vs. the abdominal region of the pig carcass burnt *with* gasoline, likely a result of the intestinal herniation. This resulted in very distinct differential decomposition patterns,

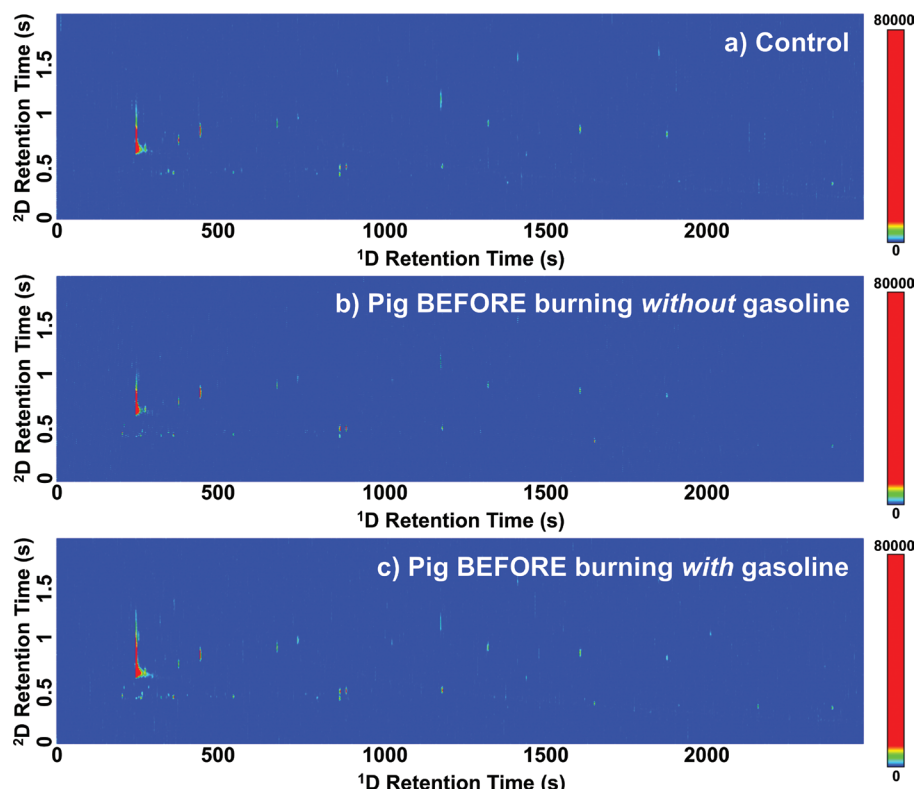


Figure 2: GCxGC-TOFMS total ion current (TIC) contour plots from Day 1: a) control, b) pig carcass before burning without gasoline, and c) pig carcass before burning with gasoline.

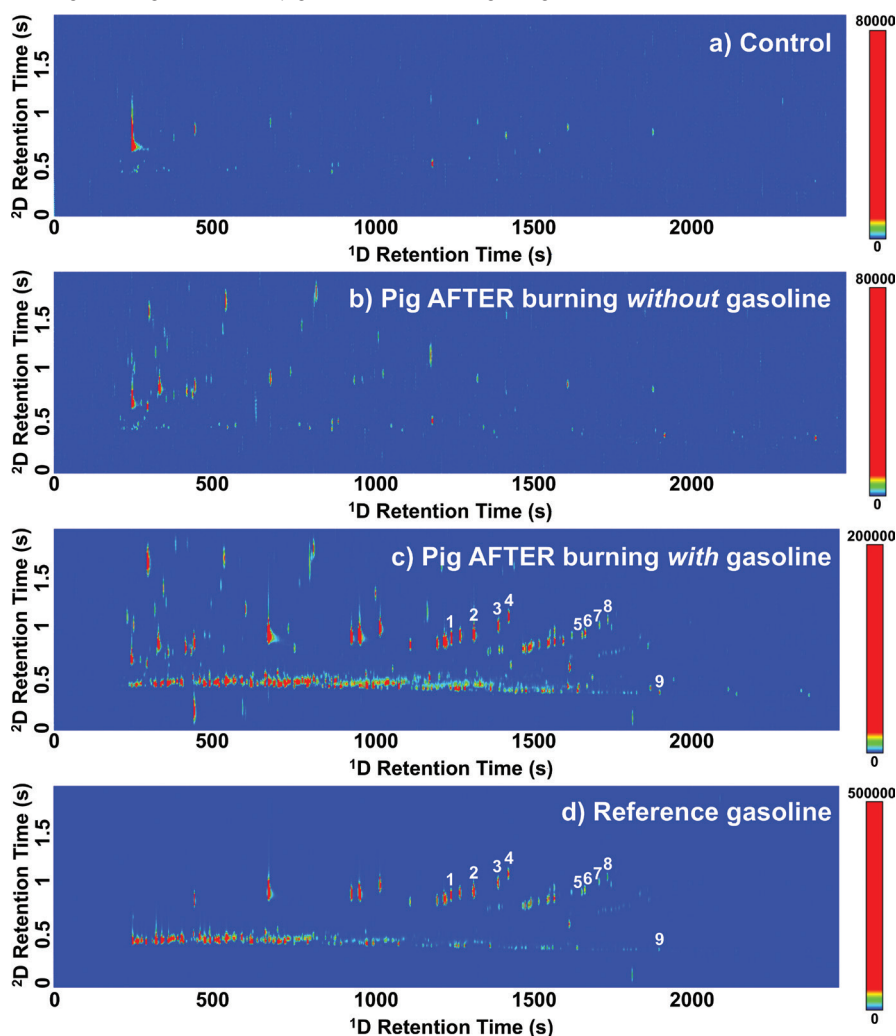
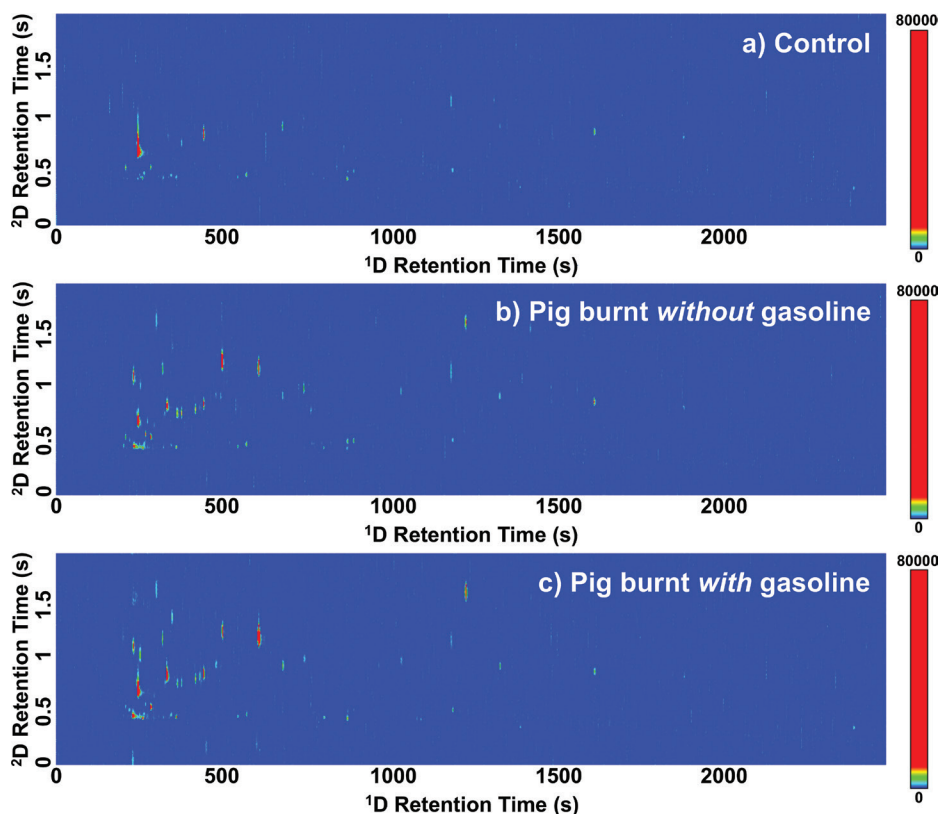


Figure 3: GCxGC-TOFMS TIC contour plots from Day 1: a) control, b) pig carcass after burning without gasoline, c) pig carcass after burning with gasoline, and d) reference gasoline. ASTM E1618-14 [22] gasoline target compounds labelled: (1) 1,3,5-trimethylbenzene; (2) 1,2,4-trimethylbenzene; (3) 1,2,3-trimethylbenzene; (4) indane; (5) 1,2,4,5-tetramethylbenzene; (6) 1,2,3,5-tetramethylbenzene; (7) 5-methylindane; (8) 4-methylindane; and (9) dodecane.



VOC profiles

On Day 1, before burning, the control site and the unburnt pig carcasses generated very similar (and simple) profiles reflective of the natural VOC profile produced by the surrounding environment (Figure 2). After burning on Day 1, the pig carcasses burnt *without* gasoline (Figure 3b) and *with* gasoline (Figure 3c) generated much more complex profiles (comprised of combustion and pyrolysis products) in comparison to the simple profile once again generated for the control site (Figure 3a). In addition to the combustion and pyrolysis products detected, the VOC profile of the pig carcass burnt *with* gasoline (Figure 3c) also tested positive for the presence of gasoline based on comparison with the reference sample (Figure 3d), and the detection and tentative identification of targeted gasoline compounds (see ASTM E1618 - 14 [22]).

On Day 9, both the pig carcass burnt *without* gasoline (Figure 4b) and the pig carcass burnt *with* gasoline (Figure 4c) generated much simpler VOC profiles than that observed after burning on Day 1. Many of the combustion and pyrolysis products detected on Day 1 after burning (Figure 3b and Figure 3c) were no longer detectable or were detected at much lower levels (Figure 4b and Figure 4c). In addition, the target compounds used for the positive detection of gasoline after burning on Day 1 (Figure 3c and Figure 3d) were also no longer detectable on Day 9 (Figure 4c), likely a result of continued weathering and microbial degradation. Nevertheless, the VOC profiles produced from the burnt pig carcasses on Day 9 were still distinct from the VOC profile produced from the control site (Figure 4a), with the two pig carcasses generating very similar profiles despite the differences in decomposition previously described.

Due to the large gap in sample collection between Day 1 and Day 9, it is unclear when many of the combustion/pyrolysis products and the gasoline target compounds were lost from the odour profile. Understanding the odour available to cadaver-detection dogs only hours after remains have been burnt vs. the odour available days, weeks, and months after burning, is essential information to consider when training cadaver-detection dogs to locate burnt remains. Therefore, future studies should consider increasing the frequency of sample collection within the first week after burning in order to better understand the changes in the profile during this period.

While many of the odour compounds produced by burnt remains are expected to

Figure 4: GCxGC-TOFMS TIC contour plots from Day 9: a) control, b) pig carcass burnt without gasoline, and c) pig carcass burnt with gasoline.

whereby the head and upper trunk regions decomposed to a greater extent in the pig carcass burnt *without* gasoline, and the torso and posterior regions decomposed to a greater extent in the pig carcass burnt *with* gasoline (Figure 1). Notably, by Day 44, minimal decomposition was observed in the limbs, posterior region, and lower abdominal region of the pig

carcass burnt *without* gasoline, while minimal decomposition was observed in the head region of the pig carcass burnt *with* gasoline; these regions instead, remained almost entirely intact at the conclusion of the trial (Figure 1). Similar patterns of differential decomposition were also observed in a study by Gruenthal et al. [4].

Table 1: List of tentatively identified class-distinguishing postmortem VOCs. Asterisks (*) and daggers (†) denote VOCs previously reported in decomposition odour research and combustion research, respectively.

#	Name	Retention Time (1D, 2D; s)	Classification
1	1-butanol*	446, 0.188	Alcohol
2	1-pentanol*	597, 0.226	Alcohol
3	1-propanol*	299, 1.654	Alcohol
4	2-methyl-1-propanol*	385, 1.840	Alcohol
5	2-butanol*	346, 1.400	Alcohol
6	3-methyl-2-butanone*	429, 0.841	Ketone
7	2,3-butanedione*	319, 1.193	Ketone
8	α-pinene*	1186, 0.519	Hydrocarbon
9	2-methyl-butanal*	435, 0.787	Aldehyde
10	3-methyl-butanal*†	417, 0.814	Aldehyde
11	ethyl butanoate*	763, 0.808	Ester
12	dimethyl sulfide*	267, 0.579	S-containing
13	dimethyl trisulfide*	1225, 1.640	S-containing
14	hexanal*†	743, 1.005	Aldehyde
15	methyl diethylcarbamodithioate	498, 1.264	S- and N-containing
16	N,N-dimethyl-methylamine*	233, 0.470	N-containing
17	2,4,6-trimethyl-octane	2604, 0.343	Hydrocarbon
18	ethyl propanoate*	539, 0.807	Ester

change based on the uncontrolled variables of combustion, it was initially hypothesised that several key decomposition products would be consistently present after burning, and that these compounds would be important for training and enhancing cadaver-detection dog recovery of burnt remains. Following the reduction in the number and abundance of combustion and pyrolysis products detected between Day 1 and Day 9, the focus turned toward establishing what, if any VOCs could consistently differentiate the burnt pig carcasses from the control site throughout the trial. To do this, Fisher ratio filtering (described in *Data processing*) was applied to the dataset tentatively identifying 18 class-distinguishing compounds (Table 1).

The 18 class-distinguishing compounds identified (Table 1) were submitted to PCA in order to provide a visual representation of the multivariate structure of the data (Figure 5). Overall, the first two principal components (i.e., PC-1 and PC-2) captured 66% of the explained variation within the dataset. All 18 of the class-distinguishing compounds identified were found to discriminate the burnt pig carcasses from the control site horizontally along PC-1, with the exception of the pig carcass burnt *with* gasoline on Day 30, which grouped near the controls. The VOC samples collected from the pig carcasses before burning were also found to group with the controls, which was expected based on the simple VOC profiles observed on Day 1 before burning (Figure 2).

Slight variation among the controls, particularly on Day 37 and Day 44, was observed vertically along PC-2. This variation was likely due to natural variation in the VOC profile produced by the surrounding environment. Variation among the burnt pig carcasses was observed to a much greater extent, occurring along both PC-1 and PC-2, revealing discrimination between the decomposition odour profiles collected on the different sampling days, a result of the dynamic nature of the decomposition process. Variability between the two pig carcasses was also observed, particularly on Day 1 after burning, as well as on Day 30 and Day 44 to a lesser extent. The greater variability between the VOC profiles produced from the pig carcasses on Day 1 after burning is hypothesised to have occurred as a result of the differences in the experimental burns and the resulting state of the remains after burning. Interestingly, the VOC profiles detected on Days 9, 16, and 37 were very similar between the two pig carcasses. Despite the difference in

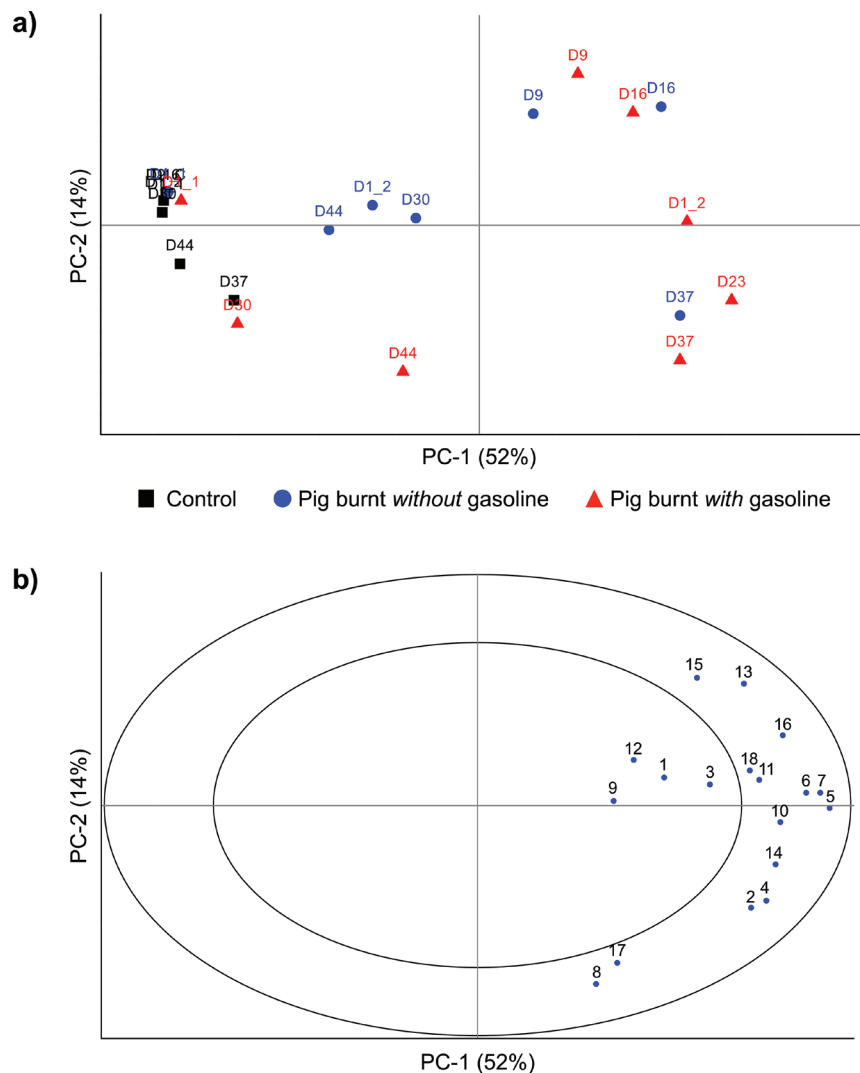


Figure 5: PCA a) scores and b) correlation loadings plots generated using pre-processed GC×GC-TOFMS peak area data for all class-distinguishing VOCs detected. Point labels in the scores plot denote the postmortem interval at the time of sample collection (D = day; D1_1 = Day 1 before burning; D1_2 = Day 1 after burning). Point labels in the loadings plot refer to compound identification as per Table 1. Note: Day 23 samples collected from the control site and the pig burnt without gasoline were lost due to instrumental difficulties.

the initial rate of decomposition observed between the two pig carcasses, and the difference in the VOC profiles detected on Day 1 after burning, the odour signatures detected after Day 1 did not appear to be overly effected by the differences in the experimental burns or the resulting state of the remains.

In total, 16 of the 18 class-distinguishing VOCs tentatively identified have previously been reported in human and/or human analogue decomposition odour research, and 2 of the 18 class-distinguishing VOCs have previously been detected in human and/or human analogue combustion research (see Table 1). Interestingly, the 2 class-distinguishing VOCs that have been previously detected in combustion research (i.e., 3-methyl-butanal and hexanal) have also been previously reported as decomposition VOCs. The class-distinguishing VOCs identified included

alcohols ($n = 5$), aldehydes ($n = 3$), ketones ($n = 2$), esters ($n = 2$), hydrocarbons ($n = 2$), nitrogen-containing compounds ($n = 2$), and sulfur-containing compounds ($n = 3$). Figure 6a displays the VOC abundance detected on each sample collection day for all 18 of the class-distinguishing VOCs identified, and Figure 6b displays the VOC abundance detected on each sample collection day for all seven of the compound classes detected. The overall low number and abundance of class-distinguishing VOCs identified is likely a result of the cooler temperatures experienced during the autumn trial [23,24].

VOC abundance was highest following burning on Day 1 for the pig carcass burnt *with* gasoline, with 15 of the 18 class-distinguishing VOCs detected. VOC abundance was nearly an order of magnitude lower for the pig carcass burnt *without* gasoline on Day 1 after burning, with only 7 of the 18 class-distinguishing

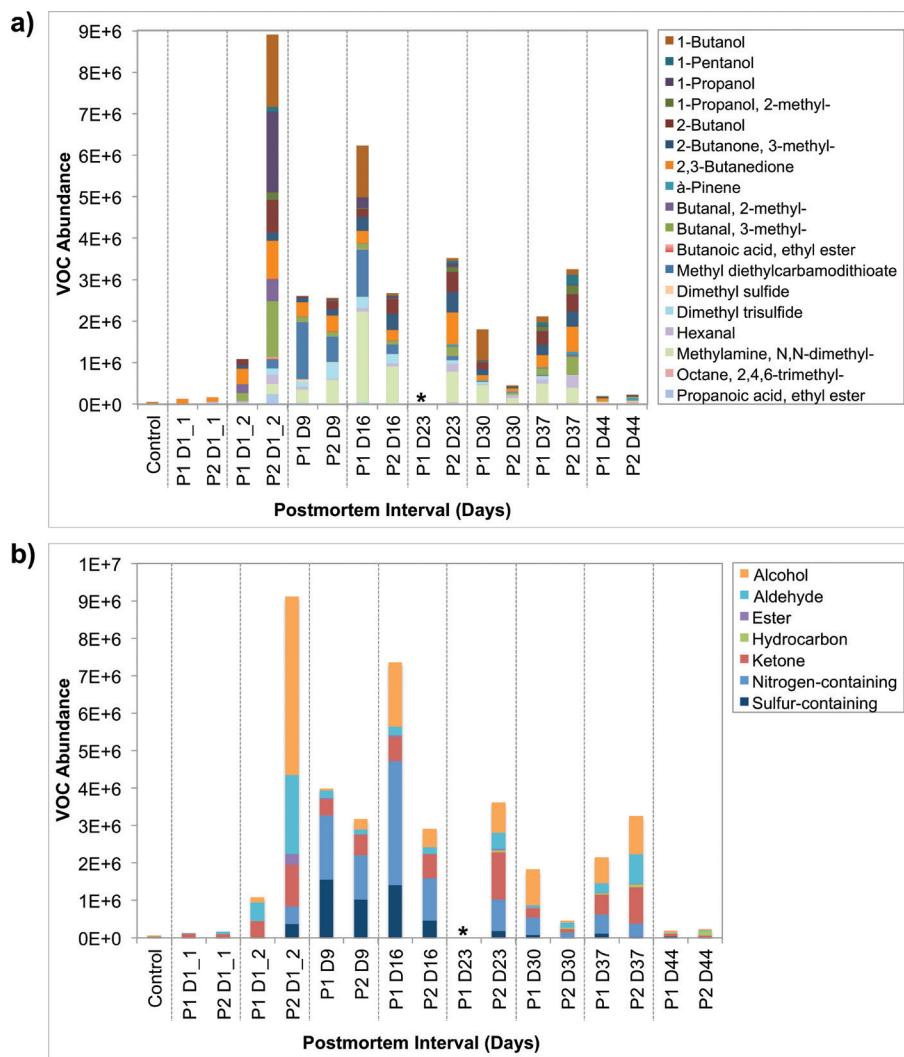


Figure 6: VOC abundance detected for a) each individual compound and b) each compound class across all postmortem intervals investigated (P1 = pig carcass burnt without gasoline; P2 = pig carcass burnt with gasoline; D = day; D1_1 = Day 1 before burning; D1_2 = Day 1 after burning). The VOC abundance for the controls was summed for each individual compound and each compound class, and then averaged for each postmortem interval. *Note: Day 23 samples collected from the control site and the pig burnt without gasoline were lost due to instrumental difficulties.

VOCs detected. It is hypothesised that the intestinal herniation observed in the pig carcass burnt *with* gasoline initiated the release of the increased abundance and number of VOCs detected following burning on Day 1. The most abundant compound classes detected for both pig carcasses on Day 1 after burning were alcohols, aldehydes, and ketones. VOC abundance was highest on Day 9 and Day 16 for the pig carcass burnt *without* gasoline when the remains were observed to be transitioning from *bloat* to *active decay*. The most abundant compound classes detected during early decomposition (i.e., on Day 9 and Day 16) for both pig carcasses were N- and S-containing compounds. VOC abundance decreased during late decomposition (i.e., on Day 30 and Day 37) for both pig carcasses, with the most abundant compound classes including alcohols, aldehydes, ketones, and

S-containing compounds. With the exception of Day 1 before burning, VOC abundance was lowest on Day 44 for both pig carcasses, once the *dry/remains* stage of decomposition was reached, with hydrocarbons and ketones dominating the profile.

The overall trends observed in VOC abundance are consistent with previous decomposition odour profiling studies [6,8,24]. The high abundance of alcohols, aldehydes, and S-containing compounds detected throughout the trial is also consistent with previous decomposition odour studies conducted at the UTS field site during cooler winter months [23], while the high abundance of aldehydes detected is consistent with previous combustion studies involving both human and animal remains [25,26]. Overall, the 18 class-distinguishing compounds identified are considered to be postmortem VOCs that are generally released naturally into

the environment through the process of decomposition; however, the detection of the postmortem VOCs on Day 1 following burning (and prior to decomposition) may be explained by the fire modification performed in this study, which may have: 1) accelerated the release of the postmortem VOCs by 'degrading' areas of the remains (e.g., skin ruptures – intestinal herniation); or 2) thermally degraded the macromolecules in the soft tissue producing the postmortem VOCs detected. It is also possible that the postmortem VOCs detected throughout the trial were produced both as combustion/pyrolysis products and as a result of decomposition, albeit in different ratios. Further research is necessary to identify the origin of the postmortem VOCs detected. Future studies should consider the addition of unburnt pig carcasses for a more direct comparison of decomposition VOCs both with and without burning modification.

Conclusions

The fire modification performed in this study, both in the absence and presence of gasoline, did not appear to influence the overall odour of decomposition. Initially, on Day 1 after burning, combustion and pyrolysis products dominated the odour profile along with the gasoline signature for the pig carcass burnt *with* gasoline. However, the combustion and pyrolysis products detected diminished over time and the gasoline signature was lost entirely by Day 9. A total of 18 tentatively identified postmortem VOCs were discovered to differentiate the burnt pig carcasses from the controls throughout the trial period, with 16 of the 18 postmortem VOCs being reported as decomposition odour VOCs in previous decomposition odour studies. Despite these discoveries, it is still important for scent-detection dogs to be exposed to training aids that replicate their target odour as closely as possible; therefore, the authors suggest that cadaver-detection dog handlers use burnt training aids when possible (and necessary) in order to ensure that any possible odour owing to combustion, pyrolysis, or the use of ignitable liquids is not masking or confusing the scent of decomposition for the dogs.

It is important to note of course that these conclusions are only relevant for the environmental and burn conditions under which this study was conducted. Given the variability that occurs in every fire, it is important for studies like this to be repeated and to be carried out under comparable

and varying conditions. This could include changing the degree of burning, the ignitable liquid(s) used, the season of sample collection, etc. Future studies should also consider including unburnt remains as controls, as well as incorporating an increased number of replicates and more frequent sampling. Although difficult due to the ethical and legal restrictions surrounding the burning and use of human remains, cadaver-detection dog trials using unburnt and burnt decomposed remains could likewise prove valuable.

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Targeted Analysis and Accurate Profiling of Volatiles and Semi-volatiles in High Complexity Samples

GC×GC-HRMS is one of the most powerful tools available to analytical chemists for the targeted analysis and accurate profiling of volatiles and semi-volatiles in samples of high complexity. This technique combines the excellent resolving power granted by two-dimensional separations with accurate mass measurements that provide a substantial advantage for identity confirmation. It is therefore not surprising that GC×GC-HRMS is increasingly more accepted in labs dealing with challenging analytical requests in very complex matrices such as, for instance, environmental and food analysis.

JSB recently investigated the use of GC×GC with Zoex thermal modulation coupled to an Agilent QTOF detector for quantification of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). These compounds are synthetic chlorinated compounds banned or restricted due to their toxicity to humans and impact on the environment but, being persistent pollutants, are still present as residues in soil, water, food etc. Their identification and quantification is of great importance for environmental and food safety since they represent a risk even at very low level. The QTOF grants wide operational flexibility allowing EI and CI acquisition, as well as MS/MS capability. In this example JSB selected the NCI to obtain higher sensitivity for the halogenated compounds. The results obtained for linearity and repeatability show that GC×GC with thermal modulation and QTOF are robust and provide performance suitable for reliable identification and quantification at low concentrations.

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