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All equal in the face of death! – Characterization of the volatile cadaveric compounds of fresh stage human corpses

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ABSTRACT

The smell associated with the decomposition of a human body has been the subject of a limited number of studies, most using body parts or other vertebrates as surrogate models. Among the limitations frequently encountered in the existing literature are the small sample size and the high variability in terms of stages of decomposition. In the present study, we collected, identified, and quantified the volatile organic compounds released by 20 human corpses at the fresh stage, using dynamic headspace collection. We also assessed the impact of some parameters on the volatilome: skin temperature, gender, age, size, postmortem interval, presence of lividities or rigidities. We found 2-heptanone to account for nearly half the scent of fresh human cadavers. Four additional compounds were also repeatedly identified: dimethyl disulfide, ethyl acetate, limonene and 3-methyl-1-butanol. The use of dynamic sampling and thermodesorption traps has allowed to increase the diversity of collected postmortem molecules, compared to previous works. However, no human specific markers were found, either because they do not exist at the fresh stage, or because they are released at trace levels. Finally, none of the tested parameters impacted the volatile profile of human corpses. We recommend performing similar assays on more advanced stages to help completing our understanding of human decomposition.

Introduction

After death, a corpse undergoes the combined action of autolysis and putrefaction, leading to drastic physical and chemical changes. Macromolecules are degraded, resulting in the emission of hundreds of different volatile organic compounds (VOCs) [1]. The characterization of the smell of decaying vertebrate is a recent research topic [2–9]. But we now have a good understanding of the biotic and abiotic factors impacting cadaveric volatilome, which include weather conditions, presence of necrophagous insects, soil types and decomposition stages [4,5,7,10–12]. Although pigs have predominantly served as surrogate human models, there have been only a few studies that directly sampled human cadavers [5,11,13–25]. Furthermore, many of these studies suffer from limited sample sizes and primarily focus on specific body parts or gravesoil rather than complete human corpses.

Five stages of decomposition have been established based on physical changes. The initial stage, known as the "fresh stage," starts immediately after death and ends when the first signs of body inflation appear [4,28]. During this stage, various physiological processes take place, resulting in the development of livor mortis and rigor mortis [29,30]. Additionally, the first necrophagous flys lay eggs on the corpse, suggesting that flies perceive cadaveric volatiles [31]. Yet, only about 20 volatile organic compounds have been identified thus far at this stage and their detection requires the utilization of bidimensional gas chromatography due to their extremely low concentrations [26,27,32,33]. Previous attempts using single separation chromatography have been unable to identify any VOCs during the fresh stage [4].

Recent research has emphasized the influence of various factors on the release of volatile compounds from deceased animals, including environmental conditions, species, soil types, and the stages of decomposition [4,10,11]. However, most of these studies have focused on human surrogates and advanced stages of decomposition. Nonetheless, a comprehensive characterization of volatiles associated with recently deceased human cadavers would have practical applications in search and rescue operations [21] and enhance our understanding of the formation of cadaveric volatile organic compounds (VOCs).

This study aims to demonstrate the potential of classical gas chromatography, utilizing a target ion methodology, for investigating early-stage human cadaveric VOCs. The impact of factors such as skin temperature, postmortem interval (PMI), and the presence of lividity and

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Table 1
Cadaver information.

Cadaver	Skin temperature	Rigidity	Lividity	Size (cm)	Age (year)	Sex	PMI (day)*
C1	18.5	A	A	186	60	M	0
C2	8.9	C	A	180	62	F	36
C3	9.5	A	A	174	64	F	0
C4	22.8	A	A	177	46	M	0
C5	24.5	B	B	177	71	M	0
C6	12.5	B	A	180	44	M	2
C7	29.1	B	A	1.8	60	M	0
C8	19	A	B	158	92	F	0
C9	13.1	B	B	175	na	M	1
C10	12.2	B	A	187	45	M	1
C11	7.2	B	A	160	76	F	11
C12	14.6	B	A	1.89	70	M	1
C13	6.3	C	A	1.84	58	M	5
C14	4.5	C	A	180	82	M	10
C15	3.8	C	A	1.84	47	M	15
C16	4.1	C	A	1.95	na	M	340
C17	na	A	A	167	33	M	9
C18	12.6	B	A	167	33	F	1
C19	8.8	A	A	90	63	M	10
C20	3.8	B	A	184	35	M	11

Rigidity (before: A, present: B, after: C); lividity (present: A, Absence: B); postmortem interval (PMI); na value are unknown. *All corpses deceased for more than 1 day were kept in a cold room since death

rigor mortis was examined to identify their influence on the emission of specific VOCs at the initial stages of decomposition. Intrinsic parameters such as age, size, and gender were also evaluated.

Material and methods

Location and presampling procedure

All samplings were conducted in a morgue located in Gilly (Belgium), under the authority of the local medical examiner (Dr. Duverger). Between November 2019 and February 2020, we were daily informed about the presence of at least one new corpse in the morgue. Upon our arrival, the corpse -laying on a metal trolley- was taken out of the cold room (4 °C) it was kept in and placed in a room at 19 °C for exactly 2 h. Then, it was introduced in the autopsy room (previously cleaned, aerated, set at 19 °C) where the sampling took place. The cadaver was provided to us inside a white plastic body bag (230 × 80 cm).

Volatile compounds sampling

To perform the VOCs sampling, we opened the zipper of the body bag a few centimeters and introduced a hydrophobic TenaxTa/Carbograph thermodesorption tube (Markes International, Llantrisant, UK). We sampled the headspace air directly inside the body bag using an air flow set at 200 mL/min (GilAir1 plus pump, Sensidyne1, St. Petersburg, Florida, USA). After 10 min of sampling, we sealed the tubes and stored them in a fridge at 4 °C to avoid desorption of the compounds. A sampling in a clean empty body bag was performed each day as a control.

While we had no access to the person's identity, we were then allowed to collect the following descriptive data from the corpse: gender, age, size, skin temperature, decomposition stage, presence of rigidities and lividities, and postmortem interval (all data were collected from the corpse after VOC sampling). Skin temperature was measured with an infrared thermometer (Etekcity®) close to the waist. Rigidity was estimated by moving the wrist join: in case no movement was allowed, the rigidity was considered present; if the join could move, then we estimated if the rigidity has ended or has not yet started based on the postmortem interval [28]. Postmortem interval was provided by the morgue manager.

Only corpses in the fresh stage were considered for the present study, for a total of 20 cadavers (Table 1). All 20 persons died inside a building. No consent was needed since our sampling was non-invasive and was exclusively performed on the VOCs leaving the corpse.

Gas chromatography analyses

Sample analyses were carried out using a gas chromatograph coupled with a mass spectrometer (QP 2020 NX, Shimadzu, Kyoto, Japan) and a thermodesorber (TD30R, Shimadzu, Kyoto, Japan). Compounds were first desorbed at 280 °C for eight minutes prior to be cryofocused by Peltier effect in a glass tube set à −20 °C. The trap was then warmed up to 270 °C and injected on the column head with a split ratio of 20. Compounds separation was performed on a Rtx-5 ms capillary column (30 m × 0.25 mm × 0.25 μm; Agilent technology; Santa Clara, California, USA). The oven was set to an initial temperature of 40 °C. The temperature increased to 100 °C with a ramp of 2 °C/min and then ramped to a final temperature of 300 °C at a rate of 10 °C/min. Helium was used as carrier gas at a constant flow rate of 1.9 mL.min^{-1}. The mass spectrometer interface temperature was held at 230 °C and the ion source was set at 200 °C. Mass spectra were collected from 35 to 500 m/z with a data acquisition time of 0.3 s. The detector voltage was set during the tuning of the mass spectrometer at 1.1 kV.

Data processing

To highlight cadaveric compounds, a post run single ion monitoring (SIM) was performed on each chromatogram. When a targeted compound was found, a specific integration method was created. We therefore obtained as many methods as targeted compounds. These methods were then applied on all chromatograms to identify the targeted compounds. These methods focused on four criteria: retention time and relative abundance of three ions. The target compounds were those gathered in Martin & Verheggen (2018). For each compound, the higher fragment of their mass spectrum was targeted using "fragment search table" in the Shimadzu postrun software. If the targeted compounds was also found in the control sampling, they were removed prior the statistical analyses.

All statistical analyses were performed using R version 3.4.2. (R Foundation for statistical computing). To evaluate the impact of the studied factors (Table 1), we conducted a permutational multivariate analyses of variance (i.e., perMANOVA) using an Euclidian distance matrix and 999 permutations ("adonis" command, R-package vegan, [34]). We adjusted p-values using Bonferroni's correction to avoid

Table 2
List of identified cadaveric compounds from 20 human corpses experiencing the fresh decomposition stage.

Chemical name	Occurrence (# corpses)	Mean	±	SD	Percentage
Sulfide					
dimethyl disulfide	18	99125.6	±	53434.9	4.4%
dimethyl trisulfide	4	547.4	±	278.7	<1%
Alkanes					
unidentified C7 alkane	8	79206.3	±	62893.9	3.5%
2.4-dimethylheptane	1	126.0	±	126.1	<1%
Ketones					
2-pentatone	3	9313.4	±	7100.6	<1%
2-hexanone	1	129282.9	±	129282.9	5.8%
2-heptanone	18	1063418.6	±	175965.0	47.9%
Aldehydes					
hexanal	3	21488.0	±	21032.2	1.0%
heptanal	4	5187.6	±	4623.5	<1%
octanal	2	385.9	±	278.0	<1%
nonanal	1	20637.1	±	20637.1	1.0%
benzaldehyde	1	4830.9	±	4830.9	<1%
Alcohols					
2-methyl-1-propanol	7	14719.5	±	7474.6	<1%
3-methyl-1-butanol	16	27113.4	±	13785.4	1.2%
benzyl alcohol	1	62003.5	±	62003.5	2.8%
Aromatics					
p-xylene	3	8058.1	±	4831.9	<1%
phenol	2	95867.0	±	78724.3	4.3%
toluene	7	174245.4	±	118602.1	7.8%
limonene	12	27717.0	±	21312.0	1.2%
styrene	3	2321.8	±	1762.9	<1%
Furans					
2-pentyl-furan	4	4526.3	±	3870.6	<1%
Esters					
ethyl acetate	10	360281.2	±	155838.3	16.2%
butyl butyrate	9	7910.6	±	4704.7	<1%

increases in type I error due to multiple testing. Homoscedasticity and normality were checked on each parameter prior the application of the permMANOVA. Regarding cadaver size, skin temperature, PMI and age, two classes were compared by using the median of the sample, since the median is not impacted by extreme values.

Results and discussion

A total of 23 cadaveric compounds were identified after removing background compounds (Table 2). The early fresh stage is usually assimilated with no cadaveric odor, especially when analyzed with a single dimension gas chromatography [4]. Comprehensive two dimensional gas chromatography allowed the identification of 20 compounds released from one-day postmortem piglet carcasses [27]. The use of targeted methods in one dimension gas chromatography, therefore, helps to reach the sensitivity of the bidimensional one on cadaver experiencing the fresh stage as we were able to detect about the same number of compounds as in GCxGC.

A single volatile compound, 2-heptanone, accounted for nearly half the volatilome of fresh human cadavers (Table 2). This molecules has been identified previously from fresh vertebrate remains [35] and has been associated with the decomposition of several human tissues (*e.g.* muscle and skin) [14,22]. Ethyl acetate is the second most abundant volatile (accounting for 16% of the collected blend) and was previously identified on putrefied human bodies [15,22,23] as well as on surrogate human models [9,27]. The other volatile compounds are released in much smaller quantities (<1% of the blend).

Only five of the 23 identified compounds were found in more than half of the sampled corpses (Table 2): dimethyl disulfide; 2-heptanone; ethyl acetate; limonene; 3-methyl-1-butanol. None of these compounds were identified during a previous assay performed in a morgue [23]. However, a scent transfer unit was used in that previous study and, according to the authors, this sampling device led to high variability across replicates. Also, the sorbent material used as a trap has specific affinities with some cadaveric molecules (according to molecular weight and polarity), as previously demonstrated with solid phase microextraction [24]. We have probably collected and identified more compounds than previous works on fresh human remains thank to the use of thermodesorption tubes, which were already validated on vertebrate remains [15]. Unfortunately, none of the compounds we identified in the present study are human specific markers. These markers have been highlighted later during the decomposition process [11,16]. If any of these markers are released during this early stage of decomposition, they were released at the trace level, below our limit of detection.

Table 3
statistical values for studied factors.

	lividity	rigidity	PMI	skin T°	sex	age	size
classes limit	na	na	2 days	9.5 °C	na	61 years	167 cm
F value	1.75	0.699	0.992	1.201	0.466	0.883	0.614
Df	1,19	3,19	1,19	1,18	1,19	1,17	1,19
p-value	0.101	0.797	0.44	0.278	0.978	0.551	0.869

None of the studied factors had a significant impact on the human cadaveric volatilome (Table 3). We expected the presence of lividity as well as occurrence of rigidity to modify the volatile emissions of a corpse. Indeed, these first postmortem modifications highlight the evolution of the decay. Lividities result from the discoloration of blood due to a depletion of oxygen. Rigidities are caused by the breakdown of adenosine triphosphate and lead the muscle to reach an irreversible state of contraction. The muscle relaxation occurs when alkaline liquid from putrefaction are released [28]. These two postmortem changes were thus expected to have an impact on the cadaveric volatilome. We raise the hypothesis that the abundance of sulfur compounds should quickly change over time after death. Sulfur compounds are byproducts of

amino acid (main component of both blood and muscle) degradation (methionine, cysteine) [36].

Surprisingly, we could not show the impact of PMI on the emission of VOCs. This is probably because corpses with higher PMI were directly stored after the deceased. Hence, all corpses stood at the early fresh stages. The storing sufficiently slowed the decomposition process, to avoid the release of additional cadaveric compounds, as already highlighted in previous studies conducted on human blood [12]. In addition, the diversity of samples probably explains the absence of statistical difference: very few corpses had a long PMI. The same argumentation applies for the temperature of the skin.

Regarding intrinsic parameters, no differences were highlighted (Table3). We expected an impact of the age of the deceased person. Indeed, elderlies release specific compounds, including dimethylsulphone, benzothiazole and nonanal [37]. Here, only nonanal was detected, and only in one corpse. No differences were shown among male and female cadavers. Our sample included only six women, all being over 50 years old, while male cadavers belonged to more varied age groups. More standardized samples could help to confirm the absence of effect. Regarding the impact of the size of the cadaver, we recommend evaluating body mass index (BMI) instead of body length. BMI gives a good indication of the ratio of macromolecules in a body, including proteins, lipids, carbohydrates. BMI could therefore be a good candidate to sort out the available corpses, especially because each tissue/organ releases its own VOCs profile while decomposing [17].

Conclusion

Our study has been conducted on a large sample size, with standardized decomposition stage and sampling methodology. Targeted ion methodology coupled with a single dimension GC–MS separation allowed the identification of 23 cadaveric volatile compounds. We found that neither age, size, gender, PMI impact the volatilome at the fresh stage. We recommend using standardized method and homogenous samples to investigate the impact of specific parameters. Performing a similar assay on more advanced stages of decomposition would be extremely informative but remains challenging in terms of sample access and size.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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