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Potential of direct immersion solid-phase microextraction to characterize dissolved volatile organic compounds released by submerged decaying rat cadavers

Marta Malevic, François Verheggen *, Clément Martin

Gembloux Agro-Bio Tech, TERRA, University of Liège, Avenue de la Faculté 2B, 5030 Gembloux, Belgium

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ABSTRACT

The decomposition process involves the degradation of carbohydrates, nucleic acids, proteins and fats, and leads to the release of volatile organic compounds (VOCs) among many other decomposition by-products. Despite the extensive literature on the VOCs emitted in the air from vertebrate corpses, there is a lack of research dedicated to aquatic decomposition. In this study, we aimed to evaluate the potential of direct immersion solid-phase microextraction gas chromatography coupled with mass spectrometry (DI-SPME/GC/MS) to characterize dissolved cadaveric VOCs. Dimethyl disulphide and indole -two compounds commonly released during decomposition- were selected to evaluate and set the optimal methodological parameters, which were found to be 10 min of collection performed under 27.5 °C and a stirring rate of 250 rpm. Using responsive surface methodology, the obtained curves highlighted the appropriate conditions for the dissolved cadaveric volatilome analysis. The method allows to trap 17 dissolved cadaveric VOCs, including commonly encountered compounds such as dimethyl disulfide, 9-hexanoic acid, dimethyl trisulfide and indole. DI-SPME/GC/MS has therefore potential for the identification of dissolved cadaveric VOCs, pending further tests are performed to optimize the method and make it capable of detecting all dissolved VOCs, through all stages of decomposition.

Introduction

The decomposition process of vertebrates involves the degradation of macromolecules (carbohydrates, nucleic acids, proteins and lipids), that leads to the release of volatile organic compounds (VOCs) among many other decomposition by-products [1-4]. Most previous studies have focused on cadavers placed in terrestrial environment, i.e. open-air or underground [2,5-7], and very few contributions have been made on submerged decomposition [8-11]. Even though the decomposition rate differs [12], underwater body decomposition follows a succession of stages similar to terrestrial ones [10,13]: the cadaver first experiences the submerged fresh stage, which starts in most cases upon its full submersion and lasts until the first signs of bloating appear. During the bloating stage, the corpse floats to the surface and a pronounced and evident decaying odour is released. The third stage is characterised by a green discoloration around the abdomen and skin sloughing due to gas and fluid pressure. In the fourth stage, the body starts to deflate and releases a much less pronounced odour. The body sinks during the fifth stage, leaving some dry skin remains floating at the surface [14].

Hundreds of cadaveric VOCs have been reported by studies dedicated to open-air or below ground decomposition, and the most complete lists of VOCs were obtained from dynamic air samplings coupled with bidimentsional gas chromatography [2,15]. The molecules detected during open-air or underground decomposition belong to almost all chemical families including ketones, nitrogen based molecules, sulphur based molecules and carboxylic acids, just to name a few [16-18]. These molecules are produced under aerobic and anaerobic conditions [19]. One study has been dedicated to the characterisation of cadaveric VOCs profile released at the water surface by immerged cadavers [26], and 41 VOCs have been identified by headspace solid-phase-microextraction (SPME). The volatile profile released by immersed bodies is less diversified than open-air decomposition probably because (1) most of the process takes place under anaerobic conditions and (2) because some compounds remain dissolved in water, therefore could not reach the headspace and be adsorbed on the SPME fiber.

The present study aims to evaluate the potential of direct immersion solid-phase microextraction, coupled with gas chromatography and mass spectrometry (DI-SPME/GC/MS), to characterize dissolved

* Corresponding author.
E-mail addresses: fverheggen@uliege.be (F. Verheggen), cmartin@uliege.be (C. Martin).

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cadaveric VOCs. We performed volatile collection on water samples having contained submerged rat cadavers -used as surrogates for human cadavers- [21], and we have selected the sampling conditions using a response surface methodology (RSM).

MATERIALS & METHODS

Rat decomposition and water sampling

Two male laboratory rats (244.5 ± 16.5 g) (*Rattus norvegicus*, Berkenhout, 1769) were reared and euthanized at the Faculty of Veterinary Medicine of the University of Liège (ethic agreement n°18- 2021). None of the rats was killed for the present experiments; they had been used in a previous one (which could not be communicated to the authors of the present research). Rats had been asphyxiated with CO_2 and kept frozen prior the experiment. Despite the potential impact of freezing on VOC emissions [20], we believe that this procedure does not impact the objective of this work (i.e. evaluate the potential of DI-SPME to characterize dissolved cadaveric VOCs). Each rat was defrosted in a hot water bath (≈ 40 °C) and left to decompose inside 15L open glass cylindrical tanks filled to the top with distilled water. A third tank, that contained no rat but filled with distilled water, was used as a control. All three tanks were placed in a room set at 18 °C. A sample of 40 ml of water was collected in each tank including the "control tank" after 30 days to maximize the quantity of cadaveric VOCs in the water. The samples were kept in 50 ml Falcon tubes in a freezer at −20 °C prior to analyses.

Initial DI-SPME sampling

To target compounds which could be used for the optimization of the sampling, DI-SPME samples were performed using polydimethylsiloxane SPME fiber coating (PDMS, Supelco, Bellefonte, PA, USA). This coating is recommended by the supplier for the analyses of chemicals in water. After a 30 min conditioning at 300 °C in the GC injector, the fiber was immersed in the water sample for 45 min. The samples were kept in a water bath set at 40 °C and stirred at 500 rpm. After sampling, the fiber was dried under a nitrogen flow for one minute at room temperature (22 °C) to avoid water reaching the mass spectrometer. The fiber was then introduced in the GC injector, and VOCs were desorbed at 275 °C under a flow of 1 ml/min of helium in split mode (split ratio 1:5). The collected compounds were separated on a gas chromatograph (QP 2020 NX, Shimadzu, Kyoto, Japan) and detected by a quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). The electron impact mode was set at 0.1 kV (source at 200 °C, transfer line at 250 °C, scanned mass range: 30 to 500 *m/z*). The temperature program used was very slow to decrease the risk of coelution as this matrix is expected to be rich in compounds: 30 °C held for 3 min, 5 °C/min until 350 °C held for 5 min. The detected chemicals were identified by interpretation of their mass spectra and comparison with spectral libraries (NIST version 2017 and FFNSC version 3).

Chemicals' preparation

Two compounds, namely dimethyl disulphide (99.8% purity, Sigma Aldrich) and indole (99% purity, Sigma Aldrich), were selected to assess the optimal values of the main experimental parameters. These chemicals are commonly reported as cadaveric compounds and were identified during the previous step. A stock solution containing the two compounds was prepared at a concentration of 100 ng/µl, in miliQ water. That concentration was chosen based on the literature [6,22] and after performing pre-test SPME analyses of two stock solutions (100 ng/µL and 1000 ng/µL). The testing conditions are provided in Supplemental Table B1. Twenty-four glass vials (V = 20 ml) were filled to the top with the stock solution (100 ng/µl) previously sealed with a cap in preparation for SPME analysis.

Data analysis

To optimize the sampling method, a response surface method (RSM) was performed. This method is based on a collection of mathematical and statistical techniques that are used to model and analyse engineering applications [19,23]. It aims at optimizing several experimental parameters (namely sample temperature, extraction duration and stirring speed) to increase the response of interest allowing a better sensitivity (i.e., the number of detected volatile compounds). RSM has already been applied in several industrial fields as well as in forensic science [23-25]. However, RSM has never been applied to improve the analysis of cadaveric VOCs. RSM is usually performed following four steps: designing a series of experiments, developing mathematical models, identifying optimal combination of parameters and representing the predictive model with 2D or 3D plots [19]. The plot can be represented by an equation: $Y = f(x_1, \ldots x_n)$, with RSM aiming at maximizing Y. To obtain RSM curves, an initial testing was conducted on 24 samples differing in conditions of stirring rate (between 200 and 400 rpm), temperature (between 23 and 33 °C) and sampling duration (between 5 and 15 min). The different tested points of the RSM are detailed in the supplementary material (Supplemental Table B1). Each of the tested points was associated with a GC/MS chromatograph. The GC method used to separate the compounds was shortened (Table A1).

Testing the optimization

Following the result obtained with the RSM, a second sampling was performed on the water where a rat was left to decompose during one month and analysed based on the previously described method (Table A1). The method was shortened to improve peak quality. A qualitative comparison was performed to determine if the optimized method allows to detect more compounds, in higher quantities.

Results & discussion

DI-SPME sampling

The preliminary analyses performed on the dissolved volatilome of thirty days submerged decaying rats allowed the identify seven compounds (Table A2). Among these, five have never been identified in the headspace of submerged bodies nor in terrestrial decaying bodies [16,24-27]. The analyses reveal that tetrahydro-indazol-4-one, DMDS, lactic acid and indole are the major compounds. After six days, the rats switch from the first stage of decomposition to the second one. The analyses were performed when rats reached the third stages to maximize the quantity of expected compounds (thirty days after the beginning of the decomposition). However, a small number of compounds was identified despite the samples being taken at a later stage of decomposition [2]. A too high stirring rate, temperature and/or sampling time could have removed compounds from the fibre resulting in the reduced number of detected compounds [30].

Table A1
Analytical parameters of the TD30R-GC/MS analysis.

Injector	GC–MS
Desorption temperature: 275 °C/ 2 min Desorption mode: splitless	**Carrier gas**: Helium **Column**: HP-5MS 30 m × 0.25 mm × 0.5 µm **Initial temperature**: 30 °C/5min **First ramp**: 5 °C/min until 90 °C **Second ramp**: 10 °C/min until 300 °C hold during 3 min **Detector**: MS **Mass scan**: *m/z* 30–500

Table A2
Peak areas of the organic compounds collected from submerged rats before and after the optimisation of the sampling method (* refers to compounds that were identified from submerged bodies by Irish et al 2019 [26]).

		Pre-optimized samplings	Optimized samplings
Aldehyde	Tetradecanal	46.505	0
Acids	Lactic acid	281.577	0
	Dodecanoïc acid	28.912	133.450
	Formic acid	0	140.072
	Tetradecanoic acid *	0	165.901
	9-Hexanoic acid *	0	2,402.985
	9-Octadecenoic acid *	0	405.476
Hydrocarbons	Pentane, 3-methyl-	0	185.999
	Hexane, 2-methyl-	0	145.201
	Phenol, 4-(1,1-dimethylpropyl)-	94.504	0
	Cyclopentane, methyl-	0	654.939
Ketones	5.9-undecadien-2-one, 6,10 dimethyl-	0	138.665
	Non-identified ketone	0	159.532
Nitrogen containing compounds	Indole*	246.535	2,225.704
	3,6,6-trimethyl-1-O-tolyl-1,5,6,7-tetrahydro-indazol-4-one	377.576	0
Sulphur containing compounds	Dimethyl disulfide *	311.075	4,010.741
	Dimethyl trisulfide *	0	195.825

Response surface methodology

RSM curves have been generated using different sampling durations, sampling temperatures and stirring rates (Fig. A3). Our data show that the stirring rate has a low impact on the adsorption of the compounds in the tested conditions. Also, they suggest temperature and collection duration to be the main parameters to focus on when using DI-SPME for forensic applications. Indeed, the adsorption of indole on the fiber follows a quadratic correlation with time and temperature, as highlighted by a single maximum on the curve. However, the curves could not highlight a single optimal combination of parameters in the case of DMDS, for which the adsorption increased with the sampling time and decreased with the rise in temperature. The RSM curves highlight a stationary point that represents a good compromise for the dissolved cadaveric volatilome analysis: duration of the sample collection = 10 min; temperature = 27.5 °C; stirring rate = 250 rpm. These parameters are in the positive slope of each DMDS RSM cuve and meet the maximum observed on indole RSM curve (Time vs temperature).

In the case of the cadaveric volatilome, the diversity of molecules and chemical families is so important that our analysis could not guarantee an optimal result for all compounds [16,17,31]. The SPME is mainly used as a qualitative method to characterize the compounds that are present in a volatilome and for cross-sample comparison. Quantification of complex samples requires laborious operations and is not often performed [32]. The stationary point observed on the RSM curve highlights appropriate conditions to sample dissolved cadaveric VOCs in water.

Testing the DI-SPME sampling

Under the selected conditions (time = 10 min; temperature = 27.5 °C; stirring rate = 250 rpm), the number of compounds trapped on the fiber almost doubled. The quantity of both indole and DMDS also increased with the new methodology. In total, 13 cadaveric compounds were identified after optimization instead of seven during pretests. The dissolved VOC profile is poor, when compared with the large lists of VOC collected in the headspace of drowned bodies [6]. Among the possible explanations: the present experiment was performed with small amounts of water, under controlled environement, and rats were used as surrogate human cadavers. Unlike pigs, rats are not the most appropriate to predict human decomposition [33,34]. Further tests performed outside laboratory settings should be carried out.

Conclusion and recommandations

In this study, we have shown that DI-SPME can be applied to characterize dissolved cadaveric compounds. After some optimizations, we managed to increase the number of VOCs directly collected from water, even though their concentrations in water were very low. This method should be optimised using a larger diversity of cadaveric compounds and a larger number of replicates. Other vertebrate species should be considered, since each species release specific compounds during their decomposition [33]. We are convinced that some cadaveric VOC remain dissolved in water and are therefore not released at the water surface, their identification remains to be done. Also, the method remains to be tested during all decomposition stages. Finally, water composition (saline water, river water, lake water) is expected to impact the decomposition and the released VOCs [28,29].

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Verheggen reports financial support was provided by University of Liege.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.forc.2023.100488.

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