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Short communication

Changes in volatile compounds of human urine as it ages: Their interaction with water

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ABSTRACT

The urinary odors are commonly perceived as unpleasant. While numerous studies have identified the volatile organic compounds (VOCs) released from urine, the odorants responsible for the urine odor are not well characterized. Furthermore, anecdotal reports suggest that the odor of aged urine is different from that of fresh urine. However, no study has yet to investigate the specific VOCs released from aged urine. In this study, we analyzed and compared the VOCs released from fresh and aged urine samples, investigating the changes in the urinary VOCs as urine aged. We found an overall decrease in concentration of many urinary VOCs, and concluded this was due to the urine evaporating as it aged. On the contrary, some highly water-soluble compounds such as short and branched-chain organic acids and trimethylamine, increased. Their increased release is most likely due to the loss of water and the subsequent release of water-soluble VOCs as urine ages. We suggest that these VOCs may contribute to the odor of the aged urine.

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1. Introduction

The urinary odors emitted from toilet restrooms and individuals suffering from either incontinence and/or metabolic disorders are commonly perceived as unpleasant. Patients suffering from incontinence often feel embarrassed of their urine odor [1]. The community would benefit from the identification and deodorization of the unpleasant urinary odorants. While numerous studies have determined the volatile organic compounds (VOCs) released from urine (reviewed in [2]), the specific compounds responsible for the urine odor are not well characterized. In fact, to our best knowledge, only a couple of studies investigated the odorants isolated from urine. Wagenstaller and Buettner [3] identified 14 urinary odorants by using gas chromatography–olfactometry and gas chromatography–mass spectrometry. Some of the odorants such as p-cresol, skatole and androstenone have fecal and urine-like odor notes, but how each odorant contributes to the overall smell of urine is unknown. Troccaz et al. [4] identified the odorants of the boiled urine and the urine incubated with microorganisms, which would be different from those of fresh urine. Additionally, anecdotal reports suggest that the odor of aged urine is different from that of fresh urine. The VOC profile in urine will be changed over time. As urine ages, its constituents are altered due to a variety of processes, as some molecules decompose and VOCs evaporate into the air. The water content in urine may influence the release of VOCs from urine as well. For example, the evaporation of water-soluble VOCs may accelerate as urine dries. Or perhaps the loss of water may cause some VOCs to bind to solid surfaces, preventing them from being released into the air. However, the investigation of the VOCs or odorants released from aged urine is very limited. Therefore, in this study, we analyzed and compared the VOCs released from fresh and aged urine samples, investigating the changes in the urinary VOCs as urine aged.

2. Materials and methods

2.1. Chemicals

Trimethylamine, acetone, ethanol, 2-butanone, 2-pentanone, 2-hexanone, 3-hexanone, 3-heptanone, 4-heptanone, 2-heptanone, 3-methylcyclopentanone, 2-nonanone, acetic acid, propylene glycol, 2- and 3-methylbutyric acids, acetamide, caprolactone,

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dimethyl sulfone, and p-cresol were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Human urine donors and urine collection

The study population for this report consisted of 6 adults (3 males and 3 females). Void urine samples were collected from each volunteer daily and stored at −20 °C. All procedures were approved by the Office of Regulatory Affairs at the University of Pennsylvania and informed consent was obtained from each urine donor before study participation.

2.3. Aging procedure of urine aliquots

The urine samples collected were combined, and a total of six 1 mL aliquots were prepared from a pooled sample. Each aliquot was placed in a 60 mL glass jar (53 mm o.d.; 47 mm height) and capped. Three of the aliquots were analyzed immediately for their VOCs [Intact]. The remaining aliquots were uncapped, placed into a fume hood, and were aged for 24 h. These 24 h-aged samples were nearly dried. The aged samples were then capped and analyzed [Aged].

2.4. Collection and analysis of volatile compounds released from urine

Each jar containing urine aliquot was extracted with a 2 cm, three-component solid phase microextraction (SPME) fiber (30 μm carboxen, 50 μm divinyl benzene, polydimethyl siloxane, Supelco Corp., Bellefonte, PA) for 30 min at room temperature. The SPME fiber containing the adsorbed VOCs was then inserted into the injection port of a Thermo ISQ single quadrupole gas chromatograph–mass spectrometer (GC–MS) (Waltham, MA) at 230 °C for 5 min. The GC–MS equipped with a Stabilwax column (30 m × 0.32 mm with 1.0 μ film thickness; Restek, Bellefonte, PA) was used for separation and analysis of the desorbed VOCs. The GC temperature program started at 60 °C for 4 min, and increased at 6 °C/min to 230 °C where the final temperature was held for 13 min. Helium was used as the carrier gas at a constant flow of 1.5 mL/min. The transfer line temperature between GC and MS was 230 °C. The mass spectrometer was operated in the electron ionization mode at 70 eV. The ion source temperature was 200 °C. The scanning frequency was 4 Hz from m/z 41 to m/z 300.

2.5. Compound identification

Compound identification was accomplished via mass spectral library comparison (The NIST11 library vested in the Thermo Xcalibur software that controlled the GC–MS and that was also used for data analysis was used for comparison) combined with manual interpretation and comparison with standard samples for the retention times and mass spectra. Pentolactone, 2-methyl-3-pentanone, 3-ethyl-2-pentanone, 3-methyl-2-hexanone, 3-methyl-2-heptanone, 6-methyl-3-heptanone, 2-methyl-4-heptanone, 4-nonanone, 3-ethylcyclopentanone, 3,5-dimethyl-2-octanone, 4-hydroxy-2-pentanone, p-menthan-3-one, 3,6-dimethylbenzofuran, and p-menth-1-en-3-one were tentatively identified using the NIST11 mass spectral library.

2.6. Data analysis

A total of 6 total ion chromatograms (TICs) were obtained: 3 for the 'intact' samples and 3 for the 'aged' samples. The resulting chromatograms and mass spectra were then analyzed by using Metabolite Differentiation and Discovery Lab (MeDDL [5]; http://meddl.cs.wright.edu/doku.php), a novel metabolite profiling software solution adapted for GC–MS data [6]. In brief, the MeDDL tool reads in lists of CDF (common data format) conversions of the raw GC–MS data files; registers peaks based on user-defined filters in terms of mass sensitivity and accuracy thresholds as well as chromatographic reproducibility tailored to the performance of the analytical platform; and performs alignment of the generated peak lists in both time and mass. Following registration and alignment, the defined result groups are processed through a logical combination of the following data filters: a fold change filter and time binning, which are described elsewhere in detail [5]. In this study, the following filter settings were used: the fold change filter that limited the peaks with 2-fold or greater change in intensity and the time binning filter using 0.1 min bins inclusive of only those peaks >10 K absolute intensity. Once each of these filters was applied to the grouped, global data set, a Boolean "AND" was added to the resulting filtered peak sets to identify their logical intersection. The cdf files converted from the raw GC–MS files for the MeDDL analysis and the resulting files are available at http://meddl.cs.wright.edu/doku.php?id=documentation.

3. Results and discussion

As shown in Fig. 1, substantial changes in the VOCs occurred when urine was aged for 24 h. The release of some VOCs decreased. When a urine aliquot was aged, for example, the major volatile constituents 4-heptanone (7) and acetone (2) decreased dramatically in the headspace as determined by the SPME collection. In contrast, the amount of some VOCs increased after the aliquot was aged. The minor constituents such as trimethylamine (1), propylene glycol (11), dimethyl sulfone (12) and pentolactone (13) in the intact urine aliquot increased after the aging process, and became the major VOCs in the chromatograms of the aged urine (Fig. 1).

To identify additional compounds that had changed levels after the urine aliquots were aged, MeDDL software was used to examine all GC–MS data. A total of 384 peaks were registered and aligned. Note that the number of registered peaks does not correspond to an equal number of individual compounds since a single compound is generally comprised of multiple peaks (ions) for the electron impact mass spectrum. The GC–MS data were then analyzed through application of the filters described in Data analysis. Results of the time-binning filter showed the presence of a minimum of 103 discrete chromatographic peaks comprising the data set. A pairwise comparison between the intact and aged urine samples for each peak was performed, and the number of binned peaks that displayed 2-fold or greater absolute intensity changes was 58. The 58 peaks were identified and a list of the identified VOCs is shown in Table 1. Note that unidentifiable or unknown compounds as well as environmental (e.g. chloroform, Texanol [a paint-derived compound]) and analytical (e.g. siloxanes, methoxy-phenyl-oxime [a SPME fiber-derived compound]) contaminants were excluded from the list. As seen in Table 1, more than two-thirds of the identified VOCs decreased as the urine aliquots were aged, which is likely due to the evaporation of the VOCs as urine ages. On the contrary, some VOCs all of which are highly water-soluble compounds increased. Their increased release is most likely due to the loss of water and the subsequent release of water soluble VOCs as urine ages. Due to the strong hydrogen bond between these compounds and water, they may not be easily released in the intact urine. When urine loses water as it dries, on the other hand, the hydrophilic compounds become dissociated from water and are facilitated to be released into the air.

In this study, we used 60 mL jars (53 mm o.d.; 47 mm height) where urine samples dried quickly due to the large surface area. Initially, 4 mL vials (15 mm o.d.; 45 mm height) were used where the evaporation of water from the urine samples was delayed. Notably,

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