Biotransformation, transmission and excretion processes of garlic odorants in humans: impact on human milk, urine and exhaled breath

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Abstract
The impact of raw garlic consumption (3 g) on the composition of human milk (n=18), urine (n=19) and exhaled breath (n=11) was explored. Urine and milk samples gathered prior to and after garlic ingestion (up to 24 h and 4 h, respectively) were analyzed by GC-MS and GC-GC-MS. Milk samples were additionally assessed by aroma profile analyses. Exhaled breath analysis was performed by PTR-MS. The compound allyl methyl sulfide was identified as garlic-derived metabolite in all three excreta. Furthermore, allyl methyl sulfoxide and allyl methyl sulfone were identified as metabolites in both urine and milk. These garlic-derived metabolites were quantified by means of stable isotope dilution assays. Two garlic-derived metabolites were identified in breath, namely allyl methyl sulfide and methanethiol. The measurements revealed inter-individual differences in metabolite concentrations and removal kinetics after consumption of 3 g of raw garlic.

Introduction
Garlic is savoured for its characteristic aroma and has been associated with beneficial health effects [1-3]. The active principles of garlic are not fully understood, although allicin and its metabolic derivatives have been proposed as active components [4]. Constituents ingested with food can be strongly modified in the body and evoke physiological effects [5]. In previous studies we identified three garlic-derived metabolites in human milk and urine, namely allyl methyl sulfide (AMS), allyl methyl sulfoxide (AMSO) and allyl methyl sulfone (AMSO²) [6, 7].These compounds have been identified as the dominant metabolites in rat liver, plasma and urine after treating rats with diallyl disulfide, a constituent compound in garlic [8]. To further characterize their biotransformation processes, the present study aimed to quantify these metabolites in human milk and urine and additionally explore the influence of raw garlic consumption on the odorant and metabolite composition of exhaled breath.

Experimental
Samples
This study was conducted in agreement with the Declaration of Helsinki. Written, informed consent was given by all volunteers prior to participation. Withdrawal from the study was possible at any time. The study (registration number 49_13B) was approved by the Ethical Committee of the Medical Faculty, Friedrich-Alexander Universität Erlangen-Nürnberg.

Milk samples were collected using mechanical or electrical breast pumps according to the mothers’ preferences. Sampling took place within the 9 to 36 weeks postpartum
lactation period. Milk samples (n=18) were collected at the times indicated in Figure 1. Sampling intervals reflected the normal lactation period of individual mothers.

3 g garlic

![Milk samples timeline](image)

**Figure 1:** Times of milk sample taking

Urine samples (n=19) were collected in brown glass bottles. Breath analysis (n=11) was performed by having individuals exhale deeply into a buffered-end-tidal (BET) sampling tube that was connected to a proton-transfer-reaction mass spectrometer (PTR-MS) for on-line analysis. Sampling times of urine and breath samples are indicated in Figure 2.

3 g garlic ingested

![Urine and breath sampling](image)

**Figure 2:** Urine and breath sampling. U: urine sample B: breath sample

**Stable isotope dilution assays (SIDA)**

Defined amounts of $^2$H$_2$-isotopically-labeled reference compounds were added to each milk and urine sample. Dichloromethane (DCM) was then added at a ratio of 1:2 (DCM/sample, v/v) to the sample. The mixture was stirred (30 min, room temperature) and then distilled via solvent-assisted flavour evaporation (SAFE; 50 °C) and subsequently concentrated to a volume of 100 µL. Analyses of AMSO and AMSO$_2$ were performed using GC-MS in selected ion monitoring (SIM) mode. The analysis of AMS was performed with GC-GC-MS. The metabolite concentrations in milk and urine samples were calculated from the intensity ratios of labeled to unlabelled compounds in the extracts.

**Creatinine content**

The creatinine content of each urine sample was determined using a creatinine kit (Labor + Technik Eberhard Lehmann GmbH, Berlin, Germany). The concentrations of the metabolites in urine samples were normalized to the creatinine content of the respective urine sample.

**Sensory analyses**

All milk samples underwent sensory analyses. These analyses took place prior to the work-up described above and were performed by a trained panel that evaluated the samples orthonasally.
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Breath gas analyses

On-line PTR- time-of-flight-MS (PTR-TOFMS) was used for monitoring the presence of garlic metabolites in exhaled breath gas [9]. Use of a BET sampling apparatus extended the analysis time of end-tidal breath and reduced the likelihood of interferences of garlic-constituents emanating from the stomach.

Results and discussion

The analyses revealed three garlic-derived metabolites in human milk and urine, namely AMS, AMSO and AMSO\(_2\). Of these three, only AMS had a garlic-/cabbage-like door whereas the other two metabolites were odourless. AMS had an impact on the aroma profile of the milk samples, whereby milk samples collected prior to garlic consumption did not exhibit the garlic-/cabbage-like door whereas samples collected after garlic intervention clearly did. Furthermore, the quantitative analyses revealed differences in concentrations and excretion kinetics of metabolites between individuals. Three examples of milk and urine sample sets are shown in Figure 3 and 4, respectively.

![Figure 3](image1.png)

Figure 3: Concentrations of garlic metabolites (left: AMS; right: AMSO and AMSO\(_2\)) in three different milk sets. pre: sample prior garlic ingestion. x h post: samples after garlic ingestion.

![Figure 4](image2.png)

Figure 4: Concentrations of garlic metabolites (left: AMS; right: AMSO and AMSO\(_2\)) in three different urine sets. pre: sample prior garlic ingestion. x h post: samples after garlic ingestion.

The highest concentrations of AMS and AMSO in the investigated milk samples were detected in the first samples taken after garlic consumption. In contrast, AMSO\(_2\) maxima were only detected in the second milk sample after garlic consumption for some
individuals. Similar results were observed in urine samples, with the highest concentrations of the garlic metabolite being detected 1-2 h after garlic intervention. By comparison, the AMSO$_2$ maxima often appeared slightly later than AMSO. In breath, the highest AMS concentration was observed between 0.5–4 h after garlic consumption (see Figure 5). Methanethiol displayed similar excretion kinetics to AMS.

![Figure 5](image)

**Figure 5:** Breath profiles of AMS (left; m/z 89.04) and methanethiol (right; m/z 49.01) for two individuals after consumption of 3 g raw garlic. t=0: time of garlic ingestion.

Overall, the duration and concentration of excretion of the metabolites exhibited large inter-individual variations, despite all individuals consuming identical quantities of raw garlic. The concentrations observed in urine samples were approximately twice or three times higher than those observed in the milk samples. Additional possible elimination routes such as via feces were not considered in this study. Nevertheless, these findings suggest that different garlic-derived metabolites are excreted via different metabolism pathways.

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**References**