Analysis of Compounds in Human Breath after Ingestion of Garlic Using Proton-Transfer-Reaction Mass Spectrometry

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After ingestion of raw garlic, the components allyl methyl sulfide (**1**), allyl methyl disulfide (**2**), diallyl sulfide (**3**), diallyl disulfide (**4**), diallyl trisulfide (**7**), dimethyl sulfide (**8**), and acetone (**9**) in the breath of a test person were analyzed over a time period of about 30 h by means of proton-transfer-reaction mass spectrometry. While the concentrations of **2**−**7** reached maxima shortly after ingestion of garlic and declined to baseline values within the next 2−3 h, concentrations of **1**, **8**, and **9** increased much more slowly and showed enhanced values even 30 h after garlic consumption. The strong increase of the concentration of acetone might be indicative of enhanced metabolism of serum cholesterol, triglycerides, and total lipids in the blood stream.

Keywords: *Breath gas analysis; garlic components; proton-transfer-reaction mass spectrometry (PTR-MS)*

INTRODUCTION

While intact garlic (*Allium sativum*) cloves show hardly any significant smell, crushed or cut garlic develops an extremely strong odor that also appears in the breath of persons who have consumed garlic (Block, 1992; Block et al., 1993). The strong odor persists for time spans up to more than a day. This phenomenon is now well understood. Within the garlic cloves, odorless alliin is stored in the mesophyll cells, well separated from an enzyme called alliinase, which is situated in the vascular bundle sheath cells (Ellmore et al., 1994). When force acts on garlic cloves, so that cells are damaged by crushing or cutting, the enzyme comes in contact with alliin, converting it to allicin, which has a typical, but not unpleasant, odor like garlic (Laakso et al., 1989). Allicin in turn is converted into rather strongly smelling organosulfides, the chemistry of which has been investigated and described in great detail by Block (1992).

The same author also has summarized the use of garlic as an important dietary constituent and as a medicine for the treatment of many disorders (Block et al., 1993) by the Egyptians, Greeks, and Romans in ancient times and ever since.

Block et al. (1993) also described early scientific investigations stimulated by the reputation of garlic as a "cure all", such as the work by Pasteur into garlic's antibacterial activity and work in 1892 by Wertheim and Semmler into the composition of distilled garlic oils (mainly diallyl disulfide). Also described are modern chromatographic investigations revealing degradation processes for allicin and other garlic thiosulfinates, particularly allyl methyl thiosulfinates, in the presence of heat or organic solvents. These analytical investigations led to a better understanding of the reasons garlic oil products show positive medical effects. For example, diallyl disulfide is known to inhibit the activation of nitrosamine, thus reducing the probability of the development of cancer of the stomach (Block, 1992).

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Ajoene, which forms by self-condensation from allicin in nonaqueous solvents, is an efficient antithrombotic agent, and allicin itself is antifungal as well as antibacterial.

Recently, a variety of sulfur- and selenium-bearing compounds were identified through the use of GC/MS techniques (Laakso et al., 1989; Minami et al., 1989; Ruiz et al., 1994) in the breath of persons after the ingestion of garlic and by an element specific technique of gas chromatography with atomic emission detection (GC-AED) (Cai et al., 1995). Among the main garlic-related compounds in the breath were allyl methyl sulfide (**1**), allyl methyl disulfide (**2**), diallyl sulfide (**3**) diallyl disulfide (**4**), 2-propenethiol (**5**), dimethyl disulfide (**6**), and diallyl trisulfide (**7**). Cai et al. (1995) show measurements taken shortly after the ingestion of garlic and at four hourly intervals thereafter, indicating a more rapid decrease with time of diallyl disulfide (**4**) than allyl methyl sulfide (**1**). Compounds **2**, **3**, and **7** were reported to decrease slowly, while 2-propenethiol (**5**) vanished rapidly.

In this work we report on the time dependence of concentrations of compounds **1**−**4** and others in the breath after consumption of garlic, measured quasi on-line in small intervals of time, over a total time period of 32 h. The distinct differences observed for several compounds yield information on the metabolism of several garlic compounds in the human body.

EXPERIMENTAL PROCEDURES

Materials and Instruments. A recently developed method called proton-transfer-reaction mass spectrometry (PTR-MS) was used (Hansel et al., 1995), which allows for fast simultaneous monitoring of the concentrations of many volatile organic compounds, including most of the ones mentioned above, present in human breath after consumption of garlic.

As the method is new and thus not yet widely known, a brief description needs to be given here. The apparatus used (a schematic drawing of which is shown in Figure 1) consists of a conventional drift tube equipped with a hollow cathode ion source which produces H_3O^+ ions with only traces of impurity ions (mainly O_2^+), so that no mass spectrometer is needed to preselect H_3O^+ before injection into the drift tube. In this way a high primary ion signal is detected at the downstream end

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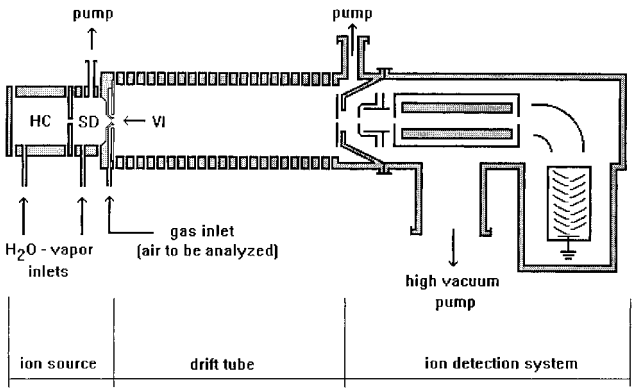

Figure 1. Schematic representation of the PTR-MS system: HC, hollow cathode; SD, drift region; VI, Venturi type inlet.

of the drift tube, which is a necessary prerequisite to achieve a high sensitivity of the system. The air to be analyzed acts itself as the buffer gas (the pressure is $\sim 10^{-1}$ Torr) in the drift tube. This is possible because H_3O^+ does not react with any of the natural components of air, as they all have lower proton affinities than H_2O. On the other hand, H_3O^+ performs proton transfer in nondissociative reactions

$$H_3O^+ + R \xrightarrow{k} RH^+ + H_2O \qquad (1)$$

with many volatile organic compounds, R, with large rate constants, k, equal to the collisional limiting values ($\cong 10^{-9}$ cm^3 s^{-1}) (McFarland et al., 1973).

The density [R] of a neutral impurity R is calculated from the count rates $i(RH^+)$ and $i(H_3O^+)$ obtained in the downstream ion detection system using the relation

$$i(RH^+) = i(H_3O^+)_0(1 - e^{-k[R]t}) \approx i(H_3O^+)_0\,[R]kt \qquad (2)$$

where t is the time the H_3O^+ ions need to pass through the drift tube. It is calculated from known mobility values of H_3O^+ in air in the usual way (McFarland et al., 1973).

An electric field E is applied along the drift tube to avoid substantial formation of cluster ions $H_3O^+(H_2O)_n$, $n = 1,2, ...$, which otherwise would be created in association reactions of H_3O^+ with water molecules present in the air to be analyzed. Increasing E and thus E/N (N being the number density of the buffer gas) leads to an increase of the relative kinetic energy, KE_{cm}, between the reactants, which is calculated, when necessary, according to standard procedures as described by McFarland et al. (1973). Usually, throughout the measurements, E/N is kept at values of 120−140 Td (1 Td = 1 Townsend = 10^{-17} V cm^2), which makes $KE_{cm} \sim 0.3$ eV, depending somewhat on the mass of the neutral reactant R.

Total reaction rate constants, k, and branching ratios for specific reactions were measured using a conventional selected ion flow drift tube, which has been described in detail elsewhere (Lindinger, 1986). The SIFDT system was operated with a helium buffer, and the reduction of the data is done as described earlier (Hansel et al., 1995). These and other measurements (Warneke et al., 1996; Praxmarer et al., 1994) have shown that in the case of exoergic proton-transfer reactions there is excellent agreement between measured reaction rate coefficients and calculated capture rate coefficients (Su and Chesnavich, 1982). The reaction rate coefficients, k, related to the components studied in the present investigation lie in the range 1.7×10^{-9} cm^3 s$^{-1} \leq k \leq 2.9 \times 10^{-9}$ cm^3 s^{-1}.

Component Identification. Checks were made by adding known amounts of various compounds to Tedlar bags filled with 3 L of air and by introducing this gas mixture to the PTR-MS system. Figure 2 shows a mass spectrum obtained from a prepared sample of a mixture of laboratory ambient air and 2.5 ± 0.5 ppm of allyl methyl sulfide (**1**). The primary ion H_3O^+ appears at mass 19, with the natural isotope $H_3{}^{18}O^+$ at mass 21 showing the expected relative intensity of 0.2% with respect to mass 19 (the natural isotopic ratio $^{18}O/^{16}O$ is 0.2%).

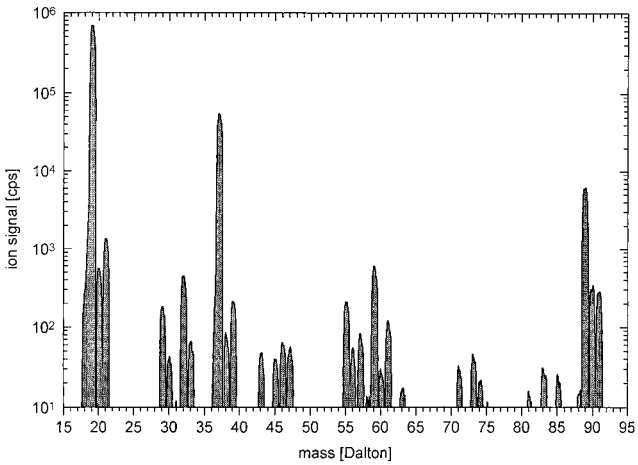

Figure 2. Mass spectrum obtained when laboratory ambient air containing ∼2.5 ppm of allyl methyl sulfide (**1**) was introduced into the PTR-MS system.

The intensities at masses 89, 90, and 91 pertain to the ion signals of protonated allyl methyl sulfide, $^{12}C_4H_9{}^{32}S^+$ (mass 89), $^{12}C_3{}^{13}CH_9{}^{32}S^+$ and $^{12}C_4H_9{}^{33}S^+$ (both having mass 90), and $^{12}C_4H_9{}^{34}S^+$ (mass 91). The relative intensities of the respective neutral isotopic species calculated from the natural abundances of the isotopes ^{13}C, ^{33}S, and ^{34}S, respectively, are 100:5.4:4.6 (mass 88:89:90). The measured count rates for the respective protonated species are 6206, 341, and 281, yielding a ratio of 100:5.5:4.5, in excellent agreement with the above calculated values. Using the ion intensities shown in Figure 2 as well as eq 2 and $k = 2.7 \times 10^{-9}$ cm^3 s^{-1} (this rate coefficient was measured for the reaction of H_3O^+ with allyl methyl sulfide), a relative density of allyl methyl sulfide of 2.37 ppm in air is obtained, in good agreement with the value of the prepared sample. The data of Figure 2 also show that there is no breakup of the protonated allyl methyl sulfide ion.

Ions at masses 37 and 55 are hydronium−water clusters $H_3O^+H_2O$ and $H_3O^+(H_2O)_2$, respectively. Mass 59 is protonated acetone, present in the laboratory ambient air typically at levels of ∼0.3 ppm. Mass 32 is the impurity primary ion O_2^+. Masses 33 and 47 are protonated methanol and ethanol, respectively. The rest of the ions with smaller intensities originate either from trace components in air or from impurity ions produced in the ion source.

Having shown that allyl methyl sulfide (**1**) is identified by the isotopic ratios of masses 89:90:91 and quantified using measured count rates of the primary ion H_3O^+ and the count rates of the product ions being protonated allyl methyl sulfide, the same procedure was applied to identify and quantify allyl methyl sulfides in human breath after consumption of garlic by a test person. As will be shown later (Figure 5), the concentration of **1** was measured as a function of time by monitoring the ion signal at mass 89 before and after consumption of garlic; to ensure that mass 89 obtained from human breath is indicative of protonated allyl methyl sulfide, the signals at masses 90 and 91 were also monitored. Figure 3 shows the respective ion signals as a function of time over a period of 9 h. The average signal ratio of masses 89:90:91 after the consumption of garlic (dashed vertical line) yields 100:5.5:4.1, which is quite close to the ratio 100:5.4:4.6 calculated for the protonated natural allyl methyl sulfide isotopes, and confirms that protonated allyl methyl sulfide was the only component detected from human breath at mass 89.

The other main components of garlic breath were investigated with the same or a similar procedure, so that unambiguous identification and exact quantification (to a degree of about ±20%) were obtained.

Human Breath Samples. Garlic was purchased at a local supermarket. A 27-year-old male test person weighing 90 kg consumed 38 g of garlic, which was cut into small pieces a few minutes before ingestion; 100 mL of water was swallowed right after that together with some bread. Breath samples having a volume of 2−3 L were collected approximately every

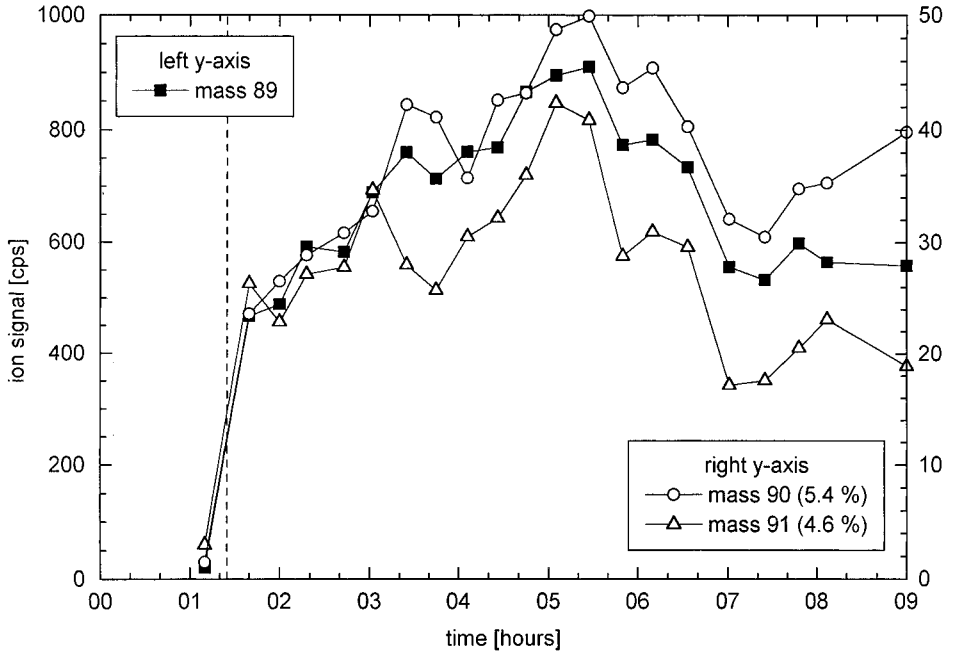

Figure 3. Ion count rates at masses 89, 90, and 91 as a function of time as obtained from the breath of a test person who ingested 38 g of garlic. The ratios of the measured count rates are indicative of protonated allyl methyl sulfide and its natural isotopes. The time 00 corresponds to 9:00 a.m. The vertical dashed line indicates the time of the ingestion of garlic.

20 min using Tedlar bags, starting about 1 h before consumption of garlic and extending for a time span of about 8 h after the intake of garlic, at which time the test person left for the night. Measurements were started again in the morning (which left an unobserved nightly time span of 14 h) and extended for about an additional 9 h.

The Tedlar sample bags were put into an oven, which was kept at a temperature of 42 °C to avoid condensation prior to injection of the breath samples into the PTR-MS system by means of the gas inlet indicated in Figure 1. The time delay between taking of the sample and analyzing it was typically <1 min.

The overall procedure described above was performed a second time with the same test person consuming about the same amount of garlic. The measurements yielded approximately the same results as in the first run. Thus, in the following only data of the first run are shown.

RESULTS AND DISCUSSION

The concentrations of those compounds in the breath of the test person were observed to change significantly after the consumption of garlic are shown as dependent on time in Figures 4 and 5. There are two distinctly different groups of compounds. Allyl methyl disulfide (**2**), diallyl sulfide (**3**), diallyl disulfide (**4**), and diallyl trisulfide (**7**) rise to a maximum concentration shortly after ingestion of garlic and decline to normal baseline values within the next 2−3 h (Figure 4). To what degree the observed baseline levels (being a few parts per billion) actually consist of impurities could not be determined unambiguously. In the case of **2**, **3**, and **7** peak concentrations are quite low, being 32, 13, and 5.5 ppb, respectively, while diallyl disulfide (**4**) reaches a peak concentration of 130 ppb. These four components, **2**−**4** and **7**, are also present in the head space air sampled from crushed garlic, as was observed presently in agreement with earlier work (Vernin et al., 1986; Laakso et al., 1989; Cai et al., 1995).

In contrast to these compounds, allyl methyl sulfide (**1**), dimethyl sulfide (**8**), and acetone (**9**) increase much more slowly after garlic ingestion (Figure 5). Compound **1** reaches a maximum of about 900 ppb after 4−5 h and

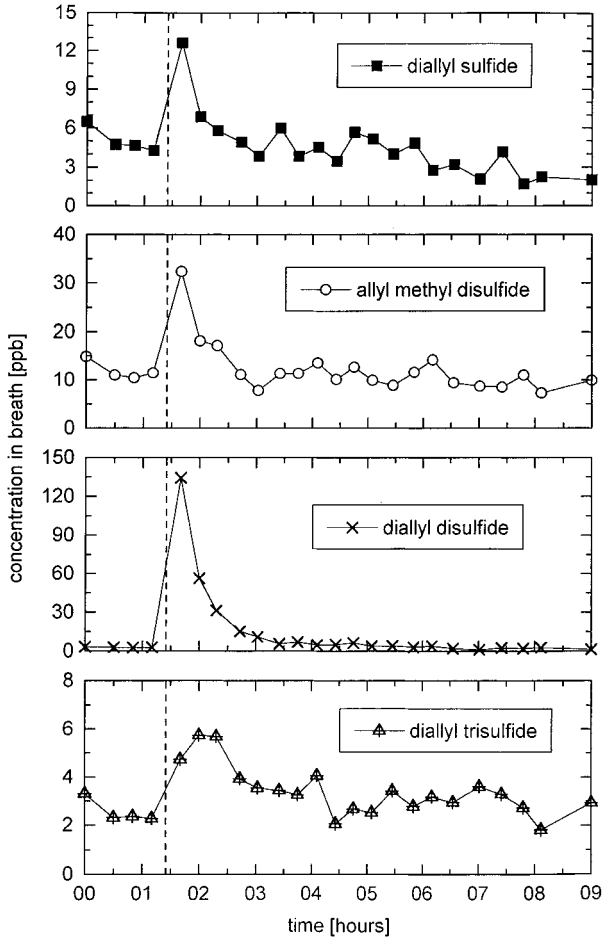

Figure 4. Variation in the concentrations of diallyl sulfide, allyl methyl disulfide, diallyl disulfide, and diallyl trisulfide in human garlic breath with time. The time 00 corresponds to 9:00 a.m. The vertical dashed line indicates the time of the ingestion of garlic.

then declines quite slowly, such that more than a day later substantial concentrations of 100−250 ppb are still detected. As **4** has an odor threshold of 0.5 ng/L ≅ 0.1

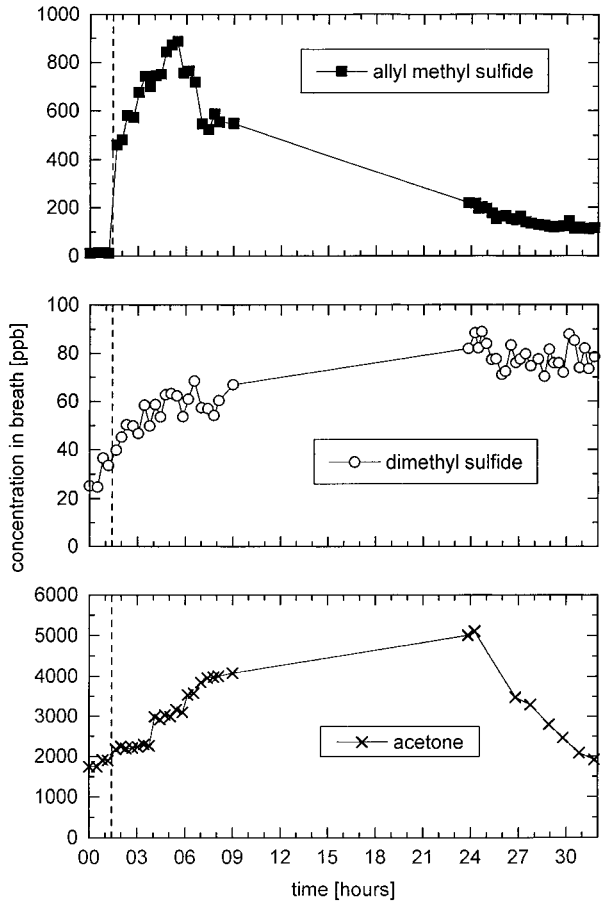

Figure 5. Variation of allyl methyl sulfide, dimethyl sulfide, and acetone in human garlic breath with time. The time 00 corresponds to 9:00 a.m. The vertical dashed line indicates the time of the ingestion of garlic.

ppb (Ruth, 1986), the sulfur-bearing compound **1**, which has much higher concentrations than this threshold, might be mainly responsible for the typical "garlic breath" persisting for a long time after ingestion of fresh garlic (Blankenhorn and Richards, 1936). While allicin, the main component in the extract from crushed garlic (Block, 1992), is observed also in the head space air of garlic, it is not present in the exhaled air of the test person. Allicin probably is metabolized very quickly in the human body as may be expected from the observation by Laakso et al. (1989) that allicin is quite unstable in a fatty oil extract.

The present data on the time dependences of the concentrations of allyl methyl sulfide (**1**) and diallyl disulfide (**4**) in garlic breath agree reasonably well with the results of Ruiz et al. (1994). These authors report **1** to reach a maximum concentration about 1 h after ingestion of garlic, while **4** reaches its maximum much earlier, declining to negligible values within about 1 h. In our case **1** reaches its maximum concentration even later (about 4 h after garlic ingestion), but **4** also vanishes within 1−2 h after garlic ingestion. The later maximum of **1** and long persistence of elevated concentrations of **1** over a time span of more than 24 h may be due to the much larger amount of garlic ingested by the present test person (38 g) as compared to the case reported by Ruiz et al. (1994) (8 g). The ratio of the maximum concentrations **1**:**4** is about 3 in the case of Ruiz et al. (1994), in fair agreement with a factor of ∼7 observed presently; however, our observed absolute maximum concentrations (∼1 ppm for **1** and ∼0.15 ppm for **4**) are quite low with respect to the ones reported by Ruiz et al. (1994).

Qualitatively the present results on the densities of **1** and **4** are similar to the ones very recently reported by Cai et al. (1995), who also observed **4** to vanish very rapidly after garlic consumption, while **1** is more persistent again. Their absolute maximum concentrations are ∼7 ppb for **1** and ∼5 ppb for **4**. These much lower concentrations compared to the present ones correspond to the small amount of garlic ingested by the test person of Cai et al. (1995) (only $\sim^1/_{12}$ of the present case).

Dimethyl sulfide is normally present in human breath in typical concentrations of 20−40 ppb. We have measured the concentration of dimethyl sulfide in 60 healthy persons, yielding an average concentration of 28 ± 8 (SD) ppb. After the consumption of garlic, it increased in the breath of the test person from 30 to about 60 ppb after 8 h and during the next day up to 90 ppb. This strongly indicates that dimethyl sulfide is in part a product of garlic metabolism. Due to its small concentration, however, the present measurements do not allow identification of its direct precursor. More remarkable is the observed increase of the acetone concentration, which rose from 1.8 to 5 ppm after 24 h. The typical range of concentrations of acetone in human breath is 1−2 ppm. Enhanced levels are observed in persons suffering from diabetes. Healthy persons show higher concentrations of acetone after fasting for more than 10−15 h or after performing strong exercise for typically more than 2−3 h. In these cases the human body has fully exploited its sugar reserves in the blood and thus has started metabolizing its fat reserves, which results in the production of acetone. In this context observations of Bakhsh and Chughtai (1984) are worth noting that levels of serum cholesterol, serum triglycerides, serum total lipids, and serum glucose increased significantly when human subjects were given a fat-rich diet for 7 days. No such increase was observed when substantial amounts of garlic were added to the same fat-rich diet. The present observation of a moderate enhancement of acetone production after ingestion of garlic may be indicative of enhanced metabolism of fatty components in the bloodstream, thus reducing the above-mentioned compounds. While acetone also was observed in the head space gas of crushed garlic, its concentration being about 10 ppm indicates too small amounts of acetone being contained in the garlic consumed by the test person to allow for the observed increase of acetone in the breath of the test person.

Cai et al. (1995) observed dimethyl selenide and other selenium-bearing compounds in concentrations significantly lower than their observed sulfur compounds. From their data we would expect concentrations of these selenium-bearing compounds to be in a regime well below 1 ppb and thus not detectable with PTR-MS at the present sensitivity.

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