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Studies of Selenium-Containing Volatiles in Roasted Coffee

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Coffee has been an important and heavily used beverage in many cultures over a long period of time. Although sulfur species have been found to be abundant constituents, no work to date has explored the presence of selenium analogues. Investigation of volatile selenium species from green coffee beans, roasted beans, and brewed coffee drink was performed using solid phase microextraction (SPME) sample preconcentration in conjunction with GC/ICP-MS. Several volatile selenium species at trace levels were detected from roasted coffee beans as well as in the steam from brewed coffee drinks. No detectable selenium (and sulfur) species, however, were found in the headspace of green beans, indicating that selenium-containing volatiles are formed during roasting, as is the case for the sulfur volatiles. Matching standards were prepared and used to identify the compounds found in coffee. Artificial supplementation of the green coffee beans with selenium before roasting was performed to further characterize the selenium-containing volatiles formed during the coffee-roasting process.

KEYWORDS: Volatile selenium species; speciation; coffee; SPME; GC/ICP-MS

INTRODUCTION

Coffee aroma is a complex mixture of volatile species. Over 800 compounds have been identified and described in the literature so far (*1*). Of these, sulfur volatiles are very important as the exquisite aroma of brewed coffee is mainly determined by volatile sulfur species. Of these, furfuryl mercaptan strongly contributes to coffee-like aroma. The mechanism of formation of volatile sulfur species in roasted coffee is unclear; however, recent studies tend to suggest that Maillard reactions between sugars and coffee bean proteins are the key precursors (*1*). **Figure 1** shows the relative variety of coffee volatiles from green and roasted coffee beans. Note that the sulfur volatiles are not present in the green coffee beans.

Many plants (and living organisms) volatilize or emit sulfur species mainly as a means of self-defense. For example, isothiocyanates in Brassicaceae plants are produced to protect them against insect attack and fungal infection. Similarly, *Allium* sulfur volatiles (disulfides, trisulfides, and thiols) are released to reveal the presence of herbivores to its natural parasitoids (*2*). In this regard, volatile sulfur species formation in coffee follows a different route as they are formed as decomposition products during the roasting of the green coffee.

Since the recent discovery of selenocysteine (SeCys) as the 21st essential amino acid for humans, the field of selenium chemistry has rapidly expanded to explore its chemical properties in biological systems. Although sulfur species have been found to be abundant constituents of coffee volatiles, no work to date has explored the presence of the selenium analogues. Despite the popularity of coffee, little is known about particular elemental forms or species in this drink. Mazzafera studied the effect of coffee supplementation with selenium; however, no conclusive results regarding its form in the coffee beans were obtained, mainly due to the lack of element-specific amino acid detection (*3*). Most of the studies have focused on the major substituents rather than on the species of a particular trace element. The present study describes the benefits of GC hyphenated with element-specific ICP-MS detection for ultratrace elemental speciation analysis in food as demonstrated with the possibility of detection and screening the ultratrace levels of selenium volatiles from roasted coffee.

Little has been done to date in elemental speciation of coffee using plasma spectrometry techniques. Changes of the total amount of sulfur volatiles were studied in roasted coffees by ICP-AES (*4*). It was found that the amount of sulfur volatiles increased in strong roast and were higher in *coffea* var. *robusta* than in *coffea* var. *arabica*. More recently Gerbersmann et al. studied the volatile sulfur species in roasted coffee with GC coupled to microwave-induced plasma atomic emission spectrometry (*5*). Very low parts-per-trillion detection levels were obtained using a capillary cryotrap. Solid-phase microextraction fibers are more commonly used for volatile extraction and preconcentration mainly because of the simplicity of the technique in comparison to cryotrapping of the analytes (*6*). Plasma mass spectrometry has shown excellent capabilities in terms of sensitivity and selectivity when coupled to GC for the determination of volatile species of metals and semimetals using SPME as sample introduction. This approach permitted the study of volatile species production in *Brassica juncea* seedlings (*7*)

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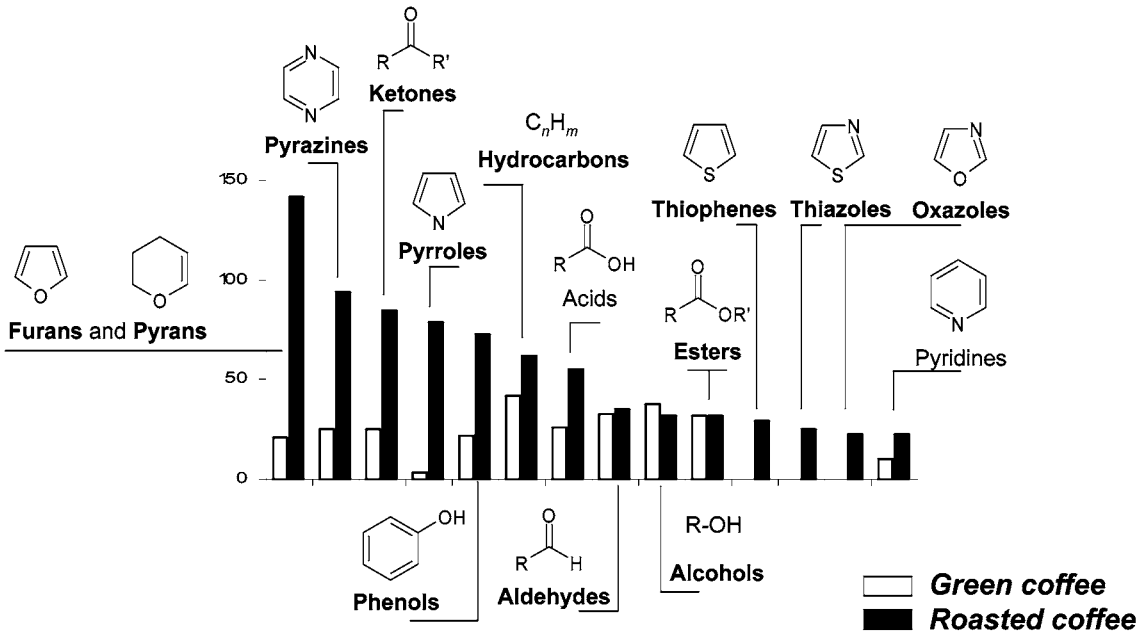


Figure 1. Variety of coffee volatiles from green and roasted coffee beans. Ordinate represents the number of different species within the class, not levels of the species.

and is now applied to the speciation of volatile Se and S compounds in coffee. The use of collision/reaction cell systems permits the monitoring of Se at its most abundant isotopes (*m*/*z* 80 and 78), providing even better performance characteristics than those reported so far in this type of application.

EXPERIMENTAL PROCEDURES

Reagents and Standards. All water was deionized (18 MΩ cm) and prepared by passage through a NanoPure treatment system (Barnstead, Boston, MA). Commercial chemicals were of analytical reagent grade and were used without further purification. Dimethyl selenide, dimethyl sulfide, and dimethyl disulfide were purchased from Fluka (Milwaukee, WI). Dimethyl diselenide, diethyl disulfide, dimethyl trisulfide, and heptacosafluorotributylamine were purchased from Sigma-Aldrich (Milwaukee, WI). Diethyl diselenide was purchased from Strem Chemicals (Newburyport, MA). The stock solutions of 1000 ppm were prepared by dilution of 2.5 μL of compound with 2500 μL of HPLC grade methanol (Fisher Scientific, Fair Lawn, NJ). Selenomethionine (SeMet), selenocystine ($SeCys_2$), and 1,4-dithio-2,3-butanediol (dithiothreitol, DTT) were obtained from Sigma-Aldrich.

The liquid Ar used to run the ICP-MS and the gases evaluated as optional gases (O_2 and N_2) were obtained from Wright Brothers (Cincinnati, OH). H_2 of 99.999% purity was used as a cell gas in the octopole reaction system.

Instrumentation. *GC Conditions.* An Agilent 6890 (Agilent Technologies, Palo Alto, CA) gas chromatograph was utilized in this work. A splitless injection mode was used, and the temperature of the injector was programmed at 220 °C. The column oven was initiated at a temperature of 75 °C and immediately ramped at 10 °C min^{-1} to a temperature of 220 °C. At such a temperature, some formation of diselenides from the selenols is expected. As found by Nielsen et al., 10% of MeSH is converted to the MeSSMe under such conditions (*8*). Reduction of the inlet port temperature, however, can result in poor thermal desorption from the SPME fiber for most of the other selenium species in the inlet liner. Helium was used as the carrier gas, and the column flow was set at a constant value of 1.5 mL min^{-1}. An HP-5 (5% phenyl, 95% methyl-polysiloxane) capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) was used for separation.

GC-TOF-MS. A Micromass GCT time-of-flight mass spectrometer (Micromass, Manchester, U.K.) coupled to the Agilent 6890N GC was used for mass spectral characterization of the synthesized reference compounds. Heptacosafluorotributylamine was used for mass calibration and as the lock mass compound (218.9856 Da). Average mass accuracy did not exceed 1 mDa. The instrument was recalibrated at any time the mass accuracy was determined to exceed this limit.

Table 1. Operating Conditions for ICP-MS

forward power	1150 W
plasma gas flow rate	15.0 L min^{-1}
carrier gas flow rate	1.00 L min^{-1}
dwell time	0.10 s per isotope
isotopes monitored	^{77}Se, ^{78}Se, ^{80}Se, ^{82}Se, ^{33}S, and ^{34}S
reaction gas	2.0 mL of H_2 min^{-1}
optional gas	5.0% N_2 relative to carrier gas

Inductively Coupled Plasma Mass Spectrometry. An Agilent 7500c ICP-MS (Agilent Technologies, Tokyo, Japan) was employed for detection. This instrument is equipped with an octapole ion guide, the Octapole Reaction System, operated in a radio frequency only mode (no mass discrimination). Implementing the reaction cell necessitates lower ion energies and narrower ion energy distribution for successful manipulation of the ions entering the cell. This is accomplished by using a platinum shield plate and bonnet (shield torch system). Instrument operating conditions are shown in **Table 1**. The Agilent Technologies GC/ICP-MS heated transfer line was used. A description of this transfer line including the connections of the optional and carrier gases is given elsewhere (*7*).

Reaction Cell. One of the main problems in Se analysis by ICP-MS always has been the interference of Ar_2^+ at *m*/*z* 78 and 80. Interferences from krypton have been reported also (*7*). Reaction cell technology recently has gained major attention as an effective tool in eliminating spectral interferences (*9*). Implementation of the reaction cell leads not only to the elimination of spectral interferences but also to the reduction of background noise, which is essential for ultratrace level analysis. Although the use of hydrogen in the reaction cell eliminates the interferences of Ar_2^+, thus allowing access to the most abundant selenium isotope, ^{80}Se, it was observed that ^{78}Se is the least affected isotope and that monitoring of *m*/*z* 78 gives the most reliable results in terms of background fluctuations and sensitivity.

Sample Preparation. Green and roasted coffee beans were purchased fresh from local specialty coffee shops. Roasted beans were ground on the same day of analysis using a conventional household coffee grinder. Green coffee beans were roasted using a household hot-air coffee roaster (Hearthware, Wheeling, IL). Additionally, the grinder and sample vials were purged with nitrogen prior to use. Coffee drink was prepared from freshly ground roasted beans using a steam-driven espresso brewing system. Coffee brew was collected in 2 mL glass vials. Afterward, the

Table 2. Characterization of the Synthesized Reference Standards

compound	T_{bp},[a] °C	mass spectra[b] (70 eV, EI+)
MeSeH		96 ($M^{\bullet+}$, 100), 93 (M – 3H, 90), 80 (M – CH_4, 80)
EtSeH		110 ($M^{\bullet+}$, 100), 108 (M – 2H, 50), 93 ($CHSe^{\bullet+}$, 20), 82 ($H_2Se^{\bullet+}$, 70)
MeSeSMe	135	142 ($M^{\bullet+}$, 100), 127 (M – $^{\bullet}CH_3$, 60), 112 ($SeS^{\bullet+}$, 15), 93 ($CHSe^{\bullet+}$, 20)
EtSeSMe	162	156 ($M^{\bullet+}$, 100), 128 (M – C_2H_4, 80), 112 ($SeS^{\bullet+}$,30), 80 ($Se^{\bullet+}$, 10)
EtSSeMe	155	156 ($M^{\bullet+}$, 100), 128 (M – C_2H_4, 80), 112 ($SeS^{\bullet+}$, 30), 93 ($CHSe^{\bullet+}$, 15)
EtSeSEt	182	170 ($M^{\bullet+}$, 100), 142 (M – C_2H_4, 55), 114 ($HSeSH^{\bullet+}$, 90)
EtSeSeMe	178	204 ($M^{\bullet+}$, 100), 189 (M – $^{\bullet}CH_3$, 8), 176 (M – C_2H_4, 70), 160 ($SeSe^{\bullet+}$, 40), 93 ($CHSe^{\bullet+}$, 30)

[a] Calculated from the retention times on the HB-5 capillary column using symmetrical selenides and diselenides as a calibration species. Estimated error: ±5 °C. [b] Based on ^{80}Se.

SPME fiber was exposed to the vial headspace (through the Teflon septum) while the drink was still hot. Samples were not stirred during extraction to avoid any oxidative loss of the analytes.

SPME. The solid-phase microextraction fibers were exposed to the headspace of the sample for 15 min. Once complete, the analytes were desorbed at 220 °C in the GC injection port using a 0.75 mm i.d. inlet liner (Supelco, Bellefonte, PA). The fiber was held in the inlet liner for 2−3 min to reduce any memory effects.

Synthesis of the Matching Standards. *Dimethylselenosulfenate (MeSeSMe)* was prepared in solution by mixing equal volumes of 1000 ppm of methanol or pentane solutions of dimethyl trisulfide and dimethyl diselenide in a closed vial. The resulting solution was allowed to equilibrate at room temperature for a few hours and, after dilution with pentane, the obtained mixture was subjected to chromatographic separation. *Diethylselenosulfenate (EtSeSEt)* was obtained by the mixing of equal volumes of diethyl disulfide and diselenide. Similarly, *ethyl methyl diselenide (MeSeSeEt)* was obtained from dimethyl diselenide and diethyl diselenide. *Methylselenol (MeSeH)* was prepared by adding a small amount of crystalline DTT to the methanol solution of dimethyl diselenide in a closed vial. After a few minutes, the resulting mixture was diluted with pentane and subjected to chromatographic characterization. Similarly, *ethylselenol (EtSeH)* was prepared from diethyl diselenide solution.

The obtained reference compounds were separated on an HP-5 column and characterized by their EI+ mass spectra using a time-of-flight mass detector (**Table 2**). Boiling points, T_{bp}, of the species were estimated from their retention times, t_R, using the linear relationship between log t_R and T_{bp} (under constant temperature ramp conditions) (*10*). Symmetrical species were used as calibrants for the boiling point estimate of the asymmetrical analogues. Results are summarized in **Table 2**. Boiling point estimates of MeSeSMe and EtSeSEt give the values of 135 ± 5 and 182 ± 5 °C, respectively. This is in close agreement with the experimental values (128−131 and 170−175 °C) obtained by Potapov et al. (*11*).

RESULTS AND DISCUSSION

SPME. SPME fibers are available commercially from Supelco. Polymeric coatings are selected on the basis of the polarity and volatility of the compounds of interest. Supelco specifically recommends the Carboxen/poly(dimethylsiloxane) (PDMS) coating for volatile analytes. This and three other coatings were compared for optimum extraction efficiency. These included the PDMS (7 and 100 μm) and polyacrylate polymeric coatings. Because the sulfur volatiles were much more abundant and because the selenium volatile compounds were expected to be similar in structure and polarity, the investigation of optimal fiber coating was conducted with the sulfur compounds. The results showed that the Carboxen/PDMS fiber extracted the sulfur volatiles to a much greater extent than any of the other fiber coatings (**Figure 2**). This agrees with the previous findings on the preconcentration of sulfur and selenium volatiles released from plants (*7*).

Selenium Volatiles from Coffee. No volatile selenium species were detected in the headspace of green coffee beans; however, there were detectable amounts of selenium-containing volatiles present in the headspace of the roasted coffee beans. Experiments were done mainly with the coffee Guatemala Antigua, for which selenium volatiles were detected also in the headspace of the coffee brew.

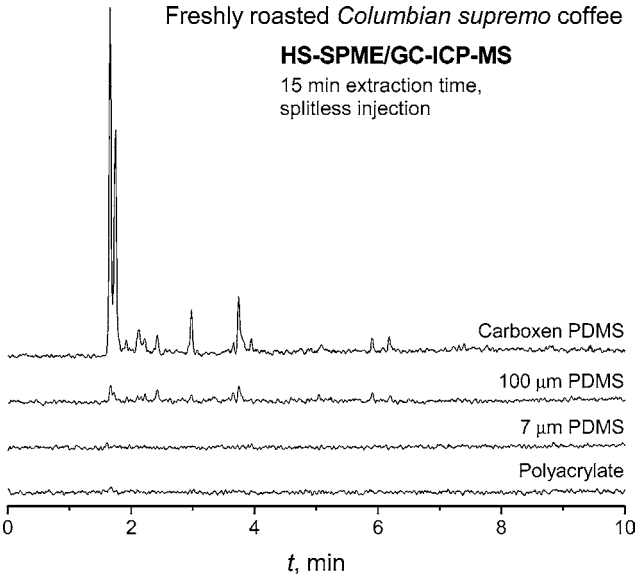


Figure 2. Effectiveness of extraction for various SPME fibers in analysis of sulfur volatiles from the headspace of coffee (^{34}S GC/ICP-MS chromatogram).

Sample stirring while exposing the SPME fiber to its headspace is found to enhance analyte transfer to the fiber. Addition of salts is also suggested for this purpose. However, in experiments with standards, aqueous sample stirring was found to favor the oxidation of diselenides, and thus analytes can be lost. Similar analyte signal decrease due to sample stirring was observed for coffee brew samples (**Figure 3**). Because of the oxidative nature of diselenides, there is a balance between the species stability and sensitivity of the method; if stirring is applied to the liquid, enhanced sensitivity may be obtained because of the increase in analyte transport to the headspace (for example, see **Figure 3**: species eluting at 7.2 min). However, this also favors the oxidative loss for some of the species (**Figure 3**, species eluting at 2−4 min), and thus sample preparation is a critical issue. As a result, it was decided to forego sample stirring, and all subsequent data in this work represent exposure of the SPME fiber to samples that were not stirred.

In Situ Preparation of Selenium Standards. The use of hyphenated techniques along with plasma spectrometric detection is becoming very advantageous for achieving elemental speciation at ultratrace levels in various biological matrices. However, only elemental information can be gathered. This

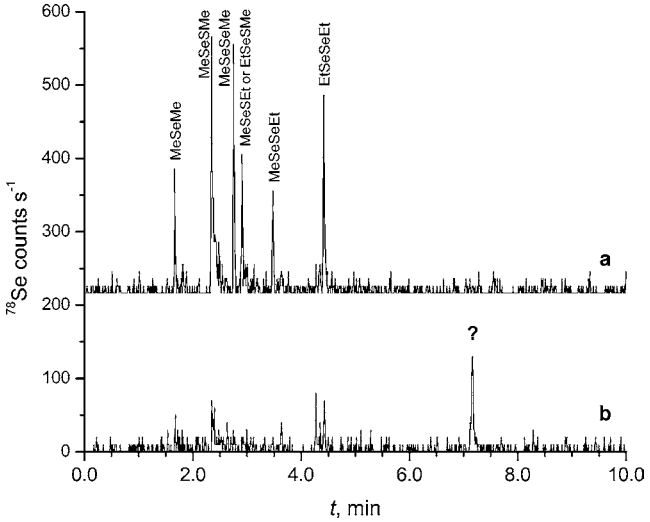


Figure 3. ^{78}Se GC/ICP-MS chromatogram showing the ultratrace levels of selenium volatiles in the headspace of regular coffee drink (Guatemala Antigua); effect of sample stirring while exposing SPME fiber to the headspace: (a) no stirring (off-set); (b) with stirring.

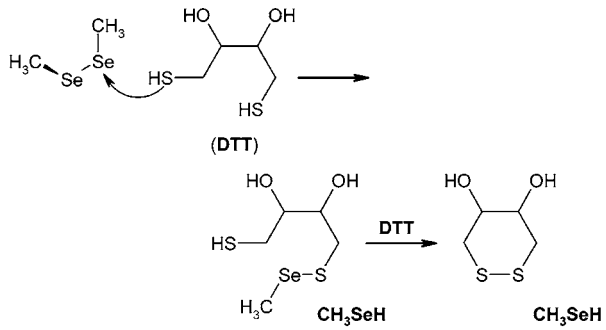
Table 3. Primary Classifications of Naturally Found Selenium Volatiles[a]

selenides	diselenides	selenosulfenates	bis(thio)selenides
HSeH	Me–SeSe–Me	Me–SeS–Me	Me–SSeS–Me
Me–SeH	Me–SeSe–Et	Me–SeS–Et	Me–SSeS–All
Me–Se–Me	Et–SeSe–Et	Me–SeS–All	All–SSeS–All
		Et–SeS–All	
		Me–SeS–CH=CHCH$_3$	

[a] Naming of species according to CAS (*19*).

precludes the identification of selenium species using atomic spectrometry techniques, such as GC/ICP-MS, unless there are available standards. The availability of selenium-containing volatile standards is limited to a small number due to their instability and infrequent use in environmental analysis, despite the growing interest in Se biospecies relative to both their toxic and health-benefit potentials. Selenium-containing volatiles of environmental interest can be classified into four main groups as summarized in **Table 3**. Symmetrical diselenides and selenides are usually available commercially and may be used as standards. They can also serve as the starting point for the preparation of other species of interest, such as selenols and selenosulfenates.

Selenols. Thiols are strong reducing agents, stronger than selenols, and thus, diselenides may be reduced to selenols by thiols. This reduction process is reversible, and thus the thiols that provide the highest equilibrium constant for this reaction are chosen. 1,4-Dithio-2,3-butanediol (DTT) is a good candidate because of the high equilibrium constant and resistance toward the oxidation from dissolved oxygen:



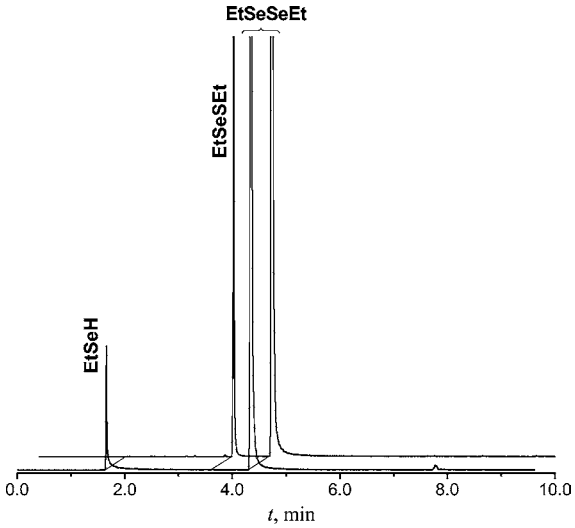
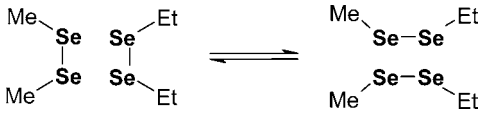


Figure 4. ^{78}Se GC/ICP-MS chromatogram revealing the formation of EtSeH from EtSeSeEt and EtSeSEt in the presence of 1,4-dithio-2,3-butanediol (DTT).

Similarly, reduction with $NaBH_4$ can be used to obtain selenols from the corresponding diselenides. **Figure 4** shows the in situ formation of ethylselenol from EtSeSeEt and EtSeSEt in the presence of DTT.

Selenosulfenates. Aliphatic selenosulfenates are easily prepared in a mixture utilizing the sulfur/selenium exchange reaction, which occurs at room temperature in aqueous or organic solvent medium:



The equilibrium is reached within a few hours at room temperature. This type of exchange is an entropy-driven process with the equilibrium constant $K \approx 4$ (*11*). Asymmetrical diselenides and selenosulfenates are prepared by mixing the corresponding symmetrical precursors. For example, Boss et al. reported the formation of all 28 asymmetrical diselenides (after 2 h) when eight symmetrical diselenides were mixed together in *n*-hexane (*12*). The yield of dimethylselenosulfenate (MeSeSMe) by mixing MeSSMe and MeSeSeMe is very small and the process is slow. It was found that the reaction of dimethyl trisulfide (MeSSSMe) and MeSeSeMe is statistically more favorable for MeSeSMe production, and this species is formed to a much greater extent when MeSSSMe and MeSeSeMe are mixed together.

Bis(thio)selenides. The first report on the natural occurrence of volatile bis(alkylthio)selenide was done by Cai et al. (*13*). MeSSeSMe was found to be present in elephant garlic. However, this class of compounds cannot be present in the headspace of roasted coffee as they are thermally unstable and rapidly undergo decomposition to the corresponding disulfides and amorphous selenium at temperatures above ~150 °C (*14*). Therefore, no further investigation of this class of species was done.

In addition to preparing seleniun- and sulfur-containing volatiles, retention times of homologues may be predicted using retention indices and boiling point correlations (*15*). Using such an approach, for example, the confusion between MeSeSMe and $MeSe(O)_2Me$ was easily resolved (*16*).

Identification of Selenium-Containing Compounds: Retention Time Matching. In the case of the acyclic selenium

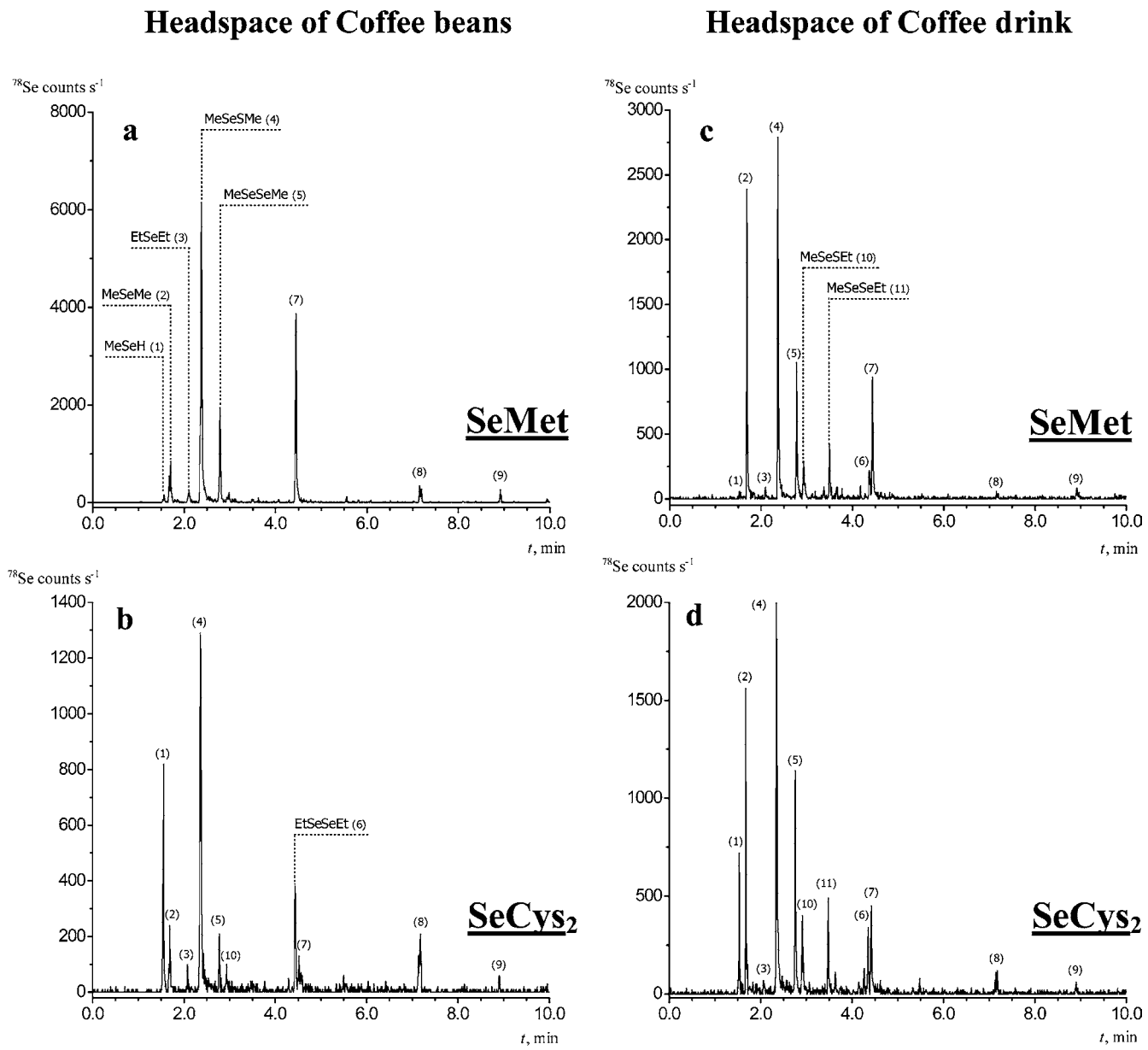


Figure 5. Supplementation of green coffee beans with selenomethionine (SeMet) and selenocystine ($SeCys_2$): (a, b) headspace of the roasted grind coffee beans; (c, d) headspace of the coffee drink prepared from the roasted beans. Peaks: (1) MeSeH; (2) MeSeMe; (3) EtSeEt; (4) MeSeSMe; (5) MeSeSeMe; (6) EtSeSeEt; (7) unknown; (8) unknown; (9) unknown; (10) MeSeSEt or MeSSeEt; (11) MeSeSeEt.

volatiles, the identification based upon retention time matching has strong reliability because of the interaction chemistry. Diselenide exchange and interaction of selenols offers an additional identification confirmation. In other words, it is unlikely there will be high levels of dimethyl selenosulfenate without the presence of dimethyl diselenide and dimethyl disulfide or methyl thiol. Moreover, element isotope specific detection allows one to confirm the correct Se isotope pattern within each of the chromatographic peaks, thus confirming the absence of isobaric interferences. The actual levels of the selenium volatiles present in the regular coffee are within the range of low parts per trillion.

A yet unidentified selenium-containing volatile (**Figure 3**) is likely to be water insoluble as it is not present in the headspace of coffee drink, whereas it is one of the most abundant Se species found in the headspace of the roasted coffee beans (data not shown). Note that this species is detected from the headspace of the stirred coffee drink. This suggests its nonoxidative nature, as the S−Se- and Se−Se-containing species are easily lost if the sample is subjected to stirring during the SPME fiber exposure (it is not detected if no stirring is applied because of the poor analyte transfer to the headspace). This species eluting at 7.2 min could be a selenium derivative of the furanone class, as recently 3-[(methylseleno)methyl]furan was found to be one of the products from the Maillard reaction in a selenomethionine−glucose model system (*17*). No further work on synthesis of this compound was done.

Supplementation of Green Coffee Beans with Selenium. Natural levels of selenium-containing volatiles are extremely low and thus the supplementation of green coffee beans with selenoamino acids was done to explore the fate of these species during the coffee-roasting process. Higher levels of the species also allow more conclusive characterization of the system. Two amino acids, selenomethionine (SeMet) and selenocystine ($SeCys_2$), were chosen as the most common Se amino acids found in the environment. Supplementation was done by overnight soaking of the green beans in 10 ppm of SeMet and 10 ppm of $SeCys_2$ aqueous solutions. Total selenium levels were

then measured in the aqueous solutions after the beans were removed, and the results obtained with ICP-MS show that ~10% of the Se was taken up, which corresponds to ~5 ppm of Se in the green coffee beans before roasting.

The main goal for the supplementation is to enhance the levels of selenium in coffee beans before their roasting. However, the skin of the bean prevents direct migration of species within the bean. To resolve this, green coffee beans were cut in half to expose the inner part of the bean. This resulted in the increase of the Se amino acids within the bean, and the roasting of the supplemented beans was then performed. No effect on the Se profile of coffee aroma was observed when regular or sliced coffee beans were roasted.

Comparison of the selenium volatiles obtained from the headspace of the drink made with regular and Se-supplemented coffee beans (**Figures 3a** and **5c**,**d**) shows generally the same profile with the only difference being the actual levels (which are ~10 times higher for supplemented coffee drinks).

As can be seen from **Figure 5**, supplementation with SeMet and $SeCys_2$ mainly gives acyclic selenium volatiles, which are primarily formed during the dehydratation process of amino acids. Interestingly, in both cases (SeMet and $SeCys_2$), the main selenium species present in the headspace of the coffee beans or coffee drink is dimethyl selenosulfenate (MeSeSMe). This species is in equilibrium with dimethyl disulfide and dimethyl diselenide, and the presence of the latter was confirmed as well. Cleavage of the Se−C bond in SeMet eventually leads to dimethyl selenide (MeSeMe), which was confirmed to be present in supplemented coffee. However, in the case of selenocystine, formation of methylselenol (MeSeH) is expected. Results shown in **Figure 5** support this hypothesis. When chromatograms obtained from the headspace of the roasted coffee beans are compared to those obtained from the headspace of the coffee brew (made from the same beans), an increase in the variety of selenium volatiles can be observed. This suggests that diselenide/disulfide and diselenide/diselenide interaction is favored in hot aqueous medium, thus giving the variety of asymmetric dichalcogenides, such as MeSeSeEt. This finding is of importance as the increase in S/Se species can contribute to the coffee aroma profile at higher selenium levels. Similarly, the formation of asymmetric disulfides and trisulfides is attributed to the reminiscent odor of aged cognac (*18*).

Several ethylated species, such as diethyl selenide (EtSeEt), diethyl diselenide (EtSeSeEt), ethylmethylselenosulfenate (MeSeSEt or EtSeSMe), and ethyl methyl diselenide (MeSeSeEt), were identified from the headspace of the supplemented coffee beans and brew. Note that these species are somehow less abundant in the environment in comparison to their methylated analogues, and only a few reports exist in the literature on the occurrence of ethylated selenium compounds. Similar to the coffee supplementation experiment, the formation of MeSeSEt was found in the headspace of garlic and onion homogenates after supplementation with selenoethionine (*13*).

In the future, work on selenium volatiles in coffee will be continued by growing coffee plants in a Se-supplemented environment. Also, synthesis and evaluation of selenofuranones that might be formed during coffee roasting will be performed.

Conclusions. The presence of volatile selenium species formed during the roasting process of green coffee beans is reported for the first time using SPME in conjunction with GC/ICP-MS. One of the advantages of GC/ICP-MS is the very low detection limits and elemental selectivity, which allows the speciation of trace elements in food. Using this technique, various selenium-containing species were characterized in the headspace of regular coffee. Dimethylselenosulfenate, dimethyl diselenide, and dimethyl selenide were the most abundant of the selenium-containing species. Artificial supplementation of the green beans was used to enrich the natural levels of selenium in the green beans. Chalcogen exchange between the disulfides and diselenides was demonstrated to occur in the coffee brew of the supplemented coffee, leading to the formation of asymmetrical diselenides and selenosulfenates.

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