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SPME–multicapillary GC coupled to different detection systems and applied to volatile organo-selenium speciation in yeast

C. Dietz, J. Sanz Landaluze, P. Ximénez-Embún, Y. Madrid-Albarrán and C. Cámara*

Department of Analytical Chemistry, Faculty of Chemistry, University Complutense de Madrid, Ciudad Universitaria, 28040 Madrid, Spain. E-mail: ccamara@quim.ucm.es; Fax: 0034 91 394 4329

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In this work the versatility of solid phase microextraction (SPME) in combination with multicapillary gas chromatography (MC–GC) was evaluated using different common detectors for organo-selenium speciation. The methods compared for detection were inductively coupled plasma mass spectrometry (ICP–MS), microwave induced plasma atomic emission spectroscopy (MIP–AES) and atomic fluorescence spectroscopy (AFS). All detectors were found to be suitable, with highest sensitivity being obtained for MIP–AES detection, with detection limits of 0.57 ng ml^{-1} for dimethyl selenide, 0.47 ng ml^{-1} for diethyl selenide and 0.19 ng ml^{-1} for dimethyl diselenide. The method was applied to the determination of volatile alkyl selenides in selenium enriched yeast samples, which revealed that the presence of inorganic selenium gives rise to at least seven different volatile species after metabolization, with dimethyl diselenide the predominant species. Commercial pasteurized yeast, containing mainly selenomethionine for its use as a food supplement, was found to be still active and produces considerable amounts of organoselenium compounds.

Introduction

Solid phase microextraction (SPME) offers numerous advantages in sample preparation for speciation analysis. It is a simple technique, easy to hyphenate with virtually any exciting detection system, sample–matrix separation can be quickly achieved, it is solvent free and sample manipulation is minimized.[1] Traditionally it is used for determination of volatile organic compounds coupled to GC. Due to its excellent analytical characteristics, coupling to ICP based instrumentation[2–4] is normally the method of choice when metal containing analytes have to be determined. The main drawback of this detector consists in its high instrumental and operational costs, which justifies research on alternative plasma sources such as microwave induced plasma[5] (MIP) and atomic fluorescence spectroscopy[6] (AFS), which may be at least one order of magnitude less expensive.

SPME has been used for inorganic selenium speciation, using either ethylation[7] or acid microwave digestion[8] for volatile species generation, coupled in both cases with GC–MS detection. Seleno-aminoacids like selenomethionine, selenoethionine and seleno-cystine were determined after derivatization using SPME and GC–ICP–MS coupling.[9] Determination of several volatile alkyl selenides and their sulfur analogues in Se-accumulating plants was carried out using SPME–GC–ICP–MS.[10] Here, the primary volatiles measured in headspace were dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe).

Regarding its clinical and environmental effects, selenium is one of the most challenging elements. Due to the narrow range from being essential to highly toxic,[11] depending on the species, oxidation state and concentration, there is an evident interest in determination of the selenium content in a wide range of environmental matrices, such as air, water, biological tissue and sediments.[12] Clear evidence for cancer chemo-preventive effects of a selenium rich diet was found.[13–15] This explains the more frequent use of selenized yeast as enriched selenium supplements in human nutrition and has led to increasing scientific interest on selenium biochemistry over the last decade. For preparation of Se-enriched yeast,[16,17] the desired levels of selenium, usually about 1000 ppm, is supplemented as selenite during a fermentation process. Uptake rate and the accumulation process are strongly influenced by the amount of dissolved oxygen in the culture medium. The yeast cream is then pasteurized and spray dried, and sometimes combined with *lactobacillus acidophilus* fermentation solubles. This process allows the selenium to interact with the yeast cell components causing transformation into more bioavailable selenium, mainly selenomethionine. Up to date, these types of samples were investigated with a variety of analytical techniques in order to establish selenium species distribution and possible pathways during metabolism. These methods frequently involve enzymatic hydrolysis,[18,19] followed by HPLC separation and mass spectrometric detection, using either electrospray ionization (ESI)[20] or ICP[21] as ionization or excitation source, respectively. The principal selenium compounds in these yeast sample extracts were identified as selenomethionine and Se-adenosyl-selenohomocysteine,[22] accounting for about 85% of the total selenium content in yeast samples. However, there were also some commercial Se-enriched yeast products detected, which can contain up to 100% of inorganic selenium.[23] Some attempts have been made to classify selenium species occurring in different fractions of yeast samples, *e.g.* in the protein fraction[24] by gel-electrophoresis ICP–MS. In aqueous yeast extract[25] using SEC–CZE–ICP–MS and in the low molecular weight fraction[26] by using HPLC–ICP–MS, a considerable number ($\approx$20) of seleno-compounds could be observed, mostly characterized by the presence of an adenosyl functional group, but the majority remain not yet definitely identified.

The present work centers on volatile alkyl selenides, such as dimethyl selenide, diethyl selenide or dimethyl diselenide. These species may result from seleno-protein degradation, bioalkylation processes or metabolism products of inorganic selenium in the context of an auto-detoxification mechanism of living yeast cells. They are not easily detected when common sample preparation methods are used. SPME should be suitable for this application as it allows monitoring volatile organoselenium concentrations in the headspace above the living organism. SPME is hyphenated with multicapillary gas chromatography (MC), thus the instrumentation becomes

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less bulky and separation is speeded up. Once optimized the parameters affecting sampling and analyte separation, the performance of different detectors such as ICP–MS, MIP–AES and AFS will be critically evaluated.

Experimental

Instrumentation

The analytical system consists in an in-house made GC injection port for SPME fiber desorption and a gas chromatographic separation unit, hyphenated to three different detection systems. These were a microwave induced plasma excitation source with atomic emission spectroscopic detection (MIP–AES), an inductively coupled plasma with mass spectrometric detection (ICP–MS) and an atomic fluorescence spectrometer (AFS).

The SPME fiber desorption unit was in-house developed in order to provide temperature control during the desorption step and to be independent of a commercial GC oven. Details of the system are given elsewhere.[27] The outlet of the desorption unit was connected to a straight 25 cm multicapillary column (BeeChrom OV-17 (50% Phenyl): 50% methylpolysiloxane, $N \approx 1000$, i.d. 40 μm, film thickness 0.2 μm). The column was housed in a stainless steel tube with graphite sealed fittings, electrically isolated by a 4 mm i.d. glass tubing which was surrounded by a coil of Nichrome wire. The latter was heated by the means of a regulated DC power supply (Dirland D-ADPS-305), modified with a set-point controller (Campini HTX031) for proper temperature control. A J-type thermocouple was placed inside the glass tubing housing the multicapillary column. The optimum fiber in terms of extraction mechanism, film thickness and polarity for SPME extraction in organoselenium determination has been previously optimized.[27] A partially crosslinked 75 μm Carboxen™/PDMS coated fiber, was found to be optimal and thus used in the ongoing experimental work. An intermediate polarity stationary phase provided the best conditions for organo-selenium separation using the multicapillary column. Parameters affecting desorption and separation in the multicapillary column were also previously established,[28] using a multivariate approach.

The MIP–AES device consists of a microwave generator (AF GMW 24-303 D, AF Analysetechnik, Tübingen, Germany) operating at a frequency of 2.45 GHz with a tunable forward power between 30 and 300 W. In the present configuration the optimum forward power was established to be 120 W. Antenna position and fine-tuning for proper coupling of the microwave energy within the cavity were optimized to obtain the maximum signal/noise ratio. Fine-tuning was found to be critical and was readjusted to a fixed value for the baseline prior to each analysis run. A TM_{010} Beenakker type cavity (Model HMW 25-471 N-W, AF Analysetechnik, Tübingen, Germany) was used, provided with ceramic discharge tubes (60 × 4 × 2 mm; length, outer/inner diameter) with tangential flow design. Horizontal plasma emission was focused to the entrance slit of the spectrophotometer (SpectraPro 300i, Acton Research Corp., MA, USA). Details of the system are given elsewhere.[28] Data recording and treatment was done with SpectraSense V4.2.7 software, provided with the instrument. Apart from argon plasma gas flow rate, an additional air flow was fed to the cavity in order to enhance the combustion of organic compounds, to improve the stability of the baseline and to enhance the lifetime the discharge tubes by acting as coolant medium.

The ICP–MS system was a Hewlett–Packard 4500 series, equipped with a Babington nebulizer, Scott double pass spray chamber and a Fassel-type quartz torch. Data evaluation was carried out with HP ChemStation software. Four isotopes, ^{77}Se, ^{82}Se, ^{32}S and ^{34}S, were monitored simultaneously and extracted ion charts were integrated manually. Ar auxiliary and plasma gas flows were, following the manufacturers recommendations, optimized in the tuning mode of the instrument, monitoring ^{59}Co, ^{115}In and ^{238}U response corresponding to a 10 μg l^{-1} tuning solution.

For AFS an atomic fluorescence spectrometer (Excalibur, P.S. Analytical Ltd., Orpington, Kent, UK), equipped with a selenium boosted discharge hollow cathode lamp (BDHCL, Photron, Victoria, Australia) was used. Primary and boosted intensities were 25 mA. The multireflectant filter and a solar blind photomultiplier tube, with a voltage of 600 V, were positioned at the right angle to excitation source. An auxiliary hydrogen flow sustained the diffusion flame in the atomization cell. Signals recording and data evaluation were done using the DataApex Chromatographic software (version 1.3.3). Details of the system are given elsewhere.[29]

Reagents and standards

Dimethyl selenide and dimethyl diselenide were obtained from Alfa Aesar (Karlsruhe, Germany). Diethyl selenide as well as the inorganic selenium species, sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4), were bought from Sigma Aldrich (Madrid, Spain). Stock solutions of the organo-selenium species of 10.000 mg l^{-1} (expressed as metal) were prepared by appropriate dilution with methanol and stored at 4 °C. Lower concentration stock solutions (100 mg l^{-1}) were prepared in a 50% mixture of methanol–water. Aqueous working solutions were prepared daily and stored on ice until use. SPME fibers were purchased from Supelco (Bellefonte, PA, USA). Deionized water was obtained from a Millipore (Bedford, MA, USA) ZMFQ 23004 Milli-Q water system. The tuning solution for ICP measurements, containing Li, Be, Mg, Co, Y, In, Ce, Bi and U in 2% HNO_3, was prepared by appropriate dilution of 1000 mg l^{-1} stock solutions (Merck, Darmstadt, Germany). Helium, argon, air and oxygen (99.99% purity) were purchased from Carburos Metalicos (Madrid, Spain). Se-enriched yeast was purchased from Pharma Nord (Precise, Vejle, Denmark). The selenium content of this material was determined as 1383.96 ± 86.7 μg g^{-1} of total selenium, basically in the form of selenomethionine in an interlaboratory study (SEAS-6), organized by the Institute of Reference Material and Measurements. Fresh baker yeast (*Saccharomyces cerevisiae*) samples were bought in a local market.

Safety note: DMSe and DMDSe have the following risk notes (R)[30] and safety (S)[31] phrases: R23/25-33-50/53; S20/21-28-45-60-61. The toxicological properties of DEtSe have not been fully investigated, the assigned RS notes are: R23/24/25, S20,24,28,36/37/39,45. All reagents should be handled with caution, highly concentrated stock solutions have to be prepared in a flow box, wearing appropriate protection (gloves, laboratory glasses, clothes).

Analytical procedure

Five ml of sample were placed together with a 6-mm stirrer in a 10 ml vial, the latter was closed with an aluminium seal and a Teflon-faced butyl septum. The vial was placed onto a magnetic stirrer. A SPME fiber, placed in a fiber assembly holder (Supelco), was passed through the septum for headspace sampling. Loaded fibers were transferred to the desorption unit for measurement directly after sampling. Experiments dealing with fresh baker's yeast, the samples were prepared by dispersing 500 mg of yeast in 5 ml of Se(IV) stock solution, at a concentration level of 20 mg l^{-1}. After closing the vials the headspace was flushed with gas in order to produce the desired atmosphere (Ar, air, O_2). The mixture was stirred at room temperature during 8–72 h prior to sampling. For experiments with high level selenium yeast 50 mg of Se-enriched yeast (SEAS-6) were simply dispersed in 5 ml of Milli-Q® water.

Table 1 Optimized parameters for organo-selenium determination using SPME–MC

SPME extraction		MC separation	
Fiber	Carboxen/PDMS 75 μm	Column type	25 cm, $N \approx 1000$, 40 μm i.d., OV-17, f: 0.2 μm
Extraction time	35 min	He carrier gas	38 ml min^{-1}
Extraction temperature	25°C	Desorption temperature	240 °C
Stirring	350 rpm	Column temperature	30 °C

Optimized SPME extraction and desorption parameters used for organoselenium analysis are summarized in Table 1. Variables affecting the detection step for use with the three different detectors are collected in Table 2.

Optimized working conditions for selenium speciation with SPME–MC–MIP–AES were adopted from a previous work.[28] A slight variation of the carrier gas flow was necessary with respect to MIP detection when using the ICP-MS detector, as now argon instead of helium had to be used as carrier gas, fundamental for proper operation of the RF plasma. The optimized parameters for sampling and separation were then used to optimize detection conditions for the remaining two detection systems.

Results and discussion

In all analytical processes, instrumental and analytical variables have to be optimized in order to obtain reliable results. Due to the large number of variables involved in the majority of common methods in speciation analysis when applied to real samples, they are normally grouped according to the different analysis steps: extraction, separation and detection. Instrumental variables affecting the detection step for the three different detectors have been partly optimized in previous[27,28] work. Thus, in the present work special emphasis was stressed on additional optimization of AFS as detection system.

Optimization of AFS detection

In order to enhance combustion yield of the hydrogen stream, a parameter that could promote cracking of organometal bonding, a multiple folded platinum wire was introduced as catalyst in three different positions with respect to the flame torch of the AFS instrument. Fluorescence response, using commercial instrumentation, is mainly influenced by reaction gas flow rate (hydrogen) and the carrier gas flow from the separation device. Other instrumental parameters are normally fixed in this type of detectors. A full factorial design with three factors at two levels and four replicates of the central point,[32] involving 12 experiments in random order was applied to establish the influence (or lack of influence) of each of these variables (hydrogen and argon flow rate, presence of platinum wire).

In each experiment a mixture of 25 ng ml^{-1} of the each selenium species tested was analyzed as described in the analytical procedure. Table 3 summarizes the factors, levels and results obtained in each experiment for the peak area corresponding to dimethyl diselenide (comparable results were obtained for each of dimethyl selenide and diethyl selenide

Table 2 Optimized instrumental parameters for organo-selenium determination using three detection systems: ICP–MS, AFS and MIP–AES

ICP–MS detection		**MIP–AES detection**	
Monitored isotopes	^{77}Se, ^{82}Se, ^{32}S , ^{34}S	MIP forward power	120 W
RF power	1257 W	Reflected power	18 W
Reflected power	0 W	Ar plasma gas	50 ml min^{-1}
Ar nebulizer gas	1.2 l min^{-1}	Air cooling gas	280 ml min^{-1}
Ar auxiliary gas	1.29 l min^{-1}	Analytical wavelength	196.0 nm
Ar plasma gas	14.8 l min^{-1}	Entrance/exit slit-width	10/40 μm
AFS detection		PMT voltage	700 V
H_2 reactive gas	120 ml min^{-1}	Integration time	50 ms
Ar carrier gas	30 ml min^{-1}	Interval time	100 ms

Table 3 Levels, response values and main and F factors obtained from the full factorial design developed to investigate the influence of the hydrogen flow (H_2), argon flow (Ar) and the presence (yes) or absence (no) of platinum wire (Pt) on the signal obtained by AFS detection (25 ng ml^{-1} of the DMSe, DEtSe and DMDSe)

	Levels[b]					
Combinations	H_2/ml min^{-1}	Ar/ml min^{-1}	Pt	DMDSe peak area	Main factor	F^a
1	− (80)	− (15)	− (yes)	6.64		
H_2	+ (120)	− (15)	− (yes)	144.34	128.37525	**14.6×10^3**
Ar	− (80)	+ (30)	− (yes)	10.59	22.19825	**4.38×10^2**
H_2–Ar	+ (120)	+ (30)	− (yes)	87.685	76.20675	**5.16×10^3**
Pt	− (80)	− (15)	+ (no)	166.98	4.41775	**17.34**
H_2–Pt	+ (120)	− (15)	+ (no)	197.90	−18.67275	**3.09×10^2**
Ar–Pt	− (80)	+ (30)	+ (no)	119.30	8.90425	**70.47**
H_2–Ar–Pt	+ (120)	+ (30)	+ (no)	228.49	−4.93025	**21.60**
$Zero_1$	0 (100)	0 (22.5)	0 (no)	158.92		
$Zero_2$	0 (100)	0 (22.5)	0 (no)	154.44		
$Zero_3$	0 (100)	0 (22.5)	0 (no)	155.068		
$Zero_4$	0 (100)	0 (22.5)	0 (no)	161.52		
$Zero_5$	0 (100)	0 (22.5)	0 (no)	159.17		

[a] Factors in bold were found to be statistically significant after ANOVA of the results ($F > 5.318$ at a 95% confidence level). [b] (+/−) indicates maximum and minimum values for the range of the respective parameter tested: 0 is the central point of the experimental design.

peak areas). The main and F factors of each variable or combination of variables were calculated by means of the YATES algorithm.[32]

Analysis of the variance (ANOVA) of the results showed that all variables have a statistically significant effect on the DMDSe signal ($F > 5.32$ at a 95% confidence level).[33] Analysis of data obtained showed that the presence of platinum wire, while producing a more stable baseline, decreased analyte response significantly, especially for DMSe and DEtSe. This effect was stronger when the wire was placed within the hydrogen flame where it became incandescent. A possible explanation for this observation is that the platinum, acting as catalyst, favors the recombination of hydrogen and OH radicals, both intermediate products during the combustion process. A high concentration of radicals, on the other hand, benefits the cracking of the organo-metal bonding of the analyte. As a result, the presence of a catalyst was found to be counterproductive. Furthermore the presence of platinum caused a notable peak broadening for all three analytes. Hence the platinum wire was removed for subsequent hydrogen and carrier gas optimization

Optimization of analyte response was carried by means of central composite design. The central composite design consisted of the points of a factorial design (2^n) augmented with six points located at $-\alpha$ and $+\alpha$ from the center in a *star* design (Table 4).

To define the response surface, and thus obtaining optimal values for the variables, the results are fitted to a general polynomial equation (eqn. (1)) where β_n are the adjustable parameters.

$$Y = \beta_0 + \beta_1 H_2 + \beta_2 Ar + \beta_{11} H_2^2 + \beta_{22} Ar^2 + \beta_{12} H_2 \cdot Ar \quad (1)$$

Fitting of data to eqn. (1) was performed using the multivariate non-linear regression analysis program NLREG,[34] and those parameters β_n with a statistical probability to be zero greater than 10% were sequentially eliminated from the model. The models finally selected for each species are able to explain 96.7% for DEtSe, 97.8% for DMDSe and 99.9% for DMSe of the total variance of data. The variable values that maximize the response obtained can be obtained easily graphically from the response surface. As can be noticed from response surfaces (Fig. 1), the hydrogen flow value that maximized the response is the same for all three species (120 ml min^{-1}) and argon flow values are different for DMDSe (30 ml min^{-1}) and DMSe and DEtSe (15 ml min^{-1}). As the response does not decrease significantly in DMSe and DEtSe (Fig. 1(b)) increasing the optimum argon flow for DMDSe (30 ml min^{-1}), this value was chosen as optimal. As can be concluded from the response surfaces an increment on hydrogen flow supposes an increment

Table 4 Variables and levels for the central composite design

	Level				
Variable	$-\alpha$	$-$	0	$+$	$+\alpha$
H_2/ml min^{-1}	70	90	100	120	130
Ar/ml min^{-1}	10	15	22.5	30	35

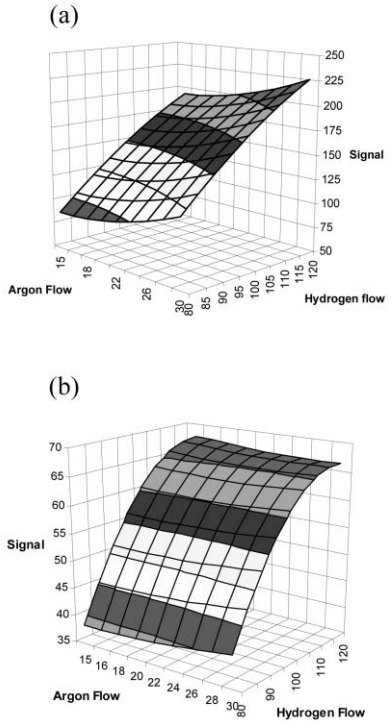

Fig. 1 Response surfaces estimated from central composite design for (a) DMDSe and (b) DEtSe with AFS detection optimization. The response surface for DMSe is very similar to DEtSe and is not shown.

on the response signal. At flow rates higher than 120 ml min^{-1} the signal/noise ratio increased significantly, thus this value was chosen as optimal for selenium speciation with AFS detection.

Figures of merit

The values for the figure of merits of the proposed method are summarized in Table 5. During the evaluation process mixed samples containing 5 ml of an aqueous stock solution of the three analytes were used. Detection limits were defined as blank signal plus three times the signal-to noise ratio and determined for a synthetic samples containing 5 ml of water as blank solution. The precision, in the case of MIP–AES measurements, was evaluated by analyzing five different samples of 15 ng ml^{-1} for each species, measured during one day and using different fibers of similar characteristics. For the remaining detectors "*n*" was also five but always the same fiber was used for evaluation. The concentration of the analyte mixture was seven-fold the detection limit (D. L.) for ICP measurements and 20 ng ml^{-1} for AFS detection.

The most sensitive detector for volatile organoselenium detection was found to be the MIP, followed by ICP–MS and then AFS. The latter was more sensitive than the ICP–MS only for DMDSe. The robustness of the method seemed to be determined by the SPME sampling step rather than by the use of different detectors. The slightly higher values obtained for standard deviation with MIP can be explained by the fact that in this case different fibers were used for the sampling

Table 5 Figures of merit for organoselenium speciation using SPME–MC coupled to MIP–AES, ICP–MS and AFS detection systems

	MIP–AES			ICP–MS			AFS		
	DMSe	DEtSe	DMDSe	DMSe	DEtSe	DMDSe	DMSe	DEtSe	DMDSe
Detection limit/ng ml^{-1}	0.57	0.47	0.19	0.76	2.07	1.33	0.88	1.55	1.33
Regression R^2	0.991	0.975	0.967	0.998	0.9964	0.980	0.997	0.989	0.994
Precision (% RSD) ($n = 5$)	7.76	7.89	7.06	11.7	4.1	4.4	4.04	5.82	5.8
Linearity range	D.L. to 100 ng ml^{-1}			D.L. to 1.5 mg l^{-1}			D.L to 1.0 mg l^{-1}		
Reproducibility R_t (% RSD)	2.08	3.85	4.36	6.15	1.69	2.82	6.81	5.59	1.21

procedure. ICP–MS was shown to be more sensitive to alterations caused by manual injection into the fiber desorption device. Most affected is the first eluting species DMSe, reproducibility for the remaining species is a little better than the values obtained by AFS. Separation of the first two eluting species, DMSe and DEtSe, was worse in AFS, possibly because of problems arising when the carrier gas was mixed with the relatively high hydrogen flow prior to detection. Reproducibility for the retention times was, in general, better the later the species eluted and never greater than 6.8%. In the cases of MIP detection the linear range can be extended by lowering the voltage applied to the photomultiplier tube, even though some sensitivity will be lost. Fig. 2 shows a typical chromatogram obtained by SPME–MIP–AES for a 25 μg l^{-1} mixed stock solution.

All investigated detectors showed sufficient sensitivity and satisfying analytical merits to be used in trace analysis of volatile organoselenium compounds with SPME sampling and MC gas chromatographic separation. ICP–MS allows multi-element determination, MIP–AES allows sequential determination without changing instrumental setup while AFS requires hollow cathode lamp and interference filter change when changing the analyte. Furthermore AFS is the technique which is most limited in the number of detectable elements. Taking further into account instrumental and operational costs, the MIP is profiling itself as the ideal detection system for this application.

Application to real samples

The analytical method developed was directly applied for determination of volatile organoselenium compounds in yeast. The sampling time was optimized at 35 min. The production of volatile species requires the assimilation of extracellular SeO_3^{2-}, the reduction to organo-seleno-compounds and subsequent methylation.[35] As fresh yeast is able to grow under either anaerobic or aerobic conditions, different atmospheres were tested in order to evaluate to what extent dissolved oxygen was affecting the production of volatile species. Time resolved evolution of selenium species was monitored in three different atmospheres; argon, air and oxygen. Species identification was carried out by comparison of retention times and spike experiments (Table 6).

Using ICP–MS the sulfur isotopes were used to detect species containing both selenium and sulfur. Up to seven different volatile organo-selenium species could be detected in these types of samples, DMDSe being the predominant species. The second most important species were DMSe and an unidentified compound, eluting after DEtSe and before DMDSe. Four minor selenium compounds could be detected, one of them DEtSe, and the remaining species being unidentified. Occurrence and concentration depends on contact time with the inorganic selenium solution or selenomethionine, respectively. There is evidence from ICP–MS measurements that the species labelled as number 6 is a sulfur-containing analogue of DMSe, most probably dimethyl selenosulfide (DMSe-S), as a sulfur peak was observed coinciding with the retention time of this compound. For the remaining species no sulfur response was observed eluting together with the selenium peaks.

Yeast was found to metabolize inorganic selenium very rapidly; 12 h after addition of inorganic selenium the dispersion had already changed in colour from light brown to reddish. This colour, typical for Se^0, may indicate that the subsequent reduction processes possibly involved in selenium assimilation by yeast and higher plants[36] are already in an advanced state. Sampling in the headspace at this point resulted in the detection of all the above mentioned species. Yeast enriched with selenomethionine gave rise to organic volatile selenium compounds, as shown in Fig. 3. In this case it seems that the selenomethionine is metabolized, indicating that this species is not the end-product of inorganic selenium metabolization by yeast. This observation is contrary to what one would expect, keeping in mind that, in this case, the yeast was exposed to a pasteurization process and should be therefore inactive.

Quantification of species could only be carried out for identified species whose standards are available, applying external calibration or standard addition. Matrix effects were negligible for determination of DMSe and DEtSe in yeast samples, when prepared following the method described in the Experimental section. For DMDSe a certain peak broadening compared with the analysis of stock solutions could be observed when running the column at room temperature. At higher temperature this problem can be avoided. As no separation problems were observed in this application, the low separation temperature was used because it permits a very simple set-up of the instrumentation. This allows the easy hyphenation between the chromatographic part of the system and different detectors tested. If any other peak is suspected to be overlapped by DMDSe, the temperature can be raised, which increases chromatographic resolution.

The slope of the calibration curves was very similar for

Table 6 Species with corresponding retention times using SPME–MC and different detectors found in enriched yeast samples

No	Rt_1/s	Rt_2/s	Rt_3/s	Identification
1	18	24	18	DMSe
2	30	48	37	Not identified
3	38	66	58	DEtSe
4	50	89	71	Not identified
5	68	130	107	Not identified
6	72	196	151	DMSe-S
7	112	252	200	DMDSe

[a] Rt_1 retention time for MIP, He carrier gas, Rt_2 for ICP–MS, Ar carrier gas, Rt_3 for AFS Ar/H_2 carrier gas.

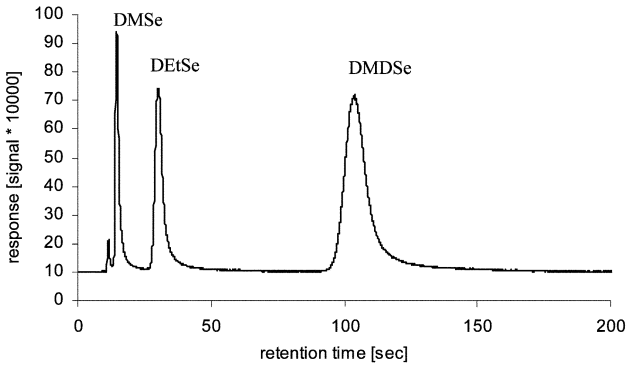

Fig. 2 Chromatogram obtained under optimized working conditions for a 25 μg l^{-1} aqueous mixed stock solution of DMSe, DEtSe and DMDSe using MC–MIP–AES.

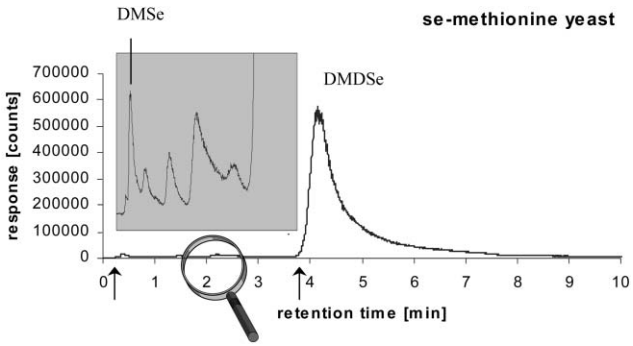

Fig. 3 Typical ICP–MS chromatogram, obtained for a commercial yeast preparation, enriched with selenomethionine after 72 h of contact in Ar atmosphere (scale in amplified area is from 0 to 20000).

DMSe and DEtSe, and somewhat higher for DMDSe. It thus seems to be reasonable assuming that a semi-quantification of the unidentified species is possible by comparing the signal obtained with those of identified species.

Some general trends for time-resolved selenium species distribution depending on the atmosphere in which the sample was kept are shown in Fig. 4. Results were evaluated for the predominant known species occurring when living yeast cells were enriched with a 20 mg l^{-1} solution of Se(IV). The values shown present the results obtained with all detectors tested. The dispersion of measured values for different sets of experiments was considerable, nevertheless general trends can be confirmed. Possible reasons for that dispersion are the fact that different batches of living organisms were used throughout the experiments and variations of laboratory temperature may have occurred, a parameter which is known to strongly affect yeast metabolism.

The endencies found were similar for selenomethionine-enriched yeast (Se-met yeast), but at higher concentration level. As can be observed from Fig. 4, the DMSe concentration rises until approximately 2 days of contact time and then levels off. This is possibly due to degradation of DMSe, forming DMDSe, a reaction pathway that can be verified using freshly prepared stock solution. Highest concentrations were found in the range 20–25 μg l^{-1} (100 μg l^{-1} for Se-met yeast), depending on the atmosphere. Taking into account the amount of yeast used (500 mg) and the dilution (5 ml), the biomass of organoselenium species determined are in the range 0.2–0.25 μg of selenium species/g of wet yeast (1.0 μg of selenium species/g of yeast for Se-met yeast). Differences observed for species evolution in Ar, air or O_2 were not very significant. This changed when the DMDSe concentration was studied. Here concentration seemed to rise until the longest contact time studied and an anaerobic environment clearly favors the production of this species. Degradation of previously formed DMSe only partly contributes to this evaluation, concentrations found were about 50 μg l^{-1} for air or Ar-atmosphere and only about 10 μg l^{-1} when the experiment was carried out in oxygen. For Se-met yeast this value raise up to 140 μg l^{-1} when air atmosphere was used. This value corresponds to 1.4 μg of selenium species/g of dry yeast.

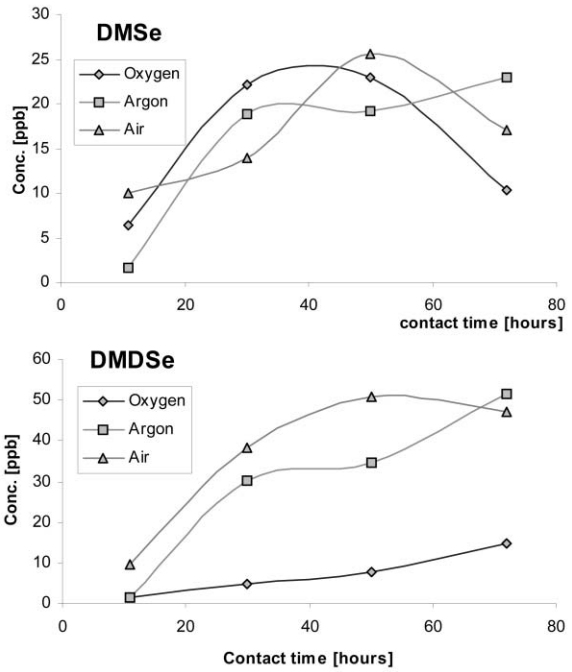

Fig. 4 Time-resolved evaluation of selenium species concentration in living yeast cells, data points shown are mean values obtained by the three detection systems (MIP–AES, ICP–MS and AFS).

Assuming that response for all volatile selenium species is within the same order of magnitude, the concentration of the unidentified species is most probably at low-ppb level. One exception is the unidentified species labelled as ''No. 4'', which seems to rise to a concentration above those obtained for DMSe, at least in the enriched living yeast cell metabolism, as shown in Fig. 5. This compound will be produced by yeast at a much lower rate when selenomethionine is metabolized.

Until now, the Se-enriched yeast producers use inorganic selenium to obtain the more bioavailable form Se-methionine, without taking into account the possible production of volatile organoselenium compounds. No evaluation of a possible loss of conversion efficiency in these processes has been carried out. Analysis of selenomethionine enriched yeast samples give rise to substantiated concerns about the quality, safety and origin of commercially available selenium containing dietary supplement products. Due to the unavailability of Certified Reference Materials for organoselenium compounds the method could not be completely validated. Nevertheless, comparison of results obtained for standard solutions using different detectors were in the range of 7% for the overall analytical process. This indicates that the method is reasonably robust. More experimental work and possibly the use of an alternative analytical technique, such as ESI-MS, will be necessary to clarify the nature of the unidentified volatile organo-selenium species.

Conclusions

Results obtained in the present work with the hyphenation of solid phase micro extraction and multicapillary gas chromatography with the three different detectors (MIP–AED, AFS and ICP–MS) demonstrates its versatility to be operated with virtually every common atomic detection system. Detection limits were in all cases in the low ppb-range, or below, for DMSe, DEtSe and DMDSe. All detectors were found to be suitable for volatile organo-selenium detection, but most straightforward analytical characteristics were obtained using MIP–AES detection. In this configuration the system can compete with highly cost intensive detectors such as ICP–MS. The multicapillary gas chromatographic column was operated at 30 °C, and as there is no need of an additional GC oven, the whole instrumentation becomes much less bulky. The technique outlines itself as the method of choice for exclusive determination of volatile organoselenium compounds as metabolism products of living organisms.

It has been demonstrated that even pasteurized yeast is able to metabolize inorganic selenium and to release organoselenium species in a short period of time. These transformations have to be taken into account when planning a campaign for the production of a new reference material. These materials for organoselenium species and additional pure compounds are still unavailable, complicating secure species identification and proper method validation. Future research will be focussed on extension of the applicability of SPME–MC instrumentation

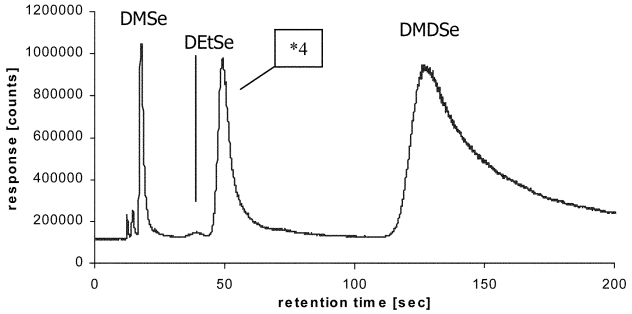

Fig. 5 MC–MIP–AES chromatogram obtained after SPME extraction using optimized working conditions for yeast enriched with inorganic selenium (20 mg l^{-1}), contact time 27 h in air.

coupled with MIP detection towards other organometallic compounds and to a further study of the yeast selenium enrichment process.

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