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Volatile organoselenium monitoring in production and gastric digestion processes of selenized yeast by solid-phase microextraction-multicapillary gas chromatography coupled microwave-induced plasma atomic emission spectrometry†

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Evolution of volatile organoselenium compounds in the production and gastric digestion of selenized yeast has been monitored. The industrial production of these kinds of material, employed as food supplements, has been simulated in a process of yeast enrichment with inorganic selenium selenium (IV) in different growth media, with variation of the pH value. The in vitro gastric digestion process was carried out with pepsin in an acid and salt mixture. Determination of volatile species of selenium was achieved coupling solid-phase microextraction (SPME) for preconcentration and sample–matrix separation and microwave-induced plasma atomic emission spectrometry, in combination with multicapillary (MC) gas chromatography for separation and detection of the selenium species. The MC column was operated at low temperatures (∼30 °C). The method was optimized, using a chemometric approach, with respect to the detection of organoselenium species such as dimethylselenide, diethylselenide and dimethyldiselenide. SPME sampling was carried out in the headspace above the corresponding solutions. Separation is fast, with a chromatogram being obtained in less than 5 min, and the detection limits were at the low parts per billion level for all species investigated. The results of the yeast enrichment process demonstrate inorganic selenium transformation into volatile organic species. The presence of inorganic selenium gave rise to at least five different volatile species after metabolization by yeast, with dimethylselenide and dimethyldiselenide being the predominant species. Commercial pasteurized yeast, containing mainly selenomethionine for use as a food supplement, and tablets were found to be still active under conditions of the simulation of the digestion process, even though producing relatively low amounts of organoselenium compounds.

KEYWORDS: yeast production; digestion; solid-phase microextraction; MIP-detection; organoselenium compounds; multicapillary column

INTRODUCTION

It is known that the behaviour of selenium compounds ranges from being essential for plants and bacteria[1] to being highly toxic,[2] with a relatively narrow tolerance band[3]. Most of the toxic effects of selenium are related to its chemical similarity to sulfur.[4] The majority of the enzymes involved in sulfur metabolism also catalyse the analogous reactions with the corresponding selenium substrates. Often, the affinity of enzymes involved in sulfur metabolism are

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similar for selenium. Usually, selenium becomes toxic at high concentrations due to the incorporation of selenium into sulfur-containing molecules, particularly the non-specific replacement of cysteine by seleno cysteine. Since clear evidence for the cancer chemo-preventive effects of a selenium-rich diet was found,[5,6] interest in selenium species determination and its biochemistry has been increasing. In producing its benefits, selenium most often works catalytically, as part of oxidative selenoenzymes such as glutathione peroxidase; however, it still remains unclear which selenium-containing molecules are active in the reduction of cancer incidence. Nevertheless, the use of selenized yeast as selenium-enriched supplements in human nutrition is on the increase. Therefore, there is an evident interest in the development of accurate and precise analytical methodologies that allow determination of the different selenium species content in yeast and a wide range of environmental matrices, such as air, water, biological tissue and sediments.[7]

Baker's yeast (S*accharomyces cerevisae*) is the most commonly used base material for the production of selenium-enriched food supplements. The principal reason is the simple enrichment process in which inorganic selenium is easily transformed to the more bioavailable selenium species. In spite of its generalized use, relatively little is known about selenium metabolism by yeast, the identity of minor species present and transformation pathways. Selenium phytovolatilization,[8] the process by which plants metabolize various inorganic or organic species of selenium (e.g. selenate, selenite, and seleno methionine (Se-Met)) into gaseous forms (e.g. dimethylselenide (DMSe)), is a potentially important technique for removing selenium from contaminated environments. The volatilization of selenium in food supplements is, on the other hand, highly undesirable. The enzymatic pathway may involve the enzyme *S*-adenosyl-L-Met : L-Met *S*-methyltransferase, responsible for the methylation of Se-Met to Se-methyl Se-Met.[9]

For modern preparation of selenium-enriched yeast,[10] selenium is supplemented as selenite during a batch fermentation process under aerobic conditions. Nutrient, optionally with a carbon and nitrogen-source supplement, is added and, after finalization of the process, the yeast is sterilized, spray dried and pelleted for further use. It has been shown that the most important parameters affecting the incorporated forms of selenium during the yeast preparation are the pH value and the dissolved oxygen level in the growth medium.[11] As a general rule, increasing the pH value favours lower selenium consumption; at the same time, a lower inorganic selenium content is obtained under these conditions in the product. Further, it could be demonstrated that an increase in oxygenation of the sample increases the uptake of selenium. When the objective of the enrichment process is to obtain the most quantitative transformation of selenium into Se-Met, the optimum enrichment procedure involves use of low concentrations of sodium selenite and aerobic conditions during the growth phase.[12]

These kinds of sample have been 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,[13,14] followed by high-performance liquid chromatography (HPLC) or ion-exchange separation[15] and mass spectrometric detection, using either electrospray ionization (ESI)[16] or ICP[17] as ionization or excitation source respectively. The principal selenium compounds in these yeast extracts were identified as Se-Met and *Se*-adenosyl-selenohomocysteine,[18] accounting for about 85% of the total selenium content in yeast samples. On the other hand, there were some commercial selenium enriched yeast products where up to 100% of the selenium content was present in the form of inorganic selenium.[19] Some attempts have been made to classify selenium species occurring in different fractions of yeast samples, e.g. in the protein fraction,[20] by gel-electrophoresis ICP mass spectrometry (MS). In the analysis of aqueous yeast extract[21] using size-exclusion chromatography (SEC)–capillary zone electrophoresis–ICP-MS and in the low-molecular-weight fraction[22] by HPLC–ICP-MS, a considerable number (~20) of selenocompounds could be observed. Those were mostly characterized by the presence of an adenosyl functional group, but the majority remain to be identified definitively. In the analysis of aqueous extracts of selenized yeast pellets using capillary electrophoresis–ICP-MS, there is some evidence for the presence of trimethylselenonium, Se-methyl Se-Met and selenocystine–Se-methylselenocysteine in addition to Se-Met.[23]

Recently, hot-water extract of *S. cerevisiae* and proteolytic digestion with protease were investigated using ion-pair liquid chromatography coupled to hydride generation atomic fluorescence spectrometry (AFS).[24] The species found depended on the extraction procedure used: only one main species could be detected in yeast samples (Se-Met) when proteolytic extraction was applied, and three unknown species were found when yeast was extracted with hot water. There was also evidence for species transformation during storage, which could only be avoided when the extracts were deep-frozen. A more systematic approach to the characterization of selenized yeast supplements involves a more sophisticated fractionation procedure, including the sequential leaching of water soluble, cell-wall bound, and membrane-protein selenium followed by a further fractionation of each extract by high-resolution SEC.[25] The basic problem in these studies is the unavailability of sufficient appropriate standards. Apart from ongoing species identification, current investigations focus on stability and reactivity[26] of the already known species, which account for ~65% of the total selenium[27] found in this kind of matrix.

The separation and isolation of volatile analytes from sample matrices is challenging, with the extraction step being the stage at which most analyte loss occurs and which introduces the largest source of error in the analytical measurement. Thus, efficient methods of extraction are continually being sought. Solid-phase microextraction

(SPME), a solvent-free sample preparation technique, has many advantages, including simplicity, portability, time consumption, and compatibility with a multicapillary (MC) GC system. The SPME technique is based on the establishment of an adsorption or absorption equilibrium of the target compounds onto a coated fused-silica fibre, which can take place in the headspace or immersed in solution.[28] The present work centres on determination of low-molecular-weight volatile selenium compounds, like DMSe, diethylselenide (DEtSe) or dimethyldiselenide (DMDSe) during the simulation of both industrial production and gastric digestion of food supplement products. These species may result from seleno-protein degradation, bioalkylation processes or metabolism products of inorganic selenium in the context of auto-detoxification mechanisms of living yeast cells, but they are not easily detected when common sample preparation methods are used. SPME allows time-resolved monitoring of volatile organoselenium concentration in the headspace above the living organism. It is hyphenated with MC-GC; thus, the instrumentation becomes less bulky and separation is speeded up. This is coupled with a highly sensitive and economic microwave-induced plasma atomic emission spectrometry (MIP-AES) detection system.

EXPERIMENTAL

Reagents and standards

All reagents used were analytical reagent grade. Deionized water was obtained from a Millipore (Bedford, MA, USA) ZMFQ 23 004 Milli-Q water system. DMSe and DMDSe were obtained from Alfa Aesar (Karlsruhe, Germany). DEtSe and the inorganic selenium species selenite (Na_2SeO_3) were bought from Sigma Aldrich (Madrid, Spain). Stock solutions of the organoselenium species of 10 000 mg l^{-1} (expressed as metal) were prepared by appropriate dilution with methanol and stored at 4 °C. Lower concentrated 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 fibres were purchased from Supelco (Bellefonte, PA, USA). Helium, argon, air and oxygen (99.99% purity) were purchased from Carburos Metalicos (Madrid, Spain). HCl (37%), NaOH, NaCl and glucose were from Merck (Darmstadt, Germany). Pepsin A from porcine stomach mucosa was obtained from Sigma Aldrich (Madrid, Spain).

Fresh baker's yeast (*S. cerevisiae*) and two different brands of selenium food supplement samples (100 mg selenium per tablet) were bought in local markets. Dry baker's yeast type II (*S. cerevisiae*) was purchased from Sigma Aldrich (Madrid, Spain). Selenium-enriched yeast was obtained from Pharma Nord (Precise, Vejle, Denmark). The total selenium content, mainly in the Se-Met form, was evaluated in an interlaboratory study (SEAS-6), and found to be 1383.9 ± 86.7 μg g^{-1}.

Safety note: DMSe and DMDSe have the following risk notes (R)[29] and safety (S)[30] 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 protective clothing (e.g. gloves, laboratory glasses, etc.).

Instrumentation

A home-made GC injection port for SPME fibre desorption coupled with a gas chromatographic separation unit was hyphenated to a MIP excitation source with optical emission spectroscopic detection (AES). The SPME fibre desorption unit was developed in house 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.[31] The outlet of the desorption unit was connected to a straight 25 cm MC (BeeChrom OV-17 (50% phenyl)–50% methylpolysiloxane, $N \approx 1000$, i.d. 40 μm, film thickness 0.2 μm).

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 tuneable forward power between 30 and 300 W. The reflected power was adjusted to a minimum after igniting the plasma. A TM_{010} Beenakker-type cavity (Model HMW 25–471 N-W, AF Analysetechnik, Tübingen, Germany) was used, provided with ceramic discharge tubes (60 mm in length, outer diameter 4 mm, inner diameter 2 mm) 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.[32] Data recording and treatment was done with SpectraSense V4.2.7 software, provided with the instrument.

Analytical procedure

Two sets of experiments were carried out with the objective of simulating the industrial production process of selenium-enriched yeast for food supplements on the one hand and the gastric digestion of such enriched yeast on the other hand.

The production process was simulated by dispersing 250 mg of fresh or dry baker's yeast in 5 ml of selenium(IV) stock solution, at different concentration levels (1–100 mg l^{-1}), in a 10 ml vial. Three different pH media were studied: slightly acid (pH 4.6), just by adding the selenium stock solution with Milli-Q® water to the desired final concentration; acid (pH 1.5), by adjusting with concentrated HCl; and alkaline (pH 12.5), by adjusting with concentrated NaOH. A 6 mm stirrer was placed inside the vial, which was then closed with an aluminium seal and a Teflon-faced butyl septum. The influence of glucose medium (70 g l^{-1}) on production was also studied. The vial was placed onto a magnetic stirrer. An SPME fibre, placed in a fibre assembly holder (Supelco), was passed through the septum for headspace

sampling. The mixture was stirred at room temperature over the period of 1–96 h prior to sampling.

In vitro gastric digestion was carried out by adding the following mixture either to 100 mg of selenium-enriched yeast or to an individual tablet (200 mg) of two different brands of commercial food supplement: 0.6 g of pepsin, 0.088 g of NaCl and concentrated HCl (pH 2) in 5 ml of Milli-Q® water, together with a 6 mm stirrer in a 10 ml vial. Further treatment was similar to that described above.

Loaded fibres were transferred to the desorption unit for measurement directly after sampling. The optimized SPME extraction, desorption and detection parameters used for organoselenium analysis are summarized in Table 1.

RESULTS AND DISCUSSION

Instrumental and analytical variable optimization was done using experimental design tools. Owing to the large number of variables involved in this analysis, they were grouped according to the different analysis steps: extraction, separation and detection. In the SPME extraction, a partially crosslinked 75 μm Carboxen™/PDMS-coated fibre was found to be the optimum fibre in terms of extraction mechanism, film thickness and polarity for organoselenium determination.[31] For gas chromatographic separation the parameters affecting desorption and separation in the MC column were also previously established[32] using a multivariate approach. Under the optimized conditions, given in Table 1, separation was found to be faster and more precise than a conventional column of similar characteristics. Instrumental variables affecting the detection step were optimized in previous works.[31,32]

Table 1. Optimized parameters for organoselenium determination using SPME-MC and MIP-AES

SPME extraction	
Fibre	Carboxen/PDMS 75 μm
Extraction time (min)	35
Extraction temperature (°C)	25
Stirring (rpm)	350
MC separation	
Column type	25 cm, $N \approx 1000$, 40 μm i.d., OV-17, f: 0.2 μm
He carrier gas (ml min^{-1})	38
Desorption temperature (°C)	240
Column temperature (°C)	30
MIP-AES detection	
MIP forward power (W)	120
Reflected power (W)	18
Ar plasma gas (ml min^{-1})	50
Air cooling gas (ml min^{-1})	280
Analytical wavelength (nm)	196.0
Entrance/exit slit-width μm	10/40
PMT voltage (V)	700
Integration time (ms)	50
Interval time (ms)	100

The values for the figures of merit of the proposed method are summarized in Table 2. During the optimization process, mixed samples containing 5 ml of 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 sample containing 5 ml of water as a blank solution. The precision was evaluated by analysing five different samples of 15 ng ml^{-1} for each species, measured during 1 day and using different fibres of the same type.

Owing 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 (MIP-AES, ICP-MS and AFS)[33] were within of 7% for the overall analytical process. This indicates that the method is reasonably robust.

Volatile organoselenium monitoring in selenized yeast samples

As explained above, the analytical method developed was directly applied for determination of volatile organoselenium compounds in a simulation of the industrial production process of selenium-enriched yeast and in a simulation of gastric digestion of this material.

Simulation of the industrial production process of selenized yeast

As *S. cerevisiae* is able to grow under either anaerobic or aerobic conditions,[11] different atmospheres (argon, air and oxygen) were tested in a previous study[32] in order to evaluate to what extent dissolved oxygen was affecting the production of volatile species. As no great difference in organoselenium production was detected, subsequent experiments were carried out under aerobic conditions, which favours the transformation of selenium into Se-Met,[12] thus reducing the amount of inorganic selenium in yeast cells.[11] The dispersion of measured values for different sets of experiments was considerable; nevertheless, general trends could be established. A possible reason for this dispersion is

Table 2. Figures of merit for organoselenium speciation using SPME-MC coupled with the MIP-AES detection system

	DMSe	DEtSe	DMDSe
Detection limit (ng ml^{-1})	0.57	0.47	0.19
Regression R^2	0.991	0.975	0.967
Precision (% RSD) ($n = 5$)	7.76	7.89	7.06
Linearity range	Detection limit to 100 ng ml^{-1}		
Reproducibility R_t (% RSD)	2.08	3.85	4.36

the fact that different batches of living organisms were used throughout the experiments. The most homogeneous results were obtained when using the baker's yeast samples from Sigma, which had previously been spray dried.

First, the influence of glucose in the growth medium (70 g l^{-1}) during production was evaluated. The results demonstrate that, in the presence of glucose, the production of volatile organoselenium compounds is about 100 times higher, clearly showing the importance of feeding the yeast cells. Consequently, glucose was used in further experiments as a carbon source supplement. Three different selenium concentrations (1, 10 and 100 mg l^{-1}) were tested to evaluate the influence on production of volatile selenium. Yeast was found to metabolize inorganic selenium very rapidly for the concentrations studied, with organovolatile species already detectable at the first sampling stage (after 1 h of incubation). Further, in the high selenium concentration samples (100 mg l^{-1}), 1 h after addition of inorganic selenium the dispersion had already changed the colour from light brown to reddish (colour of Se^0) and sampling in the headspace resulted in the detection of volatile selenium species.

Up to five different volatile organoselenium species could be detected in these kinds of sample, the first and predominant one being DMSe, normally representing between 40 and 95% of total volatile selenium (Figure 1a). Species identification was carried out by comparison of retention times and spike experiments (Table 3). When using high concentrations of inorganic selenium (100 mg l^{-1}) and longer incubation times, DMDSe was the main species detected (Figure 1b). Another important species was an unidentified compound, eluting after DEtSe and before DMDSe. It should also be commented that, when using high concentrations of selenium(IV), one important species (Table 3 and Figure 1b) is detected in chromatograms eluting before DMSe. As this coincides with the appearance of the commented reddish colour of the solution, it may be concluded that this species could be volatile Se^0, but this conclusion has to be proved with more experiments.

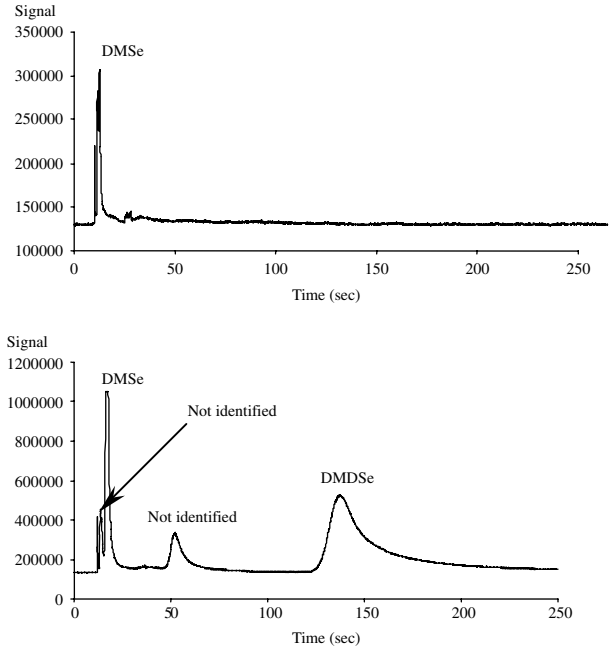

Figure 1. MC–MIP-AES chromatogram obtained after SPME extraction using optimized working conditions for yeast enriched with inorganic selenium (100 mg l^{-1}) for (a) 2 h and (b) 75 h contact time in air.

The influence of pH is low in terms of species distribution, with DMSe being the major volatile species found at all three pH values studied. But concerning the amount of species, the data clearly show that, in the neutral solutions, much greater quantities of volatile organoselenium species are produced (Figure 2). Although this has been defined as neutral because no acid or basic media is added, a solution of fresh baker's yeast with any amount of inorganic selenium is slightly acidic (pH 4.1–4.5). Suhadja *et al.*,[11] in similar experiments, indicated a lower inorganic selenium consumption when increasing the pH value from 3 to 5. As the pH values investigated in this study do not coincide with those used in that investigation, the dependence of metabolization on pH remains unclear in this range, but it can be concluded that very acidic or basic conditions do not favour the consumption of inorganic selenium.

Several problems have been detected during quantification of species, principally due to growing pressure in the vials containing living yeast cultures, to temperature variations in the laboratory and to matrix effects. Different calibration strategies were examined (standard addition, external), and comparable results were obtained; thus, the easier external calibration procedure was chosen for ongoing experiments. The external calibration was carried out by comparing the integrated peak areas obtained for mixtures of stock solutions of each of the three species with standards available with the signal obtained for real samples. The analyte volume was 5 ml throughout the experiments.

The slopes of the calibration curves were very similar for DMSe and DEtSe, and somewhat higher for DMDSe. Although quantification of species could only be carried out for identified species whose standards are available, it thus

Table 3. Species found in yeast samples enriched with inorganic selenium with corresponding retention times (R_t) using SPME-MC and MIP-AES detection

Species	R_t (s)	Identification
1	12	Not identified
2	18	DMSe
3	38	DEtSe
4	55	Not identified
5	145	DMDSe

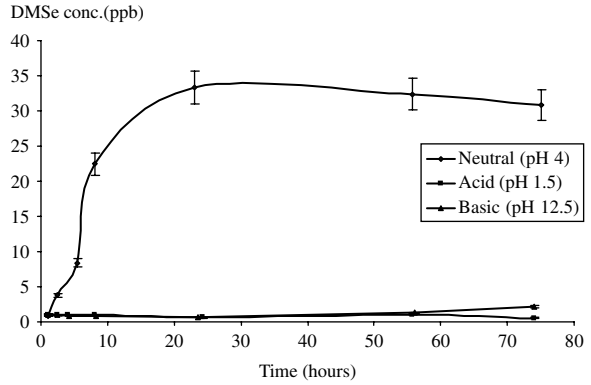

Figure 2. Time-resolved evolution of DMSe concentration in living baker's yeast cells at the three different pH values studied, using MC-MIP-AES after SPME extraction.

seems reasonable to assume that a semi-quantitative analysis of the unidentified species is possible by comparing the signal obtained with those of identified species. Following that concept, the concentration of the unidentified species is most probably at the low parts per billion level. The highest concentrations were found in the region of 200 $\mu g\,l^{-1}$, for DMDSe species when 100 $mg\,l^{-1}$ of selenium(IV) and 75 h incubation were used. This means that only 0.2% of the total selenium is transformed into volatile organoselenium species. In one set of experiments, using 1 $mg\,l^{-1}$ of selenium(IV), 200 $\mu g\,l^{-1}$ of DMSe was detected, which means that 20% of inorganic selenium was transformed into a volatile species; however, upon repeating these experiments the conversion rate was found to be lower. In general, taking into account the amount of yeast used (250 mg) and the dilution (5 ml), the absolute amount of organoselenium species determined is in the region of 0.16 μg of selenium species per gram of fresh yeast. The total amount of organovolatile species produced grew when the inorganic selenium concentration added to the yeast was increased, but, proportionally, the quantity of transformed inorganic selenium decreased. Some workers have observed a decrease in yeast growth when high concentrations of inorganic selenium are present, indicating an inhibition effect by selenium on yeast growth,[12,34] a finding that could be confirmed in this study.

When volatile organoselenium production by dry baker's yeast (purchased from Sigma) was evaluated, a similar species distribution as obtained with fresh baker's yeast was observed, but the absolute concentrations were significantly lower when a high inorganic selenium content was used. The maximum volatile organoselenium concentration obtained was 10 $\mu g\,l^{-1}$ of DMSe when 100 $mg\,l^{-1}$ of selenium(IV) and 25 h of incubation were used, and 5 $\mu g\,l^{-1}$ of DMSe were detected when 1 $mg\,l^{-1}$ of selenium(IV) was used.

Volatile organoselenium compounds during simulation of gastric digestion

Two different kinds of sample were treated: selenium-enriched yeast (purchased from Pharma Nord) and selenium-containing food supplement (purchased from local markets). Yeast enriched with selenium (containing mainly Se-Met) produced up to four different volatile organoselenium species, with DMDSe being the predominant one (Figure 3). The second most important species were DMSe and an unidentified compound, eluting after DEtSe and before DMDSe. Another, minor selenium compound could be detected, and this was identified as DEtSe. As in the other samples, the occurrence and concentration depend on the contact time, with a maximum production of almost all species at 30 h (Figure 4). Contrary to what was expected, keeping in mind that in this case the yeast was exposed to a pasteurization process, yeast enriched with selenium metabolizes the principal species, Se-Met, as shown in Figure 3, producing seleno-organovolatile compounds.

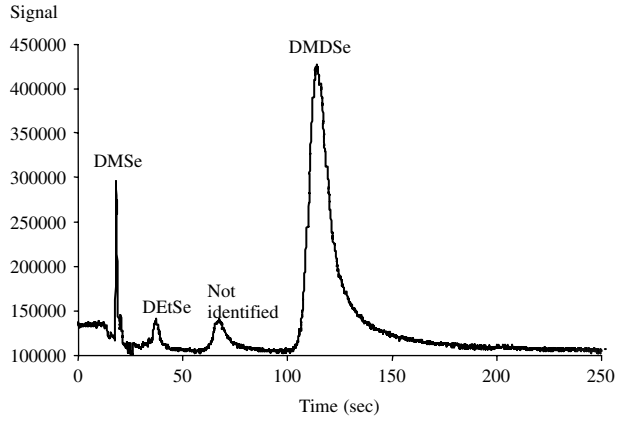

Figure 3. MC–MIP-AES chromatogram obtained after SPME extraction using optimized working conditions for commercial selenium-enriched yeast from Pharma Nord treated with pepsin for 50 h in air.

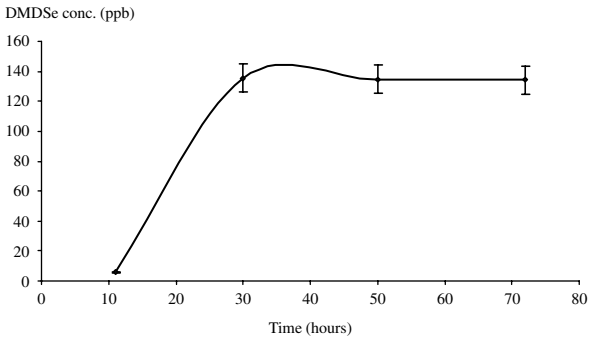

Figure 4. Time-resolved evolution of DMDSe concentration in Pharma Nord yeast using MC–MIP-AES after SPME extraction.

As in the simulation of the production process, only identified species whose standards are available could be quantified. The highest concentrations found were in the region of 140 μg l^{-1}. As indicated in the Experimental section, the total selenium content in yeast determined in an interlaboratory study is 1383.9 ± 86.7 μg g^{-1}. Taking into account the amount of yeast used (250 mg) and the dilution (5 ml), this means that only 0.0014% of the total selenium is transformed into volatile organoselenium species.

In the case of the selenium content of food supplement samples, tablets from both commercial sources proved to produce only the DMSe species (Figure 5), even when large exposure times were applied. The DMSe concentration evolution over time was similar to other species, reaching a maximum of 8–10 μg l^{-1} within the first 24 h, after which the concentration decreased. As each tablet has a mass of ~200 mg, the amount of DMSe determined was in the range 0.125–0.250 μg of selenium per gram of tablet. The total selenium content indicated by the manufacturer was of 100 mg per tablet (no species information was given); consequently, only around 0.05% of the total selenium was transformed during the simulation of digestion into volatile organoselenium species.

The difference between the organovolatile species produced by the simulation of digestion in Pharma Nord selenium-enriched yeast, with a 'certified' high Se-Met content, and the species produced by the food supplement tablets may indicate that these tablets do not originally contain the same selenium species. Other investigators, in studying six different brands of yeast-based selenium food supplement, found that only two of them had high levels of Se-Met and one of them appeared to contain all inorganic selenium, despite label claims for the content being only Se-Met.[19] More experimental work, and possibly the use of an alternative species-specific analytical technique, such as ESI-MS, will be necessary to clarify the nature of the unidentified volatile organoselenium species.

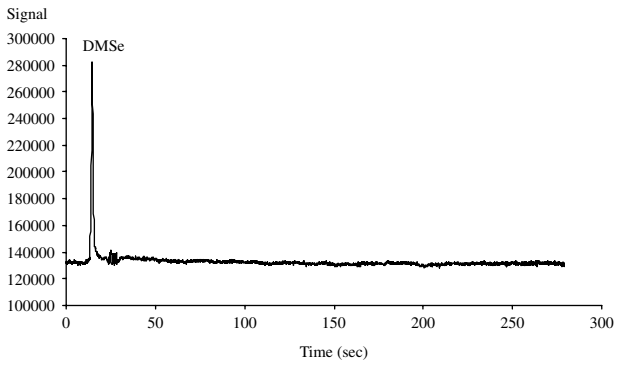

Figure 5. MC–MIP-AES chromatogram obtained after SPME extraction using optimized working conditions for selenium-containing food supplement tablets; contact time, 27 h in air.

CONCLUSIONS

Hyphenation of SPME–MC-GS with MIP-AES detection is the method of choice for exclusive determination of volatile organoselenium compounds as metabolic products of living organisms. This configuration allows the determination of volatile species that are often very difficult to determine; thus, quite a few publications are dealing with volatile organoselenium detection and much work is being done in determination and identification of other selenium compounds. Detection limits were, in all cases, in the low parts per billion range (or loss) for DMSe, DEtSe and DMDSe. The MC-GC column was operated at 30 °C, thus avoiding the need to use an additional GC oven, which makes the whole instrumental set-up much less bulky.

In the simulation of the production process, although great variability between different sets of data was obtained, yeast was found to metabolize inorganic selenium and to release organoselenium species comparatively rapidly. The main species after metabolization is DMSe in the first few hours, followed by DMDSe after the first 24 h. Although in almost all experiments the transformation of inorganic selenium to organovolatile selenium species is below 0.1%, the occasional experiment yielded up to 20%, producing an extreme decrease in reaction yield if other, more bioavailable selenium species, such as Se-Met, were aimed to be obtained. Until now, the makers of selenium-enriched yeast have used inorganic selenium to produce the more bioavailable form, i.e. Se-Met, without taking into account the possible production of volatile organoselenium compounds. The evaluation of a possible loss of conversion efficiency in these processes can help in producing better quality and safer commercial selenium-containing dietary supplement products.

On the other hand, the evaluation of organovolatile selenium compounds production during simulation of a digestion process of dietary food supplements may indicate that the selenium species present in these kinds of sample differ between the different brands. Although the volatile organoselenium production is quite low compared with the total selenium content, there is a difference between the species produced during the simulation of a digestion process of SelenoPrecise Yeast (with a high 'certified' Se-Met content) and tablets of two brands of food supplement. It is surprising that organoselenium compounds could be detected in all these kinds of sample, because, in theory, the yeast should be completely inactive after the pasteurization and drying process suffered during its production.

Reference materials for organoselenium species and more pure compounds are still unavailable, making it difficult to secure species identification and proper method validation. Future research will be focused on extension of the applicability of SPME-MC instrumentation coupled with MIP detection towards other organometallic compounds and application of other analytical techniques, such as ESI-MS or isotope-dilution–ICP-MS, both for identifying and

quantifying some organovolatile selenium compounds in further studies of the yeast selenium-enrichment process.

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