

**University of Massachusetts Amherst**

---

**From the Selected Works of Julian Tyson**

---

2009

## Speciation of selenium dietary supplements; formation of S-(methylseleno)cysteine and other selenium compounds

Julian Tyson  
Prince O Amoako  
Peter C. Uden



Available at: [https://works.bepress.com/julian\\_tyson/160/](https://works.bepress.com/julian_tyson/160/)

# Speciation of selenium dietary supplements; formation of S-(methylseleno)cysteine and other selenium compounds

Prince O. Amoako, Peter C. Uden\*, Julian F. Tyson

Department of Chemistry, University of Massachusetts Amherst, 710 N. Pleasant St., Amherst, MA 01003-9336, USA

## A B S T R A C T

Speciation of selenium is of interest because it is both essential and toxic to humans, depending on the species and the amount ingested. Following indications that selenium supplementation could reduce the incidence of some cancers, selenium-enriched yeast and other materials have been commercialized as supplements. Most dramatically however, the SELECT trial that utilized L-selenomethionine as the active supplement was terminated in 2008 and there is much debate regarding both the planning and the results of efficacy studies. Further, since dietary supplements are not regulated as pharmaceuticals, there are concerns about the quality, storage conditions, stability and selenium content in selenium supplements. Enzymatic hydrolysis enabled selenium speciation profiles to be obtained by high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and following derivatization gas chromatography with atomic emission detection (GC-AED). Coated fiber solid phase microextraction (SPME) was used to extract volatile selenium species for determination by GC-AED and GC-MS. Similar speciation patterns were observed between yeast-based supplements subject to extended storage and those heated briefly at elevated temperatures. All the yeast-based supplements and one yeast-free supplement formed S-(methylseleno)cysteine on heating. Evidence was obtained in support of the hypotheses that S-(methylseleno)cysteine is formed from a reaction between dimethyldiselenide and cysteine or cystine.

## 1. Introduction

The United States 1994 Dietary Supplement Health and Education Act (DSHEA) defines a dietary supplement as “a product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (a) a vitamin; (b) a mineral; (c) an herb or other botanical; (d) an amino acid; (e) a dietary substance for use by man to supplement the diet by increasing the total dietary intake; or (f) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (a), (b), (c), (d), or (e)” [1,2]. Many dietary supplements are sold over the counter but are not specifically regulated by the United States Food and Drugs Administration (FDA). It is estimated that less than 1% of all adverse effects of dietary supplements are reported to the FDA [3]. The usage and sales of dietary supplements in the United States continue to increase. Estimated sales increased about 100% between 1992 and 1996 [3], were \$18.8 billion in 2003 [4], \$20.3 billion in 2004 [3] and are reported to be about \$23 billion in 2007 [5].

Selenium is an essential micronutrient that plays an important role in many biological processes through the action of seleno-proteins that have an important antioxidant and detoxification function in the human body [6]. The typical dietary intake of selenium in the United States is estimated to be between 80 and 120  $\mu\text{g day}^{-1}$ , but intake is substantially less in many regions of the world [7]. Organically bound selenium is present in foods mainly as selenomethionine (SeMet), selenocystine and Se-(methyl)selenocystine while inorganic selenium occurs less frequently and in very low amounts in food [8]. Organic forms are believed to have greater bioavailability than inorganic forms that are less effective in raising selenium levels in the blood, and the form of selenium affects selenoprotein expression [9]. Selenium deficiency in diet has been linked to coronary heart disease [10], lower response to influenza infections [11], high blood pressure [12], osteoporosis [13], epilepsy [14,15], rapid progression of HIV to AIDS [16,17], leukemia [18] and other cancers [19,20]. Symptoms of selenium deficiency occur at intake below 20  $\mu\text{g day}^{-1}$  and symptoms of toxicity at more than 800  $\mu\text{g day}^{-1}$  [13]. Chronic toxicity of selenium results in selenosis, characterized by hair and nail loss and brittleness, gastrointestinal problems, skin rash, garlic breath odor, and nervous system abnormalities [21]. The Institute of Medicine of the National Academy of Sciences has set

\* Corresponding author. Tel.: +1 413 5452293; fax: +1 413 5454490.  
E-mail address: pouden@chem.umass.edu (P.C. Uden).

400  $\mu\text{g day}^{-1}$  of selenium as the tolerable upper intake level for adults [22].

Due to the low abundance of selenium in some natural and common processed foods [7], a variety of selenium-enriched materials including yeast [23,24], milk [9], broccoli [25,26], garlic [27,28], green onions [29,30], green tea [31,32] and mushrooms [33,34] have been commercialized or proposed as selenium dietary supplements. Baker's yeast (*Saccharomyces cerevisiae*) is most commonly used for the production of supplements; selenized or 'high selenium yeasts' are prepared by growing yeast in a sodium selenite-enriched medium [35–37]. The principal organoselenium species produced in selenized yeast is selenomethionine [38,39], but selenoanions and other selenoamino acids including selenocystine, Se-lanthionine and Se-(methyl)selenocysteine have been determined at levels <1% of that of selenomethionine [40]. Ouerdane and Mester have reported essentially quantitative replacement of methionine by selenomethionine in yeast grown on a SeMet-containing medium [41].

In 1996, Clark et al. [42] reported that dietary supplementation of 200  $\mu\text{g day}^{-1}$  as selenized yeast decreased the incidence and mortality of cancer (lung, colorectal, and prostate) by nearly 50%. This study excited considerable public and scientific interest in the nutritional and clinical importance of selenium, notably in respect to potential cancer mitigation, and it has stimulated a number of further trials to better evaluate its efficacy. Principal amongst these has been the Selenium and Vitamin E Cancer Prevention Trial (SELECT) in the US, Puerto Rico and Canada [43–46], and the Prevention of Cancer by Intervention with Selenium (PRECISE) trial in the UK, Denmark and Sweden [47–49]. There is now considerable debate regarding both the planning and the results of these studies. Most dramatically the SELECT trial that utilized L-selenomethionine as the active supplement, was terminated in 2008 following a conclusion that there had been no demonstrable beneficial effect on prevention of prostate cancer and there was a possible indication of increased incidence of diabetes [50]. Since selenized yeast, as employed by Clark et al. and in most supplements, was not tested in the SELECT study, it is conceivable that this difference proved important and that other selenium species observed in the 'Clark' yeast were germane to those results [51,52]. Alternatively Rayman et al. have argued that the null results of SELECT are explainable by the fact that the candidate subjects already had optimal selenoprotein concentration/activity (notably selenoprotein P) and the results of SELECT tell nothing regarding effects of selenium on advanced cancer or on men of low Se status [53–55]. They further suggest that the selenium speciation of the supplements used is of lesser importance in outcome than the candidate demographics.

Bleys et al. [56] found high serum selenium levels to be positively associated with occurrence of diabetes and Stranges et al. [57] reported long-term selenium supplementation (200  $\mu\text{g day}^{-1}$  as selenized yeast) increased the risk of type 2 diabetes. These observations have also raised concern in the nutritional selenium community.

Whatever proves to be the eventual outcome amongst nutritionists and clinicians, it is established that different ingested selenium species follow different metabolic pathways, hence the need to determine selenium species in addition to total selenium concentrations remains paramount [8]. The need to determine very low concentrations of different selenium species accurately is clear. Further the stability of selenium species in different materials during processing, packaging or storage is of concern.

In this paper we report the speciation of selenium in a range of currently available yeast- and non-yeast-based selenium supplements, with particular focus on the presence and production of S-(methylseleno)cysteine, selenomethionine selenoxide and dimethyldiselenide upon storage and thermal treatment.

## 2. Experimental materials and methods

### 2.1. Instrumentation

A Hewlett Packard (Agilent) HP 5921A atomic emission detector interfaced to an HP 5890 II gas chromatograph (GC–AED) was used to determine selenium species in the headspace and in ethyl chloroformate-derivatized extracts of the selenium supplement samples. Gas chromatographic separations were obtained on a 30-m SE-30 capillary column with an internal diameter of 0.32 mm and a stationary phase film thickness of 0.25  $\mu\text{m}$  (Supelco, Bellefonte, PA). Eluted species from the column were simultaneously monitored at the emission lines 196 nm (Se), 181 nm (S) and 193 nm (C). The helium carrier flow rate was 1  $\text{mL min}^{-1}$  and the injection split ratio was 30:1. Nitrogen gas was used as make-up gas to stabilize the hydrogen plasma. The GC oven was held at 60 °C for the first 10 min and then heated at a rate of 5 °C  $\text{min}^{-1}$  to 300 °C and held there for 2 min, giving a run time of 60 min. The injection port on the GC was kept at 250 °C. The solvent vent on the detector was set at 7 min for both volatile and derivatized selenium species. Similar procedures have been reported for the determination of volatiles in selenized yeast [58].

For GC–MS analysis, a Hewlett Packard (Agilent) HP 5989A mass spectrometer was interfaced to an HP 5890 II gas chromatograph, with a DB-5MS (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ) capillary column (J & W Scientific, Inc., Folsom, CA). The temperature program was the same one used for the GC–AED analysis, with a solvent delay of 3 min. Helium was the carrier gas at a flow rate of 1  $\text{mL min}^{-1}$ .

A PerkinElmer Elan DRC-e inductively coupled plasma mass spectrometer (PerkinElmer Sciex, Ontario, Canada) was used as HPLC detector (HPLC–ICP–MS). The operating conditions with a cross flow nebulizer and Scott spray chamber were as follows: RF power 1100 W, plasma Ar flow rate 15  $\text{L min}^{-1}$ , nebulizer Ar flow rate 0.98  $\text{L min}^{-1}$ , auxiliary gas flow rate 1.20  $\text{L min}^{-1}$ , methane collision cell gas 0.6  $\text{mL min}^{-1}$ , isotopes monitored  $^{78}\text{Se}$ ,  $^{80}\text{Se}$ ,  $^{72}\text{Ge}$ , dwell time 80 ms [52]. The HPLC system which utilized a 5- $\mu\text{m}$  Symmetry Shield<sup>TM</sup> RP-C<sub>8</sub> column (3.9 mm  $\times$  15 cm) (Waters Corporation, Milford, MA) and an ion-pairing mobile phase of 0.1% heptafluorobutanoic acid (HFBA), 1% methanol and 99% water (v/v) was described previously [40,51].

### 2.2. Reagents

All solutions were prepared in 18 M $\Omega$  cm water obtained from a Barnstead E-pure system (Barnstead, Boston, MA). Protease XIV, DL-selenomethionine, L-cysteine, methionine and Se-(methyl)selenocysteine hydrochloride were obtained from the Sigma–Aldrich Chemical Company (St. Louis, MO). The dimer of cysteine, DL-cystine, was obtained from Acros Organics/Fisher Scientific (Fair Lawn, NJ). Ethylchloroformate (ECF) (Sigma–Aldrich, St. Louis, MO), pyridine (Fisher Scientific, Fair Lawn, NJ) and ethanol (200 proof; absolute, USP grade) (Pharmco Products, Brookfield, CT) were used to derivatize enzymatic extracts for GC–AED determinations. Hexane and chloroform (Fisher Scientific, Fair Lawn, NJ) were used to extract derivatives or redissolve residues of derivatized extracts. S-(methylseleno)cysteine was synthesized and characterized as reported by Block et al. [59]. Stock solutions of the amino acids (10,000 ppm) were prepared by dissolving approximately 10 mg of the amino acid in 0.1 M HCl solution. A constant temperature oil bath was set up with silicone oil (Aldrich, Milwaukee, WI) in a glass vessel set on a hot plate and the temperature monitored with a liquid-in-glass thermometer.

### 2.3. Yeast-based and yeast-free selenium supplements

SelenoExcell<sup>TM</sup> high selenium yeast tablets (200  $\mu\text{g tablet}^{-1}$ ) were obtained from Cypress Inc. (Fresno, CA). Schiff selenized yeast

tablets (Schiff Nutrition Group, Salt Lake City, UT), Sundown<sup>®</sup> selenium with vitamin C, brewer's yeast and citrus bioflavonoids tablets (Sundown, Inc., Boca Raton, FL), Maxilife<sup>®</sup> Selenomax<sup>®</sup> high selenium yeast capsules (Twin Lab, Inc., Ronkonkoma, NY), Spring Valley<sup>®</sup> high selenium yeast tablets (Leiner Health Products, Inc., Carson, CA), Fields of Nature<sup>®</sup> high selenium yeast tablets (Fields of Nature, Division of IVC Industries, Inc., Freehold, NJ) were bought from local retail stores. Each of the selenized yeast samples was stated to contain 200 µg selenium per tablet or capsule. Representative tablets were crushed into a fine powder with a pestle and mortar after carefully removing the titanium oxide coatings, if present. Powder from representative capsules were emptied into a glass vial and mixed.

Other selenium-containing supplements, which were either yeast-free or did not have stated yeast 'status', were analyzed to compare the results to those from yeast-based samples. Seleno-6<sup>™</sup> yeast-free organically bound selenomethionine tablets, 100 µg tablet<sup>-1</sup>, (Solgar Laboratories, Leonia, NJ) and Berkeley and Jensen Men's Premium Multivitamin with Minerals and Herbs, which contains vitamins [A, B1, B2, B6, B12, C, D, E, and K] and minerals including 100 µg selenium per tablet described as "selenium amino acid chelate", (BJWC, Natick, MA) were purchased from local retail stores. LifeExtension<sup>™</sup> Se-(methyl)selenocysteine, which contains 200 µg capsule<sup>-1</sup>, and LifeExtension<sup>™</sup> super selenium complex, which contains 300 IU vitamin E plus 200 µg selenium per capsule as 50 µg as selenomethionine, 50 µg as sodium selenate, 25 µg as selenodiglutathione and 75 µg as Se-(methyl)selenocysteine, were purchased from Quality Supplements and Vitamins, Inc. (Ft. Lauderdale, FL). Both of the LifeExtension<sup>™</sup> brand capsules were yeast-free. These samples were treated in the same way as the selenized yeast samples.

## 2.4. Analytical procedure

### 2.4.1. Thermal treatment

Sample powder was heated in a closed vial at 100 °C and 0.2 g aliquots were sampled after 1, 3 and 7 days. The aliquots were taken through the enzymatic digestion procedure described below and analyzed by HPLC-ICP-MS and GC-AED. Also, 10 mg selenomethionine, 10 mg selenomethionine and 20 mg L-cysteine mixture (1:2), and 10 mg selenomethionine and 20 mg DL-cystine mixture (1:2) were separately heated in closed vials at 150 °C for 24 h. The solid products were then dissolved in 0.1 M HCl and analyzed by HPLC-ICP-MS.

### 2.5. Headspace extraction for GC analysis

A solid-phase microextraction (SPME) fiber with polydimethylsiloxane (PDMS) coating (100 µm; non-bonded phase) (Supelco, Bellefonte, PA) was introduced into the headspace through the septum of the sealed vial and the volatile analytes were extracted for 20 min. Extracted analytes were then desorbed for a minute into the injection port of the gas chromatograph.

### 2.6. Enzymatic extraction for HPLC and GC

Enzymatic digestion using Protease XIV was as reported elsewhere [52,53] and is summarized below. About 0.2 g of powdered sample was added to 0.02 g of Protease XIV in 15-mL centrifuge tube with 5 mL of water and was shaken for 24 h at room temperature, centrifuged at 3,000 g for 20 min and filtered through a 0.45-µm polypropylene filter and a 10,000 Da molecular weight cut-off filter. For HPLC-ICP-MS analysis the clear filtrate (900 µL) was mixed with 100 µL of the ion-pairing reagent (concentrated HFBA) while the filtrate had to be derivatized for GC-AED analysis.

## 2.7. Derivatization for GC analysis

Selenoamino acid standards and extracts from selenium supplements were derivatized with ethylchloroformate (ECF) using procedures developed by Hušek [60] and reported by a number of workers [61–64]. Derivatized standard solutions (1000 ppm) were prepared by treating 100 µL of the 10,000 stock solution with 1 mL of water-ethanol-pyridine mixture (60:32:8, by volume) and 50 µL of ECF and the mixture shaken, venting off any evolved CO<sub>2</sub> gas. Then 1 mL of chloroform containing 1% ECF was added to extract the derivatives into the organic phase by shaking for 30 min on a Burrell Wrist Action<sup>™</sup> shaker (Burrell Scientific, Pittsburgh, PA) and the organic (lower) phase separated using a micropipette. The extraction into the organic phase was repeated two more times and the three portions combined and evaporated to dryness under a stream of N<sub>2</sub> gas. The residue was then redissolved in 1 mL of chloroform and 1 µL injected into the gas chromatograph.

Enzymatic hydrolysates from the selenized yeast samples were also derivatized with ethylchloroformate (ECF) for GC-AED analysis. The procedure for the standard solutions was scaled up for the selenized yeast extracts. To a 2-mL portion of the extract was added 5 mL of a water-ethanol-pyridine (60:32:8 by volume) followed by 1 mL of ECF. The mixture was shaken and any evolved CO<sub>2</sub> vented. The ethylated derivatives were extracted into the organic phase with 3 mL of chloroform containing 1% ECF by shaking for 30 min. The chloroform (organic) layer was separated; the extraction procedure repeated two more times and the three portions combined. The chloroform extract solution was evaporated to dryness with a flow of N<sub>2</sub> gas and the residue redissolved in hexane or chloroform. Samples of 1 µL volume were injected.

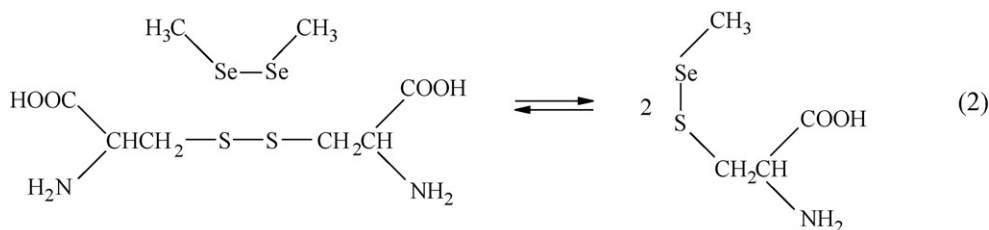
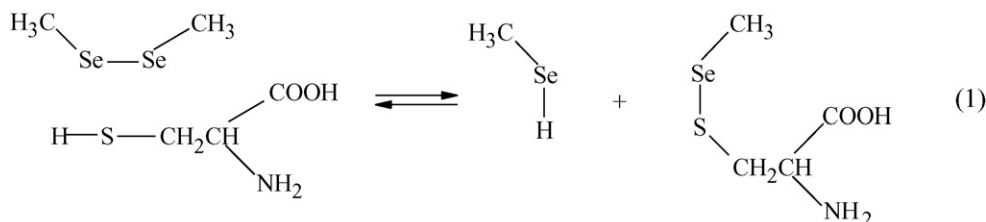
## 3. Results and discussion

The major organoselenium species in selenized yeast is selenomethionine [38–40]. In 2004, we reported [51] the presence of a sulfur-selenium amino acid, S-(methylseleno)cysteine, in archived selenized yeast samples from the Clark study [42]. Its presence was subsequently confirmed by GC-AED [52] and <sup>77</sup>Se NMR [59]. Gammelgaard et al. investigated the oxidative degradation of selenomethionine and followed the formation of selenomethionine Se-oxide [66]. The same group identified S-(methylseleno)cysteine in intestinal epithelial cell homogenates by LC-ICP-MS and LC-ESI-MS after incubation with methyl seleninic acid and showed that its formation did not require the presence of an enzymatic system but only cysteine [67]. They further identified Se-(methyl)selenocysteine and selenomethionine as metabolites of methylseleninic acid in rat hepatocytes and showed that S-(methylseleno)cysteine and S-(methylseleno)glutathione were intermediates in their formation [68].

Several research groups have investigated enzyme-catalyzed reactions as part of the sample preparation procedure for the extraction of Se species from yeast. It appears that such reactions are not entirely quantitative notably for SeCys. Dernovics and Lobinski pointed out the poor quality of analytical data concerning the presence and the concentration of SeCys in yeast [69]. They studied a well-defined mixture of SeCys-containing di- and tri-peptides, isolated from yeast, by derivatization and proteolytic digestion followed by 2D (size-exclusion followed by ion-pairing reversed-phase) HPLC-ICP-MS and ES-MS/MS. The SeCys-containing oligopeptides had a remarkable resistance to proteolytic digestion and quantitative liberation of SeCys was therefore not possible. This behavior is presumably reflected in that of cysteine itself and may be germane to the mechanisms postulated below regarding thermal treatment.

Since the selenized yeast incorporates selenomethionine as the major component, it could be inferred that S-(methylseleno)cysteine was formed from the degradation of selenomethionine. We have previously reported a similar speciation pattern on subjecting selenized yeast samples to brief heating at elevated temperature [52]. In the present study, selenium species in some commercially available selenium supplements are determined. Dimethyldiselenide has been reported as the principal volatile selenium species in the headspace of selenized yeast, probably resulting from the degradation of selenomethionine [58]. In the present study, two hypotheses for the formation of S-(methylseleno)cysteine are proposed:

- (i) a reaction between dimethyldiselenide, a degradation by-product of selenomethionine, and cysteine, which is naturally present in yeast, as shown without a proposed mechanism in reaction (1) and
- (ii) a reaction between dimethyldiselenide and cystine, the oxidation dimer of cysteine, as shown without mechanism in reaction (2)



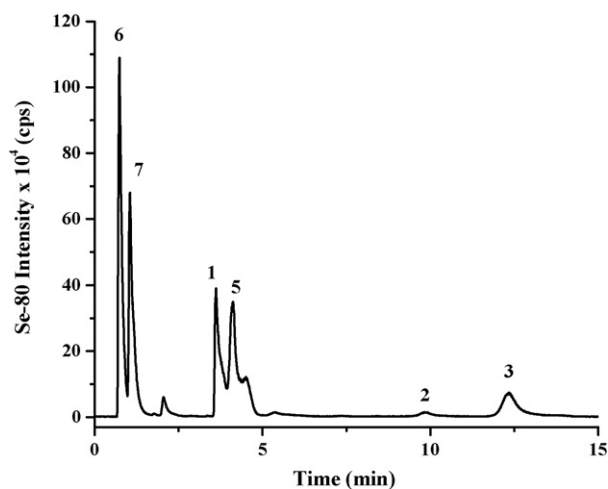
Samples of both yeast-based and yeast-free commercially available selenium supplements were subjected to brief heating. It is emphasized that the thermal treatments employed here are not designed to simulate any long-term stability studies of selenized yeast as have been reported earlier. However, since the products produced under brief thermal stress conditions are shown to correlate with those observed after lengthy storage, it is believed that the thermal treatment provides valuable information. Any volatile selenium species given off were sampled by solid-phase microextraction (SPME) and determined by gas chromatography with atomic emission detection (GC-AED) and GC-mass spectrometry (GC-MS). After enzymatic extraction of heated samples, selenium species were determined by HPLC-ICP-MS and by GC-AED. The speciation patterns were compared with respect to the formation of S-(methylseleno)cysteine. To determine the selenium species in supplements enzymatic hydrolysis, followed by extraction, was used to release more than 90% of the total selenium present [53]. The selenium species in the extract can then be determined by HPLC-ICP-MS, after ion-pairing, and GC-AED, after derivatization. The selenium species observed in this study are shown in Table 1. The derivatization procedure used converts both selenomethionine and selenomethionine selenoxide to the same derivative that appears on the GC-AED chromatograms as a single peak. Identification of selenium species was by retention time matching, but in the case of dimethyldiselenide, direct confirmation was obtained by GC-MS. Table 2 shows selenium species identified in commercially

**Table 1**  
Targeted selenium compounds.

Selenium compound	Molecular formula
1. Selenomethionine Se-oxide hydrate	$\text{CH}_3\text{Se}(\text{OH})_2\text{CH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
2. S-(methylseleno)cysteine	$\text{CH}_3\text{SeSCH}_2\text{CH}(\text{NH}_2)\text{COOH}$
3. Selenomethionine	$\text{CH}_3\text{SeCH}_2\text{CH}_2\text{CH}(\text{NH}_2)\text{COOH}$
4. Dimethyldiselenide	$(\text{CH}_3)_2\text{Se}_2$
5. Se-(methyl)selenocysteine	$\text{CH}_3\text{SeCH}_2\text{CH}(\text{NH}_2)\text{COOH}$
6. Selenate	$\text{SeO}_4^{2-}$
7. Selenite	$\text{SeO}_3^{2-}$
8. Selenodiglutathione	$\text{C}_{20}\text{H}_{32}\text{N}_6\text{O}_{12}\text{S}_2\text{Se}$

available selenium supplements before they were subjected to thermal treatment. Fig. 1 shows the HPLC-ICP-MS chromatogram of the Life Extension Super Selenium Complex capsules. Comparisons with results for selenium supplements utilizing these HPLC conditions [40,51,52] confirm the peak identifications and show a complex selenium speciation including inorganic and organic compounds. The former (selenate and selenite) are quantifiable since the both elute after the void volume and no other anionic selenium species have ever been observed under these conditions.

In contrast, the Berkeley and Jensen brand was found to contain only anionic selenium. The ambiguous label of selenium in this brand as "selenium amino acid chelate" could mislead consumers to believe that this tablet contains selenoaminoacids. Under the



**Fig. 1.** HPLC-ICP-MS chromatogram of the enzymatic extracts of the LifeExtension™ Super Selenium complex capsules. selenomethionine Se-oxide 1; S-(methylseleno)cysteine 2; selenomethionine 3; Se-(methyl)selenocysteine 5; Selenate 6; and selenite 7.

**Table 2**

Summary of selenium species identified in some commercially available selenium supplements by GC–AED and HPLC–ICP–MS.<sup>a</sup>

Brand name	Manufacturer's label	Species identified
SelenoExcell™	High selenium yeast	2, 3
Schiff	Selenized yeast	1 <sup>a</sup> , 2, 3
Sundown®	Selenium with vitamin C, brewer's yeast and citrus bioflavonoids	2, 3
Maxilife® Selenomax®	High yeast selenium	2, 3
Spring Valley®	High selenium yeast	2, 3
Fields of Nature®	High selenium yeast	2, 3
Seleno-6™	Yeast-free organically bound selenomethionine	1 <sup>a</sup> , 3
LifeExtension™	Se-(methyl)selenocysteine [yeast-free]	5
LifeExtension™	Selenomethionine, sodium selenate, selenodiglutathione, Se-(methyl)selenocysteine plus vitamin E [yeast-free]	1 <sup>a</sup> , 2, 3, 5, 7 <sup>a</sup>
Berkeley and Jensen	Selenium as amino acid chelate with vitamins and other minerals	6 <sup>a</sup> , 7 <sup>a</sup>

1 is selenomethionine Se-oxide; 2 is S-(methylseleno)cysteine; 3 is selenomethionine; 4 is dimethyldiselenide; 5 is Se-(methyl)selenocysteine; 6 is selenate; 7 is selenite.

<sup>a</sup> Species identified only by HPLC–ICP–MS.

**Table 3**

Peak area percent of selenium compounds in enzymatic extracts of Schiff high selenium yeast tablets by HPLC–ICP–MS.<sup>a</sup>

Selenium species	Freshly opened (n = 2)	Stored for 1 month <sup>b</sup>
Selenomethionine Se-O	25	46.46 ± 1.58
S-(methylseleno)cysteine	1	1.32 ± 0.02
Selenomethionine	72	49.23 ± 1.59
Sum	98	97
Unidentified	2	3

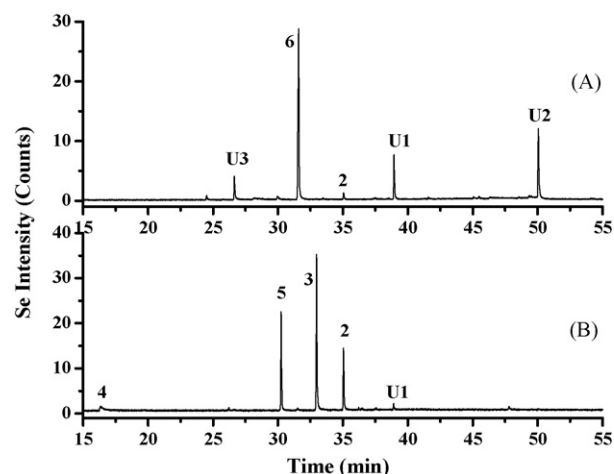
<sup>a</sup> The % selenium distributions (mean ± standard error) are expressed in terms of total selenium species eluting from the column.

<sup>b</sup> n = 3.

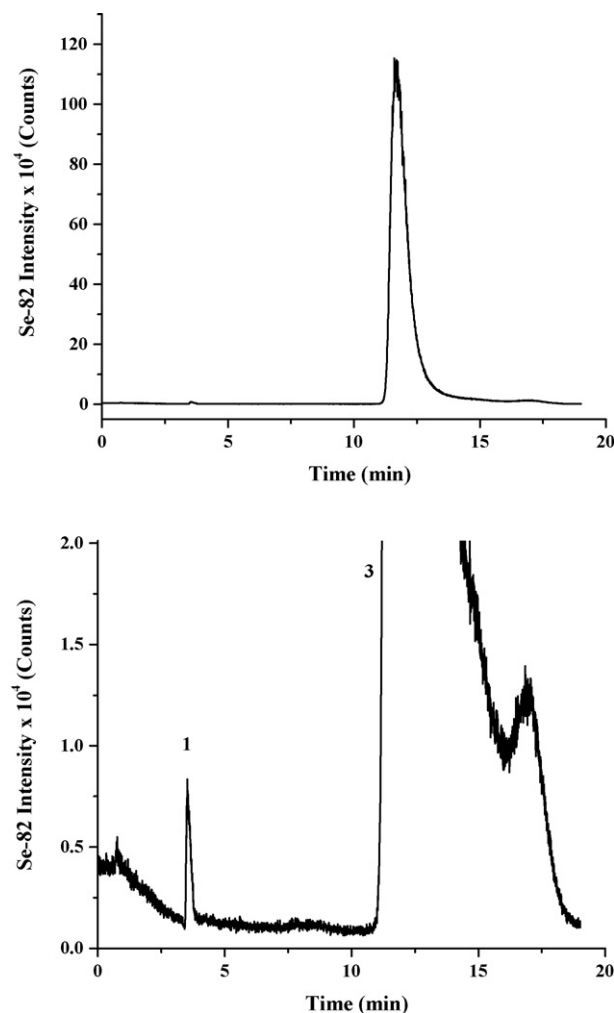
DSHEA, only manufacturers of dietary supplements are responsible for their correct labeling and safety [1,65]. This means that, unlike drugs, dietary supplements do not need the approval of the Food and Drug Administration (FDA) before entering the market, possibly exposing consumers to incorrect or ambiguous labeling.

All the selenium supplement samples, except for the Berkeley and Jensen brand, formed dimethyldiselenide in the headspace when heated at 100 °C for a day, as sampled by SPME and determined by GC–AED. The presence of dimethyldiselenide was also confirmed by SPME–GC–MS. In all the selenized yeast samples the amount of selenomethionine decreased relatively on heating over time and there was a relative increase in the amounts of S-(methylseleno)cysteine, and selenomethionine Se-oxide.

The increase in the amount of this sulfur–selenium amino acid could be explained as being formed while selenomethionine degrades, which is consistent with our hypotheses. Dimethyldiselenide, formed on degradation of selenomethionine would react with cysteine or cystine, naturally present in yeast, to form S-(methylseleno)cysteine. Alternately, dimethyldiselenide would react with the available thiol groups or disulfide links to form products that released S-(methylseleno)cysteine on hydrolysis. A similar speciation pattern has been reported on archived yeast samples and was observed in the Schiff brand selenized yeast tablets stored at room temperature as shown in Table 3 [52]. This is important to note because, while all the selenium supplement supplements had expiry dates on the bottles, none of them had a date of manufacture. Consumers have no way of telling how long the supplements have been on the shelf and the whether the pre-



**Fig. 2.** GC–AED chromatograms of the LifeExtension™ Super Selenium Complex capsules heated at 100 °C for 24 h showing (A) the sulfur trace and (B) the selenium trace. 2 is S-(methylseleno)cysteine; 3 is selenomethionine (including selenomethionine Se-oxide); 4 is dimethyldiselenide; 5 is Se-(methyl)selenocysteine; 6 is selenate; 7 is selenite; and U1, U2 and U3 are unidentified peaks.

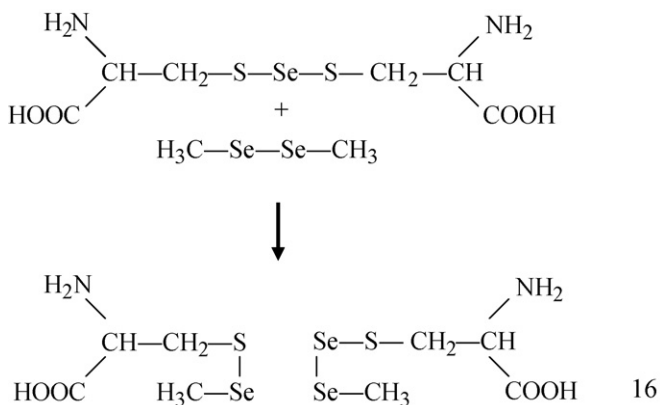


**Fig. 3.** HPLC–ICP–MS chromatograms of selenomethionine heated at 150 °C for 24 h, upper: full scale, lower: zoom. 1 is selenomethionine Se-oxide; 3 is selenomethionine.

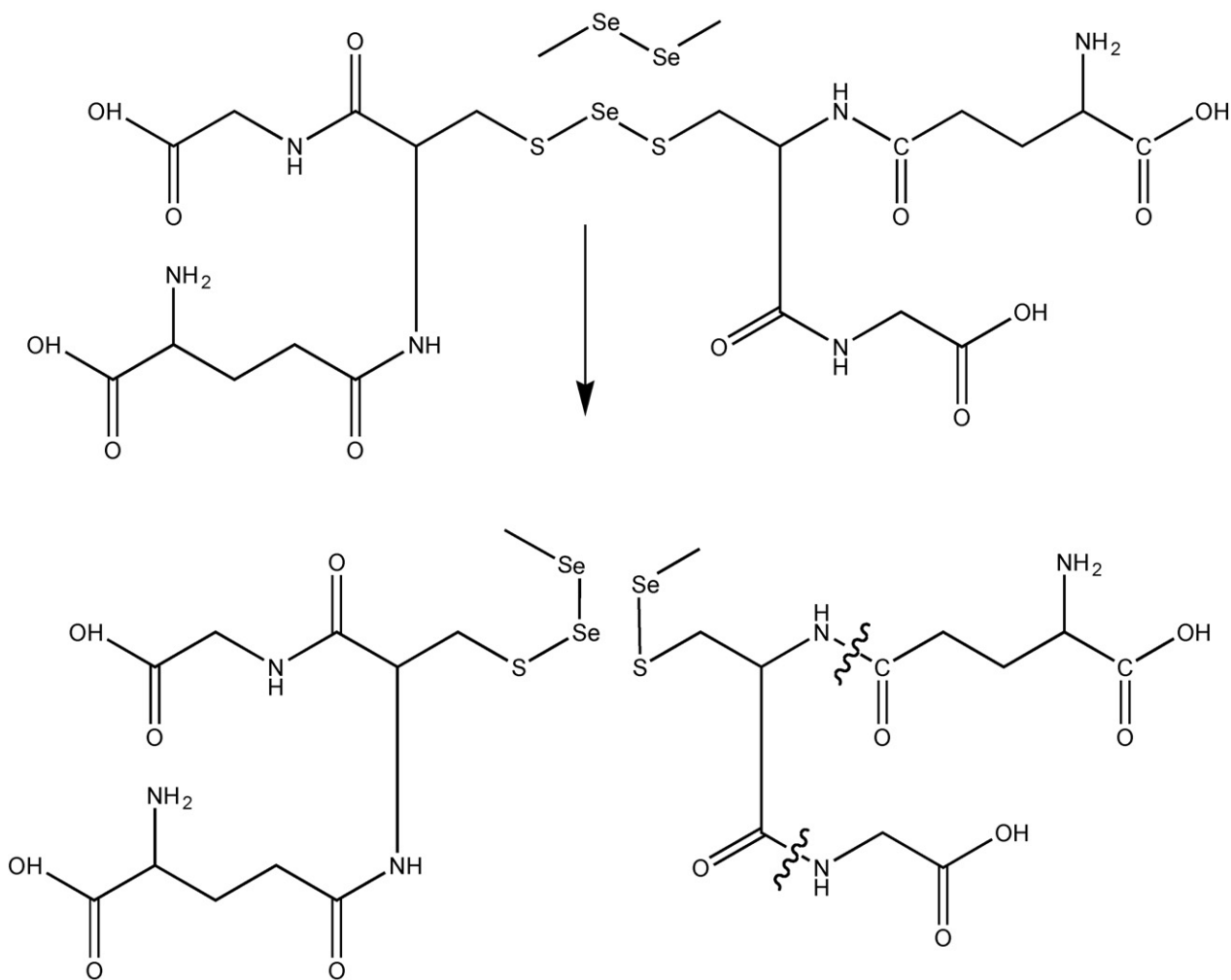
sumed active agent, selenomethionine has degraded to form other selenium species. No study has yet been made to evaluate the nutritional benefit or toxicity of *S*-(methylseleno)cysteine.

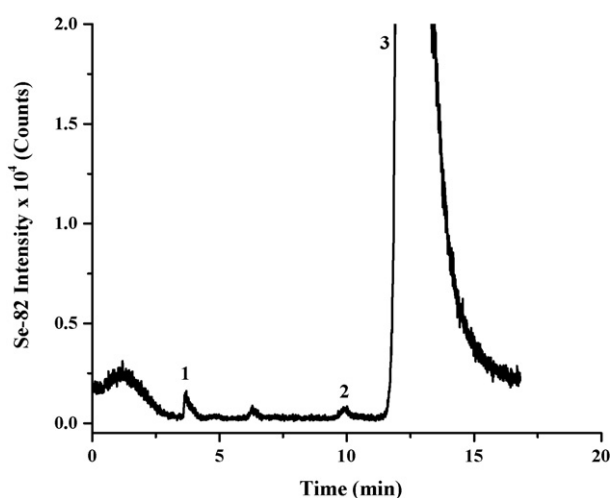
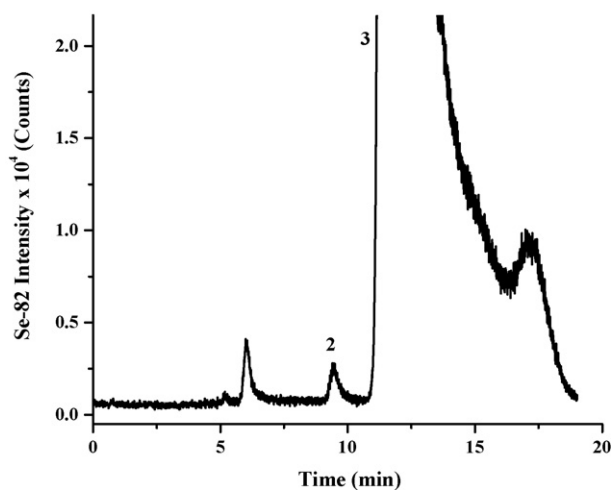
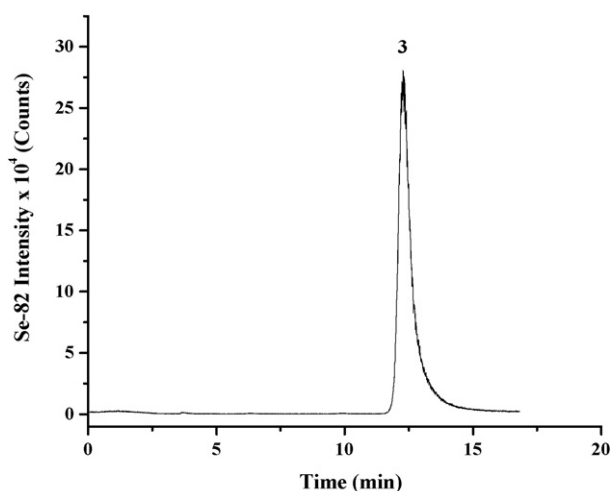
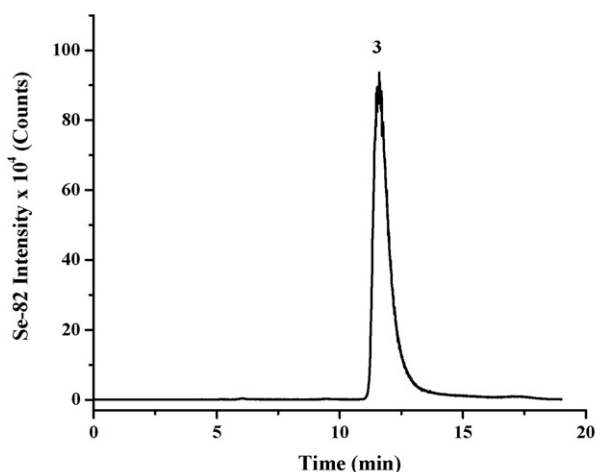
For the yeast-free supplements containing selenomethionine or *Se*-(methyl)selenocysteine, dimethyldiselenide was also formed in their headspace on heating at 100°C. However, *S*-(methylseleno)cysteine was found to form only in the Life Extension Super Selenium Complex as shown in Fig. 2. This was somewhat unexpected as this brand is yeast free and does not have a sulfur source from cysteine or cystine. However, it may be that selenodiglutathione, a bis-selenium adduct with two glutathione (a tripeptide of glycine, cysteine and  $\gamma$ -glutamic acid) molecules, which contains the *S*-*Se*-*S* linkage, is the only source of sulfur necessary for the formation of this *Se*-*S* amino acid in this yeast-free supplement. The scheme below shows one possible route to the formation of *S*-(methylseleno)cysteine. The upper part shows the reactants (selenodiglutathione and dimethyldiselenide). After reaction as shown to form the two products, hydrolysis of the amide bonds, as shown by the wavy lines, results in the formation of one molecule of *S*-(methylseleno)cysteine, one molecule of glycine and one molecule of  $\gamma$ -glutamic acid. It can be seen that the molecule containing the *-S*-*Se*-*Se*-*CH*<sub>3</sub> moiety could undergo a further reaction with dimethyldiselenide to form, after hydrolysis of the amide bonds, additional molecules of *S*-(methylseleno)cysteine, glycine and  $\gamma$ -glutamic acid, together with  $\text{CH}_3(\text{Se})_3\text{CH}_3$ .

Enzymatic hydrolysis of any unreacted selenodiglutathione would produce glycine,  $\gamma$ -glutamic acid and the dimeric selenodiglutathione, which retains the *S*-*Se*-*S* link. This could then react with dimethyldiselenide as shown below.



It also possible that the dimethyldiselenide reacts with molecules containing the *Se*-*Se*-*S* linkage to give a further molecule of *S*-(methylseleno)cysteine and  $\text{CH}_3(\text{Se})_3\text{CH}_3$ .





**Fig. 4.** HPLC-ICP-MS chromatograms of selenomethionine and L-cysteine mixture (1:2) heated at 150 °C for 24 h, upper: full scale, lower: zoom. 2 is S-(methylseleno)cysteine; and 3 is selenomethionine.

**Fig. 5.** HPLC-ICP-MS chromatograms of selenomethionine and cystine mixture (1:2) heated at 150 °C for 24 h, upper: full scale, lower: zoom. 1 is selenomethionine Se-oxide; 2 is S-(methylseleno)cysteine; and 3 is selenomethionine.

The GC-AED chromatograms of the derivatized extracts of the LifeExtension™ Super Selenium complex capsules in Fig. 2 show that U1 is a Se-S compound with a retention time of 38.9 min on both the selenium and sulfur traces. U1 may possibly have the structure (before derivatization) of  $\text{CH}_3\text{SSeSCH}_2\text{CH}(\text{COOH})\text{NH}_2$  based on the hypothesized reaction mechanism. However, the positive identification of U1 requires further study. No evidence of methylselenol was found although its volatility (b.p. ca. 10 °C) could preclude gas chromatographic retention.

To further test our hypotheses for the formation of S-(methylseleno)cysteine in selenized yeast, pure selenomethionine and selenomethionine-cysteine and selenomethionine-cystine mixtures were heated at 150 °C for 24 h in closed vials. No selenium species were determined in the headspace when pure selenomethionine was heated at 100 °C. This could be due to little or no degradation occurring in the pure selenomethionine on heating at 100 °C. However, dimethyldiselenide was observed to form in the headspace after heating at 150 °C for a day. As a result, 150 °C was selected as the temperature to heat the amino acid and their mixtures. This observation suggests that pure selenomethionine is more thermally stable than selenomethionine formed in selenized yeast. After heating the sample at 150 °C for 24 h, the solid products were dissolved in 0.1 M HCl and analyzed by HPLC-ICP-MS. Both pure selenomethionine and the mixtures had changed color from white to brown after heating, but only the pure selenomethionine heated was wholly soluble. The mixtures had some insoluble

particles that were filtered off through a 0.45- $\mu\text{m}$  filter prior to analysis. The insoluble particles may be degradation by-products of cysteine or cystine. Figs. 3–5 show that only the selenomethionine plus cysteine, and selenomethionine plus cystine mixtures formed S-(methylseleno)cysteine. This was predictable as only the mixtures had sources of sulfur (cysteine and cystine). This provides some evidence in support of our hypotheses for the formation of S-(methylseleno)cysteine.

#### 4. Conclusions

Selenium speciation and stability in dietary supplements are important because the nutritional benefit or toxicity of selenium depends in part on the selenium species ingested and this remains imperfectly characterized and broadly sample dependent. Consumers have no way of determining the selenium speciation of supplements and there is no indication on how long they have been on the shelf. From the present studies it may be surmised that further regulation should be enacted for the dietary supplement industry to protect and inform consumers. This is even most important to prevent ambiguous labeling of these products.

All the selenized yeast samples examined formed S-(methylseleno)cysteine on heating over time. On the contrary, the LifeExtension™ Super Selenium Complex capsules, which



contains selenodiglutathione as one of selenium species, was the only yeast-free supplement that formed S-(methylseleno)cysteine on heating. We see evidence in these thermal studies to support our hypotheses that yeast-based selenomethionine and Se-(methyl)selenocysteine degrade to form dimethyldiselenide, which reacts with cysteine or cystine, if present, to form S-(methylseleno)cysteine. The proposed reaction mechanism for this formation, we believe, is similar to that which takes place in archived yeast samples. In the absence of cysteine or cystine, as in yeast-free selenium supplements, selenodiglutathione may react with dimethyldiselenide to form S-(methylseleno)cysteine.

There have been two schools of thought as to which is a more effective anticancer agent, selenomethionine or Se-(methyl)selenocysteine [70]. In the present study, both selenomethionine and Se-(methyl)selenocysteine were shown to degrade on heating to form dimethyldiselenide, which could mean that both selenium species follow a similar metabolic pathway in the human body.

## Acknowledgement

We thank the Procter and Gamble Company for support.

## References

- [1] US Public Law 103-417, 103rd Congress, October 25, 1994, Dietary Supplement Health and Education Act of 1994.
- [2] E.A. Yetley, Multivitamin and multimineral dietary supplements: definitions, characterization, bioavailability, and drug interactions, *Am. J. Clin. Nutr.* 85 (2007) 269S–276S.
- [3] J.Y. Woo, Adverse event monitoring and multivitamin–multimineral dietary supplements, *Am. J. Clin. Nutr.* 85 (2007) 323S–324S.
- [4] K. Randimer, B. Bindewald, J. Hughes, B. Ervin, C. Swanson, M.F. Picciano, Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999–2000, *Am. J. Epidemiol.* 160 (2004) 339–349.
- [5] NIH State-of-the-Science Panel, National Institutes of Health state-of-the-science conference statement: multivitamin/mineral supplements and chronic disease prevention, *Ann. Intern. Med.* 145 (2006) 364–371.
- [6] T. Ferri, G. Favero, M. Francesconi, Selenium speciation in foods: preliminary results on potatoes, *Microchem. J.* 85 (2007) 222–227.
- [7] J.E. Spallholz, L.M. Boylan, M.M. Rhaman, Environmental hypothesis: is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India? *Sci. Total Environ.* 323 (2004) 21–32.
- [8] L. Letavayová, V. Vlčková, J. Brozmannová, Selenium: from cancer prevention to DNA damage, *Toxicology* 227 (2006) 1–14.
- [9] G. Ravn-Haren, S. Bügel, B.N. Krath, T. Hoac, J. Stagsted, K. Jørgensen, J.R. Bresson, E.H. Larsen, L.O. Dragsted, A short-term intervention trial with selenate, selenium-enriched yeast and selenium-enriched milk: effects on oxidative defence regulation, *Br. J. Nutr.* 99 (2008) 883–892.
- [10] G. Flores-Mateo, A. Navas-Acien, R. Pastor-Barruso, E. Guallar, Selenium and coronary heart disease: a meta-analysis, *Am. J. Clin. Nutr.* 84 (2006) 762–763.
- [11] I. Jaspers, W. Zhang, L.E. Brighton, J.L. Carson, M. Styblo, M.A. Beck, Selenium deficiency alters epithelial cell morphology and responses to influenza, *Free Rad. Biol. Med.* 42 (2007) 1826–1837.
- [12] T.S. Nawrot, J.A. Staessen, H.A. Roels, E.D. Hond, L. Thijs, R.H. Fagard, A.F. Dominiczak, H.A. Struijker-Boudier, Blood pressure and blood selenium: a cross-sectional and longitudinal population study, *Eur. Heart J.* 28 (2007) 628–633.
- [13] R. Ebert, F. Jakob, Selenium deficiency as a putative risk factor for osteoporosis, *Int. Cong. Ser.* 1297 (2007) 158–164.
- [14] M.R. Ashrafi, S. Shams, M. Nouri, M. Mohseni, R. Shabani, M.S. Yekaninejad, N. Chegini, A. Khodadad, R. Safaralizadeh, A probable causative factor for an old problem: selenium and glutathione peroxidase appear to play important roles in epilepsy pathogenesis, *Epilepsia* 48 (2007) 1750–1755.
- [15] S.L. Volpe, J.L. Schall, P.R. Gallagher, V.A. Stallings, A.G.C. Bergqvist, Nutrient intake of children with intractable epilepsy compared with health children, *J. Am. Diet. Assoc.* 107 (2007) 1014–1018.
- [16] A. Campa, G. Shor-Posner, M. Baum, Selenium status and the human immunodeficiency virus, *J. Am. Diet. Assoc.* 100 (2000) 418–418.
- [17] J.D. Bogden, J.M. Oleske, The essential trace minerals, immunity, and progression of HIV-1 infection, *Nutr. Res.* 27 (2007) 69–77.
- [18] I.T. Ozgen, A. Dagdemir, M. Elli, R. Saraymen, F.G. Pinarli, T. Fisgin, D. Albayrak, S. Acar, Hair selenium status in children with leukemia and lymphoma, *J. Pediatr. Hematol. Oncol.* 29 (2007) 519–522.
- [19] C. Ip, Lessons from basic research in selenium and cancer prevention, *J. Nutr.* 128 (1998) 1845–1854.
- [20] U. Peters, C.B. Foster, N. Chatterjee, A. Schatzkin, D. Reding, G.L. Andriole, E.D. Crawford, S. Sturup, S.J. Chanock, R.B. Hayes, Serum selenium and risk of prostate cancer—a nested case-control study, *Am. J. Clin. Nutr.* 85 (2007) 209–217.
- [21] S.B. Goldhaber, Trace element risk assessment: essentiality vs. toxicity, *Regul. Toxicol. Pharmacol.* 38 (2003) 232–242.
- [22] Institute of Medicine, Selenium, in: *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*, National Academy Press, Washington, DC, 2000, pp. 284–324.
- [23] S. McSheehy, L. Yang, R. Sturgeon, Z. Mester, Determination of methionine and selenomethionine in selenium-enriched yeast by species-specific isotope dilution with liquid chromatography-mass spectrometry and inductively coupled plasma mass spectrometry detection, *Anal. Chem.* 77 (2005) 344–349.
- [24] Y.F. Li, C. Chen, B. Li, Q. Wang, J. Wang, Y. Gao, Y. Zhao, Z. Chai, Simultaneous speciation of selenium and mercury in human urine samples from long-term mercury-exposed populations with supplementation of selenium-enriched yeast by HPLC-ICP-MS, *J. Anal. Atom. Spectrom.* 22 (2007) 925–930.
- [25] Z. Pedrero, D. Elvira, C. Cámara, Y. Madrid, Selenium transformation studies during broccoli (*Brassica oleracea*) growing process by liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS), *Anal. Chim. Acta* 596 (2007) 251–256.
- [26] M.W. Farnham, A.J. Hale, M.A. Grusak, J.W. Finley, Genotypic and environmental effects on selenium concentration of broccoli heads grown without supplemental selenium fertilizer, *Plant Breeding* 126 (2007) 195–200.
- [27] Y. Dong, D. Lisk, E. Block, C. Ip, Characterization of the biological activity of  $\gamma$ -glutamyl-Se-methylselenocysteine: a novel, naturally occurring anticancer agent from garlic, *Cancer Res.* 61 (2001) 2923–2928.
- [28] Y. Ogra, K. Ishiwata, Y. Iwashita, K.T. Suzuki, Simultaneous speciation of selenium and sulfur species in selenized odorless garlic (*Allium sativum* L. Shiro) and shallot (*Allium ascalonicum*) by HPLC-inductively coupled plasma-(octopole reaction system)-mass spectrometry and electrospray ionisation–tandem mass spectrometry, *J. Chromatogr. A* 1093 (2005) 118–125.
- [29] E. Kápolna, P. Fodor, Bioavailability of selenium from selenium-enriched green onions (*Allium fistulosum*) and chives (*Allium schoenoprasum*) after 'in vitro' gastrointestinal digestion, *Int. J. Food Sci. Nutr.* 58 (2007) 282–296.
- [30] M. Shah, J. Mejjia, J.A. Caruso, Relative mass defect filtering of high-resolution mass spectra for exploring minor selenium volatiles in selenium-enriched green onions, *Anal. Chem.* 79 (2007) 846–853.
- [31] J. Xu, F. Yang, X. An, Q. Hu, Anticarcinogenic activity of selenium-enriched green tea extracts in vivo, *J. Agric. Food Chem.* 55 (2007) 5349–5353.
- [32] F. Yu, J. Sheng, J. Xu, X. An, Q. Hu, Antioxidant activities of crude tea polyphenols, polysaccharides and proteins of selenium-enriched tea and regular green tea, *Eur. Food Res. Technol.* 225 (2007) 843–848.
- [33] Y. Ogra, K. Ishiwata, J.R. Encinar, R. Lobinski, K.T. Suzuki, Speciation of selenium in selenium-enriched shiitake mushroom, *Lentinula edodes*, *Anal. Bioanal. Chem.* 379 (2004) 861–866.
- [34] V.D. Huerta, M.L.F. Sanchez, A. Sanz-Medel, An attempt to differentiate HPLC-ICP-MS selenium speciation in natural and selenized Agaricus mushrooms using different species extraction procedures, *Anal. Bioanal. Chem.* 384 (2007) 902–907.
- [35] J.S. Landaluz, C. Dietz, Y. Madrid, C. Cámara, 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, *Appl. Organomet. Chem.* 18 (2004) 606–613.
- [36] C.A. Ponce de León, M.M. Bayón, C. Paquin, J.A. Caruso, Selenium incorporation into *Saccharomyces cerevisiae* cells: a study of different incorporation methods, *J. Appl. Microbiol.* 92 (2002) 602–610.
- [37] J.R. Dainty, T.E. Fox, Modeling in nutrition. The metabolism of selenium, copper, zinc and calcium using stable isotopes in humans, in: R. Cornelis (Ed.), *Handbook of Elemental Speciation II: Species in the Environment, Food, Medicine & Occupational Health*, Wiley & Sons, Ltd., Chichester, England, 2005, pp. 690–710.
- [38] M. Kotrebai, M. Birringer, J.F. Tyson, E. Block, P.C. Uden, Identification of the principal selenium compounds in selenium-enriched natural sample extracts by ion-pair liquid chromatography with inductively coupled plasma- and electrospray ionization-mass spectrometric detection, *Anal. Commun.* 36 (1999) 249–252.
- [39] B. İsciöğlü, E. Henden, Determination of selenoamino acids by gas chromatography-mass spectrometry, *Anal. Chim. Acta* 505 (2004) 101–106.
- [40] M. Kotrebai, M. Birringer, J.F. Tyson, E. Block, P.C. Uden, Selenium speciation in enriched and natural samples by HPLC-ICP-MS and HPLC-ESI-MS with perfluorinated carboxylic acid ion-pairing agents, *Analyst* 125 (2000) 71–78.
- [41] L. Ouerdane, Z. Mester, Production and characterization of fully selenomethionine-labeled *Saccharomyces cerevisiae*, *J. Agric. Food Chem.* 56 (2008) 11792–11799.
- [42] L.C. Clark, G.F. Combs, B.W. Turnbull, E.H. Slate, D.K. Chalker, J. Chow, L.S. Davis, R.A. Glover, G.F. Graham, E.G. Gross, A. Krongrad, J.L. Lesher, H.K. Park, B.B. Sanders, C.L. Smith, J.R. Taylor, Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin, *J. Am. Med. Assoc.* 276 (1996) 1957–1963.
- [43] SELECT Home Page, <http://www.crab.org/select/> (accessed 24.04.08).
- [44] E.A. Klein, S.M. Lippman, I.M. Thompson, P.J. Goodman, D. Albanes, P.R. Taylor, C. Coltman, The selenium and vitamin E cancer prevention trial, *World J. Urol.* 21 (2003) 21–27.
- [45] S.M. Lippman, P.J. Goodman, E.A. Klein, H.L. Parnes, I.M. Thompson, A.R. Kristal, R.M. Santella, J.L. Probstfield, C.M. Moinpour, D. Albanes, P.R. Taylor, L.M.

- Minasian, A. Hoque, S.M. Thomas, J.J. Crowley, J.M. Gaziano, J.L. Stanford, E.D. Cook, N.E. Fleshner, M.M. Lieber, P.J. Walther, F.R. Khuri, D.D. Karp, G.G. Schwartz, L.G. Ford, C.A. Coltman, Designing the selenium and vitamin E cancer prevention trial (SELECT), *J. Natl. Cancer Inst.* 97 (2005) 94–102.
- [46] P. Greenwald, D. Anderson, S.A. Nelson, P.R. Taylor, Clinical trials of vitamin and mineral supplements for cancer prevention, *Am. J. Clin. Nutr.* 85 (2007) 314S–317S.
- [47] M.P. Rayman, The importance of selenium to human health, *Lancet* 356 (2000) 233–241.
- [48] E.H. Larsen, M. Hansen, H. Paulin, S. Moesgaard, M. Reid, M. Rayman, Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies, *J. AOAC Int.* 87 (2004) 225–232.
- [49] E.H. Larsen, R. Lobinski, K. Burger-Meÿer, M. Hansen, R. Ruzik, L. Mazurowska, P.H. Rasmussen, J.J. Sloth, O. Scholten, C. Kik, Uptake and speciation of selenium in garlic cultivated in soil amended with symbiotic fungi (mycorrhiza) and selenate, *Anal. Bioanal. Chem.* 385 (2006) 1098–1108.
- [50] S.M. Lippman, E.A. Klein, P.J. Goodman, et al., Effect of selenium and vitamin E on risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT), *JAMA* 301 (2009) 39–51.
- [51] P.C. Uden, H.T. Boakye, C. Kahakachchi, R. Hafezi, P. Nolibos, E. Block, S. Johnson, J.F. Tyson, Element selective characterization of stability and reactivity of selenium species in selenized yeast, *J. Anal. Atom. Spectrom.* 19 (2004) 65–73.
- [52] P.O. Amoako, C.L. Kahakachchi, E.N. Dodova, P.C. Uden, J.F. Tyson, Speciation, quantification and stability of selenomethionine, S-(methylseleno)cysteine and selenomethionine Se-oxide in yeast-based nutritional supplements, *J. Anal. Atom. Spectrom.* 22 (2007) 938–946.
- [53] M.L. Cooper, H.O. Adami, H. Gronberg, F. Wiklund, M.P. Rayman, Interaction between nucleotide polymorphisms in selenoprotein P and mitochondrial superoxide dismutase determines prostate cancer risk, *Cancer Res.* 64 (2008) 10171–10177.
- [54] M.P. Rayman, Selenoproteins and human health: insights from epidemiological data. *Biochem. Biophys. Acta* 2009 Mar 25 [Epub ahead of print].
- [55] M.P. Rayman, G.F. Combs Jr, D.J. Waters, Response to reference 50, Letter to JAMA, March 2009 (personal communication).
- [56] J. Bleys, A. Navas-Acien, E. Guallar, Serum selenium and diabetes in U.S. adults, *Diabetes Care* 30 (2007) 829–834.
- [57] S. Stranges, J.R. Marshall, R. Natarajan, R.P. Donahue, M. Trevisan, G.F. Combs, F.P. Cappuccio, A. Ceriello, M.E. Reid, Effects of long-term selenium supplementation on the incidence of type 2 diabetes, *Ann. Intern. Med.* 147 (2007) 217–223.
- [58] C. Dietz, J.S. Landaluze, P. Ximénez-Embún, Y. Madrid-Albarrán, C. Cámara, SPME-multicapillary GC coupled to different detection systems and applied to volatile organoselenium speciation in yeast, *J. Anal. Atom. Spectrom.* 19 (2004) 260–266.
- [59] E. Block, R.S. Glass, N.E. Jacobsen, S. Johnson, C. Kahakachchi, R. Kamiński, A. Skowrońska, H.T. Boakye, J.F. Tyson, P.C. Uden, Identification and synthesis of a novel selenium-sulfur amino acid found in selenized yeast: rapid indirect detection NMR methods for characterizing low-level organoselenium compounds in complex matrices, *J. Agric. Food Chem.* 52 (2004) 3761–3771.
- [60] P. Hušek, Rapid derivatization and gas chromatographic determination of amino acids, *J. Chromatogr.* 552 (1991) 289–299.
- [61] G. Haberhauer-Troyer, G. Álvarez-Llamas, E. Zitting, P. Rodríguez-González, E. Rosenberg, A. Sanz-Medel, Comparison of different chloroformates for the derivatization of selenoamino acids for gas chromatographic analysis, *J. Chromatogr. A* 1015 (2003) 1–10.
- [62] Y.C. Fiamegos, C.G. Nanos, C.D. Stalikas, Ultrasonic assisted derivatization reaction of amino acids prior to their determination in urine by using single-drop microextraction in conjunction with gas chromatography, *J. Chromatogr. B* 813 (2004) 89–94.
- [63] P. Hušek, P. Simek, Alkyl chloroformates in sample derivatization strategies for GC analysis. Review on a decade use of the reagents as esterifying agents, *Curr. Pharm. Anal.* 2 (2006) 23–43.
- [64] F.R. Zaggout, S.J. Gaskel, J.S. Hajjaj, Identification of ethylchloroformate derivatives of amino acids and hydrolysis of myoglobin and small peptides using gas chromatography-mass spectrometry, *Asian J. Chem.* 18 (2006) 1366–1376.
- [65] Overview of Dietary Supplements, US Food and Drug Administration Center for Food Safety and Applied Nutrition, 2001, <http://www.cfsan.fda.gov/~dms/dsoview.html#regulate> (accessed 12.05.08).
- [66] B. Gammelgaard, C. Cornet, J. Olsen, L. Bendahl, S.H. Hansen, Combination of LC-ICP-MS, LC-MS and NMR for investigation of the oxidative degradation of selenomethionine, *Talanta* 59 (2003) 1165–1171.
- [67] C. Gabel-Jensen, K. Lunoe, K.G. Madsen, J. Bendix, C. Cornett, S. Sturup, H.R. Hansen, B. Gammelgaard, Separation and identification of the selenium-sulfur amino acid S-(methylseleno)cysteine in intestinal epithelial cell homogenates by LC-ICP-MS and LC-ESI-MS after incubation with methylseleninic acid, *J. Anal. Atom. Spectrom.* 23 (2008) 727–732.
- [68] C. Gabel-Jensen, J. Odgaard, C. Skonberg, L. Badolo, B. Gammelgaard, LC-ICP-MS and LC-ESI-MS<sup>n</sup> identification of Se-methylselenocysteine and selenomethionine as metabolites of methylseleninic acid in rat hepatocytes, *J. Anal. Atom. Spectrom.* 24 (2009) 69–75.
- [69] M. Dermovics, R. Lobinski, Characterization of the selenocysteine-containing metabolome in selenium-rich yeast, *J. Anal. Atom. Spectrom.* 23 (2008) 744–751.
- [70] H.G. Infante, G. O'Connor, M. Rayman, R. Wahlen, J. Entwisle, P. Norris, R. Hearn, T. Catterick, Selenium speciation analysis of selenium-enriched supplements by HPLC with ultrasonic nebulization ICP-MS and electrospray MS/MS detection, *J. Anal. Atom. Spectrom.* 19 (2004) 1529–1538.