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Analytical methodology

Characterization of selenium speciation in selenium-enriched button mushrooms (*Agaricus bisporus*) and selenized yeasts (dietary supplement) using x-ray absorption near-edge structure (XANES) spectroscopy

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ABSTRACT

Selenium is an essential trace element for which dietary intake is not sufficient in many parts of the world such as in Europe. Yeast and mushrooms may accumulate considerable amounts of selenium, but the chemical form in mushrooms has not been elucidated yet. Thus, we determined the selenium speciation of selenium-enriched button mushrooms in comparison to that of selenized yeast via Se K-edge XANES spectroscopy. Quantitative analysis of the XANES spectra revealed that the selenium in selenized yeast is mainly present as seleno-methionine but that in selenium-enriched button mushrooms, it is present predominantly as *Se*-methyl-L-seleno-cysteine. As this form is highly bioavailable and directly enters the selenium metabolic pool, selenium-enriched mushrooms may be a good food choice to improve selenium intake.

1. Introduction

Dietary selenium intake is currently insufficient in up to 1 billion people all over the world. The prevalence of selenium deficiency is expected to further increase due to increasing selenium losses from soil [1]. This situation is critical because selenium acts as cofactor of many enzymes (e.g., glutathione peroxidases, deiodases) and thus plays a key role in protection from free radicals as well as in the regulation of growth, development and metabolism [2]. Selenium deprivation is associated with impaired antioxidant protection and with disturbances in cellular differentiation and immune responses, which might favor oxidative stress, infections and chronic diseases such as cancer [3,4]. Meta-analyses of epidemiological studies have revealed that a sufficient intake of selenium may protect against breast, lung and prostate cancer [5] as well as cardiovascular diseases [6].

Foods from animal origin such as meat and fish are the major dietary sources of selenium. Most plant foods are comparably low in selenium except for vegetables of the *Allium* and *Brassicaceae* family such as onions and broccoli, which may accumulate considerable amounts of selenium [7]. Interestingly, mushrooms cultivated on a selenium-fortified substrate may also accumulate large amounts of selenium in the form of organic selenium or as inorganic selenite. However, only a few selenium-containing compounds in mushrooms have been identified so far [8].

Dietary supplements such as selenized yeast, produced by fermenting *Saccharomyces cerevisiae* in selenium-rich media, are considered to be a possible source of organic food-form selenium, e.g., Amoako et al. [9]. Virtually all of the selenium in yeast occurs as structurally related substitutes for sulfur in the amino acid methionine, thus forming seleno-methionine via the same pathways and enzymes

Abbreviations: XANES, X-ray absorption near-edge spectroscopy

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that are used to form sulfur-containing methionine. Owing to its similarity to S-containing methionine, seleno-methionine becomes incorporated into yeast proteins [10].

Mushrooms are of great interest as a dietary source of selenium, but the exact chemical speciation of organic selenium in selenium-enriched mushrooms has not been clarified yet. Total selenium content in mushrooms and yeasts can be easily determined by gas chomatography (GC), atomic absorption spectrometry (AAS) or hydride generation–atomic fluorescence spectroscopy (AFS) (e.g., [11–14]. Furthermore, selenium speciation in selenium-enriched mushrooms and/or selenized yeasts were recently characterized by high pressure liquid chromatography- inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) [9,15,16], LC-ESI-MS [14], and by size-exclusion chromatography-inductively coupled plasma-mass spectrometry (SEC-ICP-MS) [17–19]. Investigations with liquid chromatography-electrospray ionization-mass spectrometry have shown—in contrast to investigations by HPLC—that organic selenium in caps and stalks of selenized *Agaricus bisporus* predominantly occurs as selenocysteine [14].

However, all of these methods applied so far have one main disadvantage: selenium speciation cannot be investigated *in situ* on the basis on natural samples. Therefore, chemical changes in the selenium speciation during preparation and extraction (such as by heating) cannot be ruled out. For example, Bird et al. [15] found more than 20 selenium-containing species (also very “unlikely” selenium compounds) in hot water and enzymatic hydrolysis extracts of selenized yeast using HPLC–ICP-MS (trifluoroacetic acid was used as an ion-pairing agent in a water–methanol phase). Knowing the exact chemical form of selenium and how selenium is included in the organic matter is crucial for its bioavailability and usage in metabolism [10], and thus, nondestructive analytical methods are necessary to characterize selenium speciation in selenium-enriched mushrooms and selenized yeasts.

X-ray absorption near-edge structure (XANES) spectroscopy using synchrotron radiation is the method of choice to characterize selenium speciation with knowledge of its speciation important for understanding its metabolism and biological significance [20] on the basis of native samples. This method elucidates the electronic and geometric structure of the environment of the absorbing (here: selenium) atoms, e.g., whether the valence of the excited atom and the electronegativity of neighboring atoms or coordination geometry around the excited atom are also in higher coordination shells. The information about the actual chemical binding of an element can often be deduced by comparing the XANES spectrum of the sample of interest with the spectra of suitable model (reference) compounds (‘fingerprint’ analysis). Furthermore, often, a quantitative analysis of spectra by linear combination fitting using suitable reference compounds can be performed, leading to a quantitative description of a single elemental species within the speciation (e.g., [21,22,23]). XANES spectroscopy at the selenium Kedge using synchrotron radiation has been successfully used to determine the speciation of selenium in different areas of biological and agricultural research (e.g., [24,25,26]). More details on the application of XANES to investigate biological significance *in situ* as well as a description of the advantages of XANES spectroscopy are shown by Prange and Modrow [27].

This study focused on two questions: (1) What is the exact chemical speciation of selenium in selenium-enriched button mushrooms *in situ*? (2) Are there any differences in selenium speciation between the microfungi (yeasts) and the macrofungi (mushrooms) when cultivated on selenite-containing substrate/medium? Does selenium in selenized yeasts and selenium-enriched mushrooms only occur as organic selenium or additionally as an inorganic selenium species?

2. Materials and methods

2.1. Preparation of mushroom and yeast samples

White button mushrooms (*Agaricus bisporus*) grown on a fortified breeding ground containing 10 mg/kg of selenium saline (selenite) were freeze-dried after harvest. The freeze-dried button mushroom samples contained total selenium between 0.35–2.86 mg/kg as determined by using a Jobin-Yvon ICP-OES instrument. Selenized yeast (Spring Valley™ Selenium) were purchased in Baton Rouge, LA, from a grocery store (Walmart), and Se K-edge XANES spectra were measured in fluorescence mode using the material as received. The selenized yeast contained 200 mcg (µg) selenium/tablet with the following additional ingredients: brewer’s yeast, cellulose, croscarmellose, vegetable stearic acid, silica and vegetable magnesium stearate (specification according to the supplier’s data sheet).

Non-selenium-enriched freeze-dried button mushrooms (*Agaricus bisporus*) were used as a negative control. For XANES spectroscopy measurements, the freeze-dried samples were ground finely and put on a self-adhesive Kapton® film (type 7010) purchased from CWC Klebetechnik (Frankenthal, Germany) and used for measurements in fluorescence mode (see below).

2.2. XANES-spectroscopy experimental

XANES spectra were recorded at the INE beamline using synchrotron radiation of the Angströmquelle Karlsruhe (ANKA) synchrotron of the Karlsruhe Institute of Technology (KIT), Karlsruhe, Germany. The storage ring was operated at 2.5 GeV with electron currents between 200 and 150 mA. The synchrotron radiation was monochromatized by a modified Lemonnier-type double-crystal monochromator [28] that was equipped with Ge (422) crystals. The monochromatic flux rate per second was 2×10^{10} photons (at 150 mA). The measurements were carried out in fluorescence mode for the reference and mushroom/yeast samples using a 5 element LEGe (Canberra-Eurisys) detector. Furthermore, for comparison reasons, the reference samples were additionally measured in standard transmission mode with ionization chambers (under argon). Further details on the INE beamline and on the experimental setup were published previously [29]. The spectra were scanned with step widths of 0.5 eV between 12,620 and 12,720 eV and with an integration time of 3 s per data point. XANES data were normalized and analyzed using the ATHENA program of the IFFEFIT package [30].

For energy calibration of the spectra, the Se Kedge XANES spectrum of the gray elemental selenium was used as a ‘secondary standard’ setting for the maximum of the first peak of the spectrum (‘white line’) to an energy of 12,658 eV. According to the step width, this value is reproducible to ± 1 eV.

2.3. Reference compounds

Potassium selenate, potassium selenite and selenourea were purchased from Alfa Aesar (Karlsruhe, Germany); seleno-DL-methionine, glutamyl-Se-methyl-L-selenocysteine, *Se*-methyl-L-selenocysteine, and methylselenic acid were purchased from PharmaSe Inc. (Lubbock, TX, USA); and L-selenocysteine and selenium (grey selenium; metal) were purchased from Sigma-Aldrich (Taufkirchen, Germany). All reference compounds were purchased in the highest purity available (A.C.S. or reagent grade). The samples were ground into a fine powder and put on a self-adhesive Kapton® film (type 7010) purchased from CWC Klebetechnik (Frankenthal, Germany). The thickness and homogeneity of the samples were optimized to avoid possible self-absorption (fluorescence mode) and possible thickness- as well as pinhole-effects (transmission mode).

2.4. Quantitative analysis

Spectra of the mushroom and yeast samples were recorded three times in fluorescence mode using the 5-element detector (see above), yielding 15 spectra for each sample. For subsequent quantitative analysis using linear combination fitting (LCF), the 15 spectra were averaged/merged, normalized and analyzed using the ATHENA program of

Table 1
Results of the least-square fitting of the selenium K-edge spectra of selenium-enriched botton mushrooms and selenized yeast with 3 reference spectra. The error of the numbers given in the table is ≤ ± 10%. (cf. Materials and Methods and (21)).

Percentage contribution of selenium speciation			
Sample	*Se*-(methyl-L-seleno)cysteine	seleno-DL-methionine	potassium selenite
Selenium-enriched *Agaricus bisporus*	55 (53)[a]	35 (36)[a]	10 (11)[a]
Selenized yeast *Saccharomyces cerevisiae*	18 (10)[a]	75 (75)[a]	7 (15)[a]

[a] Results for 1st derivative as control are shown in parentheses.

the IFFEFIT package [30]. The least square fitting process was carried out in 3 steps. In a first step for both samples (the selenized yeast and the selenium-enriched mushrooms), the best reference spectra (fluorescence vs. transmission) for selenomethionine and the best reference compounds/spectra for selenocysteine were determined. In a second step, sample spectra were fitted using all combinations of 3 from 5 best reference spectra (selenite and 2 spectra each for selenocysteine and selenomethionine). For both samples, best fits were obtained with the same set of 3 reference compounds: Se-(methyl-L-seleno)cysteine, seleno-DL-methionine and potassium selenite. The corresponding results are summarized in Table 1, and the corresponding spectra of the samples and the best fits are shown in Fig. 2. In many cases the 1st derivatives show a more pronounced fine structure than the original absorption spectra and are therefore very sensitive for quantitative analyses using a least square fitting process. Hence, for checking the consistency of the original fitting process, in a third step, the 1st derivatives of the sample spectra were fitted using the corresponding derivative spectra of the reference compounds. The 1st derivatives of the sample spectra were fitted similar to the absorption spectra by using again all combinations of 3 from the derivative spectra of the 5 reference spectra used for fitting the absorption spectra. The results of this process agree quite well with those obtained by fitting the original spectra (best fits obtained by the same 3 reference compounds!) and they are also included in Table 1. The results of this fitting agreed quite well with those obtained by fitting the original spectra, and they are also included in Table 1.

Based on various quantitative analyses carried out by the authors (e.g. also as “blind testing” experiments of defined mixtures of sulfur compounds) the error of the percentage contributions for the compounds in the linear combination fitting (Table 1) can be roughly estimated to ± 10% (according to Prange et al. [21] and refences therein).

3. Results and discussion

The aim of the present study was to investigate the exact chemical speciation of selenium in selenized yeast and in selenium-enriched mushrooms. To the best of our knowledge, this is the first application of XANES to selenium-enriched button mushrooms to investigate possible differences in the chemical form in which selenium is incorporated in yeast (grown with selenite in broth by fermentation) versus that in mushrooms (cultivated on selenite-containing substrate). For this analysis, a broad variety of reference compounds for selenium (different species) were measured and their respective relevance tested (Fig. 1). In general, these compounds are used only as representatives for a given class of an atomic environment of the selenium atom. For example, gray selenium is used as indicator for the presence of selenium (0) in general, although it was intuitively evident that the metal, grey selenium, was definitely not present in the samples under investigation. In Fig. 2, the

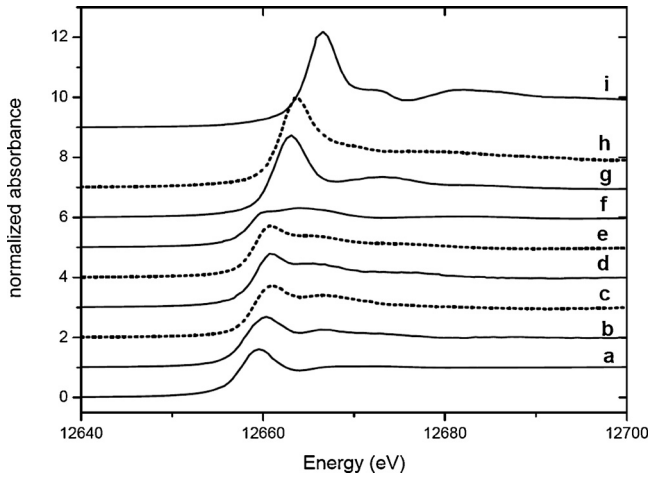

Fig. 1. Selenium K-edge XANES spectra of different reference compounds: (a) elemental selenium (gray), (b) L-selenocysteine, (c) *Se*-methyl-L-selenocysteine, (d) glutamyl-*Se*-methyl-L-selenocysteine, (e) seleno-DL-methionine, (f) selenourea, (g) methylselenic acid (h) potassium selenite and (i) potassium selenate. (a.u., arbitrary units).

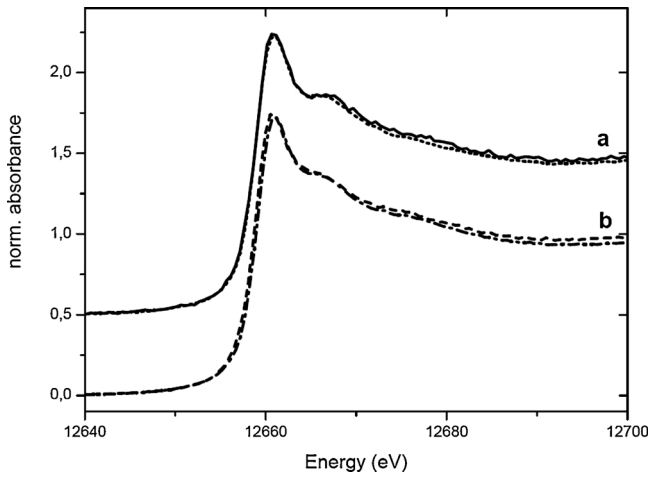

Fig. 2. Selenium K-edge XANES spectra of (a) selenium-enriched *Agaricus bisporus* (solid line) and corresponding ATHENA fits (dotted line), (b) selenized yeast (*Saccharomyces cerevisiae*, Spring Valley™) (dashed line), and corresponding ATHENA fit (dashed-dotted line)). (a.u., arbitrary units.).

Se K-edge XANES spectra of selenized yeast (a) and selenium-enriched mushrooms, grown on selenite (b), are shown together with their accompanying ATHENA fits. The spectra of the samples showed a maximum intensity at 12,663 eV, corresponding to organic selenium. Therefore, upon uptake of selenite, selenium was mainly incorporated into the organic molecules in the form of seleno-amino acids; a minimal selection of reference compounds (seleno-DL-methionine, *Se*-methyl-L-selenocysteine, selenite) was sufficient to fit the spectra (Table 1). Interestingly, the ratio of different seleno-amino acids in selenized yeasts compared to that of selenium-enriched mushrooms was significantly different. *in situ,* XANES revealed two different speciations (Table 1): selenium in selenized yeast was mainly present as seleno-methionine, in selenium-enriched button mushrooms it predominantly occurred as *Se*-methylseleno)cysteine, which supports the results by Maseko et al. [14] and contradicts the prior results obtained by HPLC [15]. It has been shown *in situ* that button mushrooms take up selenite from the substrate and incorporate it into *Se*-(methylseleno)cysteine, which is the selenium form with the highest bioavailability [20]. Furthermore, according to Whanger et al. [31] and Dong et al. [32], *Se*-methyl-L-selenocysteine and γ-glutamyl-*Se*-(methylseleno)cysteine are the most

effective anticancer selenium agents. From this point of view, the speciation of selenium in selenium-enriched mushrooms is superior to seleno-methionine provided by selenized yeasts and superior to dietary supplements containing inorganic selenium.

Both yeasts and button mushrooms were freeze-dried, but they had a completely different speciation. A change in speciation of selenium by freeze-drying might be assumed. However, Moreno et al. [33] have evaluated and shown the stability of selenium species in freeze-dried biological samples oysters over a period of 12 months. Therefore, the speciation results presented here for the freeze-dried samples can be assumed to correspond to native samples. In the yeast as well as in the button mushrooms, a small amount of selenite was also detectable (close to the error limit), which might be a result of "unincorporated" selenite taken up during growth. A further oxidation to selenite or reduction of selenite to elemental selenium as well as any other inorganic form of selenium was not observed when comparing the results (especially regarding the positions of the white lines indicating the "formal" oxidation state) with the reference compounds (Fig. 1), as has also been observed for different bacteria and molds [24]. In contrast, button mushrooms cultivated on substrate (not enriched with selenite) and yeasts (*Saccharomyces cerevisiae*) grown without selenite used as negative controls led to a very weak and noisy spectra when investigated by Se K-XANES (data not shown), indicating that selenium was only present in trace amounts.

According to the European Food Safety Authority (EFSA), adults can achieve an adequate selenium supply by an intake of 70 µg selenium per day. This dose ensures levelling off of the plasma selenoprotein P (SePP) concentration, which reflects saturation of the functional selenium body pool, thus indicating the fulfilment of the selenium requirement [34]. The German, Austrian and Swiss nutrition societies recommend a daily selenium intake of 60 µg for women and of 70 µg for men to obtain saturation of SePP, taking into account sex-specific differences in reference body weight [35]. The Institute of Medicine (USA) [36] considers 55 µg of selenium per day to be adequate to meet selenium requirements, based on the dose that is necessary to maximize glutathione peroxidase activity. However, this biomarker is less valuable than SePP is to define the selenium requirement [34]. While the average selenium intake by adults in the United States is 109 µg per day, it ranges from 31.0 to 65.6 µg/day in European countries, thus leading to much higher serum selenium concentrations in the United States (Maryland: 1.69 to 2.15 µmol/l; South Dakota 2.17 to 2.50 µmol/l) compared to those in Europe (0.66 to 1.69 µmol/l) [36]. A single portion (200 g) of selenium-enriched button mushrooms would provide approximately 20 µg of selenium, an amount that would considerably contribute to closing the gap between existing and recommended selenium intake in selenium-deficient regions, without exceeding the tolerable upper intake level for adults set at 300 µg/d [34] and 400 µg/d [36]. Seleno-methionine, which is ingested from selenized yeast, is highly bioavailable, but it is covalently bound in methionine-containing proteins and has to be catabolized to make selenium available to the metabolic pool. In contrast, selenocysteine enters directly into the selenium metabolic pool and is incorporated in selenoproteins with catalytic function [37].

Although *Se*-methyl-L-selenocysteine is an excellent source for selenium supplementation and is the best bioavailable form, it is actually not allowed as a dietary supplement in the EU (in contrast to the US). Since 2009, the European Union has released a series of decrees with a list of selenium compounds allowed to be used, which includes inorganic selenium forms, selenized yeast, L-selenomethionine and its hydroxy analog. *Se*-methyl-L-selenocysteine, which occurs in the selenium-enriched button mushroom, is not listed, although this chemical form of selenium is a good and healthy alternative (instead of dietary supplements) for human selenium supplementation on the basis of an enriched food (mushrooms). Therefore, it can be suggested that the EU list should be amended to include *Se*-methyl-L-selenocysteine.

In conclusion, button mushrooms cultivated on selenium-fortified substrate provided organic selenium in the form of *Se*-methyl-L-selenocysteine, which directly enters the metabolic selenium pool, in contrast to seleno-methionine ingested by yeast. Thus, button mushrooms present a valuable selenium source and is an interesting organic alternative to seleno-methionine from the dietary supplement of selenium enriched yeast.

Conflict of interest

The authors declare that there are no conflict of interest.

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References

[1] G.D. Jones, B. Droz, P. Greve, P. Gottschalk, D. Poffet, S.P. McGrath, S.I. Seneviratne, P. Smith, L.H. Winkel, Selenium deficiency risk predicted to increase under future climate change, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) 2848–2853.
[2] M. Kieliszek, S. Błażejak, Current knowledge on the importance of selenium in food for living organisms: a review, Molecules 21 (2016) 609.
[3] G.F. Combs, Selenium in global food systems, Br. J. Nutr. 85 (2001) 517–554.
[4] H. Steinbrenner, B. Speckmann, L.O. Klotz, Selenoproteins: antioxidant selenoenzymes and beyond, Arch. Biochem. Biophys. 595 (2016) 113–119.
[5] X. Cai, C. Wang, W. Yu, W. Fan, S. Wang, N. Shen, P. Wu, X. Li, F. Wang, Selenium exposure and cancer risk: an updated meta-analysis and meta-regression, Sci. Rep. 6 (2016) 19213.
[6] X. Zhang, C. Liu, J. Guo, Y. Song, Selenium status and cardiovascular diseases: meta-analysis of prospective observational studies and randomized controlled trials, Eur. J. Clin. Nutr. 70 (2016) 162–169.
[7] M. Roman, P. Jitaru, C. Barbante, Selenium biochemistry and its role for human health, Metallomics 6 (2014) 25–54.
[8] J. Falandysz, Selenium in edible mushrooms, J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev 26 (2008) 256–299.
[9] P.O. Amoako, P.C. Uden, J.F. Tyson, Speciation of selenium dietary supplements; formation of S-(methylseleno)cysteine and other selenium compounds, Anal. Chim. Acta 652 (2009) 315–323.
[10] G.N. Schrauzer, Selenium and selenium-antagonistic elements in nutritional cancer prevention, Crit. Rev. Biotechnol. 29 (2009) 10–17.
[11] T. Stijve, Selenium content of mushooms, Z. Lebensm. Unters.-Forsch. 164 (1977) 201–203.
[12] J.P. Diaz-Alarcon, M. Navarro-Alarcon, H. Lopez-Garcia de la Serrana, M.C. Lopez-Martinez, Determination of selenium levels in vegetables and fruits by hybride generation atomic absorption spectrometry, J. Agric. Food Chem. 42 (1994) 2848–2851.
[13] M. Tuzen, E. Sesli, M. Soylak, Trace element levels of mushroom species from East Black Sea region of Turkey, Food Control 18 (2007) 806–810.
[14] T. Maseko, D.L. Callahan, F.R. Dunshea, A. Doronila, S.D. Kolev, K. Ng, Chemical characterization and speciation of organic selenium in cultivated selenium-enriched Agaricus bisporus, Food Chem. 141 (2013) 3681–3687.
[15] S.M. Bird, P.C. Uden, J.F. Tyson, E. Block, E. Denoyer, Speciation of selenoamino acids and organoselenium compounds in selenium-enriched yeast using high-performance liquid chromatography-inductively coupled plasma mass spectrometry, J. Anal. Atom. Spectr. 12 (1997) 785–788.
[16] V. Díaz Huerta, M.L. Fernández Sánchez, A. Sanz-Medel, An attempt to differentiate HPLC-ICP-MS selenium speciation in natural and selenised *Agaricus* mushrooms using different species extraction procedures, Anal. Bioanal. Chem. 384 (2006) 902–907.
[17] V. Gergely, K.M. Kubachka, S. Mounicou, P. Fodor, J.A. Caruso, Selenium speciation in *Agaricus bisporus* and *Lentinula edodes* mushroom proteins using multi-dimensional chromatography coupled to inductively coupled plasma mass spectrometry, J. Chromatogr. A 1101 (2006) 94–102.
[18] P. Bhatia, F. Aureli, M. D'Amato, R. Prakash, S.S. Cameotra, T.P. Nagaraja, F. Cubadda, Selenium bioaccessibility and speciation in biofortified *Pleurotus* mushrooms grown on selenium-rich agricultural residues, Food Chem. 140 (2013) 225–230.
[19] Y. Liu, C. Li, X. Luo, G. Han, S. Xu, F. Niu, X. Hu, H. Wu, H. Zhang, Characterization

of selenium-enriched mycelia of *Catathelasma ventricosum* and their anti-hyperglycemic and antioxidant properties, J. Agric. Food Chem. 63 (2015) 562–568.
[20] C.M. Weekley, H.H. Harris, Which form is that? The importance of selenium speciation and metabolism in the prevention and treatment of disease, Chem. Soc. Rev. 42 (2013) 8870–8894.
[21] A. Prange, R. Chauvistré, H. Modrow, J. Hormes, H.G. Trüper, C. Dahl, Quantitative speciation of sulfur in bacterial sulfur globules: X-ray absorption spectroscopy reveals at least three different species of sulfur, Microbiology-UK 148 (2002) 267–276.
[22] A. Prange, B. Birzele, J. Krämer, H. Modrow, R. Chauvistré, J. Hormes, P. Köhler, Characterization of sulfur speciation in low molecular weight subunits of glutenin after reoxidation with potassium iodate and potassium bromate at different pH values using X-ray absorption near-edge structure (XANES) spectroscopy, J. Agric. Food Chem. 51 (2003) 7431–7438.
[23] U. Zanzen, G.L. Bovenkamp-Langlois, W. Klysubun, J. Hormes, A. Prange, The interaction of interaction of cooper ions with *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*—an X-ray absorption near edge structure (XANES) spectroscopy study, Arch. Microbiol. 200 (2018) 401–412.
[24] A. Prange, B. Birzele, J. Hormes, H. Modrow, Investigation of different human pathogenic and food contaminating bacteria and moulds grown on selenite/selenate and tellurite/tellurate by X-ray absorption spectroscopy, Food Control 16 (2005) 723–728.
[25] D. Thavajarah, A. Vanderberg, G.N. George, I.J. Pickering, Chemical form of selenium in naturally selenium-rich lentils (*Lens culinaris* L.) from Sasketchewan, J. Agric. Food Chem. 55 (2007) 7337–7341.
[26] N.V. Dolgova, S. Nehzati, S. Choudhury, T.C. MacDonald, N.R. Regnier, A.M. Crawford, O. Ponomarenko, G.N. George, I.J. Pickering, X-ray spectroscopy and imaging of selenium in living systems, Biochim. Biophys. Acta (2018) in press.
[27] A. Prange, H. Modrow, X-ray absorption spectroscopy and its application in biological, agricultural and environmental research, Rev. Environ. Sci. Biotechnol. 1 (2002) 259–276.
[28] M. Lemonnier, O. Collet, C. Depautex, J.-M. Esteva, D. Raoux, High vacuum two crystal soft X-ray monochromator, Nucl. Instr. Meth. 152 (1978) 109–111.
[29] J. Rothe, S. Butorin, K. Dardenne, M.A. Denecke, B. Kienzler, M. Löble, V. Metz, A. Seibert, M. Steppert, T. Vitova, C. Walther, H. Geckeis, The INE beamline for actinide science at, ANKA. Rev. Sci. Instrum. 83 (2012) 043105-1-13.
[30] B. Ravel, M. Newville, ATHENA, ARTEMIS, HEPHAESTUS: data analysis for xray absorption spectroscopy using IFEFFIT, J. Synch. Radic. 12 (2005) 537–541.
[31] P.D. Whanger, C. Ip, C.E. Poland, P.C. Uden, G. Welbaum, Tumorigenesis, metabolism, speciation, bioavailability, and tissue deposition of selenium in selenium enriched ramps (*Allium tricoccum*), J. Agric. Food Chem. 48 (2000) 5723–5730.
[32] Y. Dong, D. Lisk, E. Block, C. Ip, Characterization of the biological activity of γ-glutamyl-Se-methylselenocysteine: a novel, naturally occurring anticancer agent from garlic, Cancer Res. 61 (2001) 2923–2928.
[33] P. Moreno, M.A. Quijano, A.M. Gutiérrez, M.C. Pérez-Conde, C. Cámara, Stability of total selenium and selenium species in lyophilised oysters and in their enzymatic extracts, Anal. Bioanal. Chem. 374 (2002) 466–476.
[34] European Food Safety Authority. Scientific opinion on dietary reference values for selenium, EFSA J. 12 (2014) 3846.
[35] A.P. Kipp, D. Strohm, R. Brigelius-Flohé, L. Schomburg, A. Bechthold, E. Leschik-Bonnet, H. Heseker, German Nutrition Society (DGE). Revised reference values for selenium intake, J. Trace Elem. Med. Biol. 32 (2015) 195–199.
[36] Institute of Medicine (USA), Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids, The National Academies Press, Washington, DC, 2000.
[37] R.A. Sunde, Selenium, et al., C.A. Ross (Ed.), Modern Nutrition in Health and Disease, 11th ed., Lippincott Williams & Wilkens, Philadelphia, 2014.