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Evaluation of candidate reference material obtained from selenium-enriched sprouts for the purpose of selenium speciation analysis

Małgorzata Bodnar*, Piotr Konieczka

Department of Analytical Chemistry, Chemical Faculty, Gdansk University of Technology, 11/12 Gabriela Narutowicza Street, Gdańsk 80-233, Poland

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ABSTRACT

Selenium is an essential element in animals and humans. Diet supplementation with selenium-rich vegetables has better chemoprotective effect than Se (IV) and SeMet. The production of selenium-enriched vegetables requires a preliminary stage in soil preparation, which may subsequently pose a risk of soil contamination. It seems that the sprouting of seeds under controlled conditions would be a good solution for delivering SeMetSeCys to the human organism. The certified reference material is necessary in order to follow the safety rules during the application of selenium-enriched foods in cancer prevention. Such reference material will contribute to the development of research methods for determining specific species of selenium. Therefore, we undertook an effort to produce the appropriate reference material for Se speciation analysis by using Se-enriched sprouts. Six types of sprouts, mainly from the family Brassicaceae, were selected for the study. The sprouts grown in both media accumulate significant amounts of selenium. However, only the plants cultivated in the medium containing Na_2SeO_3 are capable of converting inorganic selenium into SeMetSeCys and SeMet. The sprouts of broccoli and white mustard grown in the medium containing 10 mg/L Na_2SeO_3 were selected as potential candidates for certified reference materials.

1. Introduction

Seeking in-depth knowledge about the composition of plants and animals is a trend in analytical chemistry. Such knowledge enables the production of healthy food and influences the production processes of nutritionally enhanced, health improving food products (Wierzbicka, Bulska, Pyrzyńska, Wysocka, & Zachara, 2007). The undertaken efforts are not only aimed at determining the total content of a given element, but also at identifying its species. This, in turn, broadens the knowledge about the metabolism of elements in plants and animals, including their bioavailability. In the case of selenium, it is crucial to know which form of the element is present in a given product due to the narrow range between the harmful dose (>400 μg/day) and recommended dietary allowance (55 μg/day) (Bendich, 2001). The advantageous properties of selenium depend on its chemical form, as follows:

- SeMet is recognized as the most assimilable form of selenium. Also, the results of a study on supplementing diet with Se-enriched yeast (mainly containing SeMet) showed a significant decrease in the prevalence of prostate cancer among the study participants (Germ & Stibilj, 2007).
- SeCys is most frequently found in selenium-containing proteins such as, glutathione peroxidase (displaying antioxidant properties), iodothyronine deiodinase and thioredoxin reductase (the latter regulates the proper functioning of thyroid). It is noteworthy that until now 25 selenium-containing proteins have been discovered in the human organism (Germ & Stibilj, 2007).
- The third important organoselenium compound is SeMetSeCys, which possibly has chemopreventive properties (Abdulah, Miyazaki, Nakazawa, & Koyama, 2005; Medina, Thompson, Garther, & Ip, 2001; Cao, Durrani, & Rustum, 2004; Yeo et al., 2002; Lee et al., 2006).

List of abbreviation: ANOVA, analysis of variance; CRM, certified reference material; CV, coefficient of variation; DW, dry weight; GF-AAS, graphite furnace atomic absorption spectrometry; HPLC, high performance liquid chromatography; ICP MS, inductively coupled plasma Mass spectrometry; Met, methionine; PE, polyethylene; SeCys, selenocysteine; SeMet, selenomethionine; SeMetSeCys, selenomethylselenocysteine; ɣ-glutamyl-MetSeCys, ɣ-glutamyl-selenomethylselenocysteine; SeMetO, Selenomethionine-Se-oxide; QA/QC, quality assurance/quality control; $tRNA^{Met}$, methionine transfer RNA.

* Corresponding author.
E-mail address: bodnar.gosiaa@gmail.com (M. Bodnar).

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Selenium is an essential inorganic component in animals and humans, which offers health benefits (as described in (Bodnar, Konieczka, & Namieśnik, 2012)), including protection against cancer. It has been confirmed that selenium protects against different types of cancer, e.g. bowel (Finley & Davis, 2001; Kune & Watson, 2006; Finley, Davis, & Feng, 2000), breast (Unni, Koul, Young, & Sinha, 2005), skin (Clark et al., 1996), prostate (Combs, 2004) and lung cancer (Zhuo, Smith, & Steinmaus, 2004; Li et al., 2006). Recent studies on the relationship between the consumption of selenium and cancer prevention were mainly focused on the chemical form of selenium, Se-enriched foods, and *in vitro* effect of monomethylated organoselenium (Ip, Thompson, Zhu, & Garther, 2000). Many of these studies were based on the application of dietary selenite or selenomethionine. The obtained results demonstrated that selenite has stronger chemoprotective effect than SeMet (Wachowicz, Żbikowska, & Nowak, 2001). Other investigations were aimed at showing that dietary supplementation with Se-enriched vegetables such as garlic and broccoli has stronger chemoprotective effect than selenite and SeMet (Finley & Davis, 2001). The aforementioned vegetables accumulate inorganic species of selenium and then convert them into organic forms, mainly SeMetSeCys.

Contrary to SeMet, SeMetSeCys is a non-proteinogenic amino acid characterized by low accumulation in the human body. SeMetSeCys is one of the main precursors of volatile methylselenol (CH_3SeH), an active form of selenium, which is responsible for a decrease in the number of some cancer cases. In the human body, SeMetSeCys is directly metabolized to methylselenol by the action of β-lyase. This distinguishes SeMetSeCys from other Se-containing compounds (including selenomethionine) which have to undergo a number of metabolic steps in order to reach the volatile Se form (a detailed description of Se metabolism in plants is given in (Bodnar et al., 2012)). SeMetSeCys mainly occurs in Se-enriched plants from the genera *Allium* and *Brassica*, e.g. broccoli, onion, garlic and mustard greens as well as plants from the genus *Astragalus* known as Se-hyperaccumulators (Ellis & Salt, 2003).

Cultivation of Se-enriched plants can be an effective way of producing Se-rich foods and thus of increasing the advantages associated with the consumption of such plants (Zhu, Pilon-Smiths, Zhao, Williams & Mehrangm 2009). However, the production of Se-enriched vegetables (e.g. broccoli, garlic and onion) requires the preliminary soil preparation which involves soil enrichment with inorganic salts of selenium. Additional fertilization of soil may lead to its contamination and, in turn, to the transfer of harmful compounds to the food chain (Sugihara et al., 2004). Additionally, in the case of boiled Se-enriched vegetables, the important selenium compounds are transformed into other organoselenium compounds and selenate, while SeMetSeCys can be mainly found in the water in which they were boiled. Therefore, in order to deliver SeMetSeCys to the human body, vegetables should be consumed uncooked (e.g. in salads), or as a soup (Pedrero, Elvira, Camara, & Madrid, 2007).

Considering all the conclusions from the worldwide research studies, germinating plants in selenium-rich medium under controlled conditions is a good way of supplying SeMetSeCys to the human body (Abdulah et al., 2009; Avila et al., 2013; Pedrero et al., 2007). Sprouts not only contain a significant amount of vitamins and minerals, but also do not need to be prepared (boiled or baked) prior to consumption. Therefore, the probability of volatilization of some health-enhancing selenium compounds, particularly the methylated species, is low.

For this reason, an effort was undertaken to evaluate the candidate reference material for the purpose of selenium speciation analysis by using selenium-enriched sprouts. Most of available reference materials are only certified for the total selenium content. Therefore it seems important to select substances which would possibly fulfill the requirements posed for candidate reference materials with regard to stability, homogeneity, and biological and chemical resistance. Until now, only two Se-enriched products and isotopically enriched selenomethionine material are used in the selenium speciation analysis and selenomethionine determination (EVISA website). These three materials (see Table 1), which are available under the trade names SELM-1 (Mester et al., 2006), ERM-BC210a (ERM_certificate) and LGC-7330 (LGCStandards website).

The aim of this study was to produce reference materials for the Se speciation analysis (particularly SeMetSeCys determination) by using selenium-enriched sprouts. It should be underlined that this is a pioneering effort, and its positive result will facilitate the development of analytical methods for the determination of selenium as well as broaden the knowledge about the beneficial influence of SeMetSeCys on living organisms.

2. Materials and methods

2.1. Chemicals

Chemicals and reagents were of analytical grade. Solutions were prepared with deionized water from a Milli-Q water purification system unit (Merck Millipore, Darmstadt, Germany) in the case of total selenium determination or were used without further purification in the case of selenium speciation. Sprout seeds were purchased from BIOS (Pszczyna, Poland) or PNOS (Ożarów Mazowiecki, Poland). Solutions for sprouts germination were prepared by dissolving sodium selenite (Sigma–Aldrich, Munich, Germany) in tap water. Multiwave acid digestion of samples was carried out with HNO_3 (Suprapur, 65%, Merck, Darmstadt, Germany). For total Se determination samples were diluted in deionized water from a Milli-Q. For matrix modification $Pd(NO_3)_2 + Mg(NO_3)_2$ in 1% HNO_3 (SCP Science, Marktobendorf, Germany) were used.

Stock standard solutions of selenomethionine (SeMet), selenomethylselenocysteine (SeMetSeCys), γ-glutamyl-seleno-methylselenocysteine (γ-glutamyl-MetSeCys) purchased from Sigma–Aldrich (Munich, Germany) were prepared by dissolving each in hydrogen peroxide (30%, POCh, Gliwice, Poland). Sodium selenite (Na_2SeO_3) solution was purchased from Merck (Darmstadt, Germany). Selenomethionine-Se-oxide (SeMetO) was achieved by adding excess of oxidizing agent, 2.5 ml of H_2O_2 to 25 ml aliquot of a 0.1 mol/L HCl solution of selenomethionine (50 mg/L Se) and left overnight in the dark.

Enzymatic hydrolysis was performed by using a nonspecific enzyme, protease type XIV from *Streptomyces griseus* and lipase type VII from *Candida rugosa* purchased from Sigma–Aldrich (Munich, Germany). Tris–HCl solution was prepared with Trizma hydrochloride base (Sigma–Aldrich, Munich, Germany) dissolved in water, and the required pH was adjusted with 37% HCl. Selenium species separation by anionic-exchange chromatography was

Table 1
Certified reference materials for total selenium and selenomethionine determination [Mester et al., 2006; ERM_certificate; LGCStandards website].

CRM name	Matrix	Certified value[a]	Producer
ERM – BC210a	Wheat flour	Se: 17.23 ± 0.91 SeMet: 27.4 ± 2.6 [mg/kg DW]	LGC
SELM-1	Se-enriched yeast	Se: 2059 ± 64 SeMet: 3448 ± 146 Met: 5758 ± 277 [mg/kg]	NRC
LGC-7330	^{76}Se-enriched selenium	99.8% + 0.2/-3.1	LGC

[a] values are given in the original version from the certificate.