Abstract  Selenium has been identified as an antioxidant of importance in the diet. Accurate determination of its chemical forms depends on the availability of suitable reference materials (RMs). Two candidate reference materials for determination of selenomethionine (Semet) in food-related materials, a standard wheat gluten sample (NIST RM 8418 Wheat Gluten) and a commercial selenium enriched yeast, have been examined by use of a gas chromatography–isotope dilution mass spectrometry (IDMS) procedure, after treatment of the matrix with 0.1 mol L\(^{-1}\) hydrochloric acid containing stannous chloride, addition of CNBr, and extraction with chloroform. This procedure results in cleavage of the CH\(_3\) Se group to form volatile CH\(_3\) SeCN. Addition of isotopically enriched \(^{74}\)Semet to an analytical sample enables estimation of the naturally occurring protein-bound \(^{80}\)Semet by IDMS without a protein-digestion process.

We found that the Wheat Gluten RM contains a significant amount of Semet as a portion of its assigned value of 2.58 \(\mu\)g Se\(_{\text{total}}\) g\(^{-1}\). Commercial selenium yeast tablets are labeled as containing an elevated level of “organic selenium”, usually as Semet. The sample we investigated contained 210 \(\mu\)g Se\(_{\text{total}}\) g\(^{-1}\) sample as determined separately by IDMS, measuring elemental selenium after digestion. 73\% of this total (153\(\pm\)21 \(\mu\)g Se\(_{\text{Semet}}\) g\(^{-1}\); \(n=23\)) was present as Semet. Thus, these two materials contain significant amounts of their total selenium content as Semet and would be good candidates for further study and characterization as reference materials for determining this important food component.

The CNBr reaction used will also enable the determination of Se-(methyl)selenocysteine, the biological role of which is of recent interest. In addition to matrix RMs for Semet, it is important to have standard materials of the pure substance. We have examined a sample of a candidate standard material of selenomethionine being prepared by the USP. It was confirmed that this material is pure selenomethionine.

Introduction  Selenium (Se) has recently been identified as an important nutrient in the diet because of its antioxidant properties [1]. Se is found in many food items, with grain products having high concentrations. Se concentrations in grain can vary widely from locale to locale, depending on the Se content of the soil. Other sources of Se are seafood (especially tuna), liver, and, to a lesser extent, meats and eggs. The Se content of most food items is not well established. Se is found in a variety of chemical forms, either inorganic or as organic forms incorporated in protein-bound amino acids, e.g. selenomethionine (Semet), the Se analog of the sulfur-containing amino acid methionine (Fig. 1).

Organically bound selenium, as selenomethionine, is better adsorbed than inorganic selenium compounds [2]. Thus, there is considerable interest in its metabolic and biological role. There is also increasing interest in the biological role of another selenoamino acid, Se-(methyl)se-
lenocysteine (Mesecys) [3, 4] (Fig. 2). Mesecys occurs at much lower abundance (approximately 1–2% the level of Semet), but is hypothesized to be much more active in key biological roles.

The US Department of Agriculture in its Dietary Guidelines for Americans encourages the populace to eat “plenty of grain products, vegetables and fruits” [5]. In addition to the fiber, macronutrients and other components, grain products are considered to be a good source of Se in the diet. With the current emphasis on antioxidants, it is quite easy to recognize the importance of being able to quantify the content of both total Se and its organic forms in foods.

We have developed a procedure for accurately determining the Semet content of food materials; it is based upon the textbook reaction of cyanogen bromide (CNBr) with methionine, containing the CH₃S-functional group, to form the volatile compound CH₃SCN [6]. The chemistry for Semet is similar, with CH₃SeCN being formed (Fig. 3). This volatile species can be detected by GC–MS. We have also shown that this CNBr reaction occurs with the CH₃Se-containing species Mesecys, producing the same CH₃SeCN moiety.

When isotopically enriched ⁷⁴Se labeled Semet (Fig. 4) is added to an analytical sample and left to react with CNBr, estimation of protein-bound Semet is possible by isotope-dilution techniques. If, however, this procedure is to be used by different laboratories, it is imperative to have a way of determining the validity of laboratories’ results by use of suitable reference materials (RMs) in which the Semet concentration is known and has been verified. In the course of our method development studies, we examined several materials that could be considered as candidate standard samples under development by USP.

Experimental

Candidate reference materials

The wheat gluten sample (RM 8418 Wheat Gluten) was obtained from the National Institute of Standards and Technology, Gaithersburg, MD, USA, and has an assigned value for total selenium content of 2.58 µg g⁻¹. A sample of a commercial product labeled to contain “selenium yeast” in tablet form was obtained at a local grocery store. Label claim was for 200 µg Se g⁻¹. During the course of our studies, we also obtained a sample of pure selenomethionine, a candidate standard sample under development by USP.

Isotope-labeled selenomethionine. ⁷⁴Se-labeled selenomethionine stock solution (10⁻³ mol L⁻¹) was obtained from C. Veillon, Nutrient Requirements and Functions Laboratory, USDA, ARS, BHNRC, Beltsville, MD 20705, USA. This solution had been calibrated for ⁷⁴Se by reverse isotope dilution using pure Se metal (99.99%). Calibration of this labeled solution was checked against gravimetrically prepared stock solutions of pure Semet standards from a commercial source (CAS #3211–76–5, Aldrich # 47394–4). Thus, the calibration for these measurements is ultimately traceable to the pure Se metal material.

Preparation of solutions and samples

To ensure accurate and precise quantification, the weights of all additions for reagents, samples, solutions, etc., were determined gravimetrically.

Sample preparation. Graduated 5-mL v-vials (Wheaton, #986299) were weighed. Standard, Wheat Gluten or yeast sample was weighed into a sample vial with HCl (0.1 mol L⁻¹, 1 mL) containing 2% by weight stannous chloride. An appropriate aliquot of the ⁷⁴Semet label was added and vortex-mixed to ensure complete incorporation of sample into the aqueous solution. For yeast samples, aliquots of the ⁷⁴Semet labeled stock solution were added directly to the test portions. Vials were held at 36–37 °C for 24 h to ensure complete mixing of the protein material with the labeled spike. CNBr (200 µL) (Aldrich, CAS# 506–68–3) was added, vortex-mixed, then heated at 36–37 °C for 24 h. To extract the product, CH₃SeCN, chloroform (CHCl₃, 1 mL) was added on day three, vortex-mixed, and the mixture was then left to stand at 20 °C until phase separation was complete. The organic phase was separated and 1 µL aliquots injected into the GC–MS for analysis. For the spiked acid stability test, 1 mL of the Mesecys solution, prepared as described below, was used as the sample instead of the yeast or gluten.

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\text{CH}_3\text{Se}-\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2
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**Fig. 2** Selenium (methyl)selenocysteine (Mesecys)

**Fig. 3** Reaction of CNBr with selenomethionine

**Fig. 4** Selenomethionine isotopically enriched with ⁷⁴Se