Determination of Selenium in Fresh Fish from Southeastern Spain for Calculation of Daily Dietary Intake

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Hydride generation atomic absorption spectrometry was used to determine the total Se content in the edible muscular tissue of the 31 most common species of fresh fish from the Mediterranean area of Motril (southeastern Spain). The samples were digested in nitric acid. The Se^{6+} in the digested solutions was prereduced to Se^{4+} by exothermic reaction in HCl solution. Determination of selenium was carried out by the standard addition method. Results for standard reference material (BCR mussel tissue) agreed closely with the certified values. Detection limit was 2.83 ng. Reproducibility expressed as relative standard deviation ranged from 4.00% to 4.71% . A summary of results is reported ranging from 0.150 to 2.014 μg/g for 31 species of fish. Considering the average daily individual consumption of fresh fish in Andalusia (southern Spain), the daily dietary intake of Se supplied by this source is 15.78 μg/day for an adult.

INTRODUCTION

It is well-known that selenium is essential for human life and is supplied by food. Toxic action is known as well as deficiency syndromes, and the optimal range of beneficial action seems to be small. While Se is an essential dietary element in minute quantities, it is also toxic to most organisms at higher concentrations (Arora et al., 1975; Banuelos and Meek, 1989).

Although food is the main source of Se for man, the dietary selenium intake principally depends on the origin of the foodstuffs. Thus, animal products (meat and fish) tend to be richer in Se than plant materials (Ari et al., 1990; Jurado Chacón et al., 1990); both ultimately reflect the soil levels. The average safe and adequate daily dietary intake of selenium is from 50 to 200 μg (National Research Council, 1989), with average levels of 70 μg/day for an adult man and 55 μg/day for an adult woman (Levander, 1991).

We analyzed the Se concentrations in 31 different species of fish for human consumption from the Motril (southeastern Spain) fishing area. As fish is one of the major sources of selenium and since the concentration varies considerably depending on the geographic fishing area, we attempted to determine the levels of this element in the fish from the chosen area. Similarly, in view of the food consumption tables for Spain drawn up by the Ministry of Agriculture, Fisheries and Food, which show that Spain has one of the highest fish consumptions in the world, we attempted to determine the daily amount of Se provided by fish products from the chosen area.

This study was undertaken as a result of the lack of studies in southeastern Spain on the intake of Se in the daily diet. The determination of Se contents in fish products was carried out to calculate the average daily dietary intake of Se, as the high consumption of fish products in the area constitutes one of the main sources of Se.

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MATERIALS AND METHODS

Apparatus. Mineralization was performed in an Invester sand beaker. A Selecta thermostated bath was then used in the reduction to Se^{4+}. All atomic measurements were made with a Perkin-Elmer Model 1100B atomic absorption spectrometer equipped with a Perkin-Elmer MHS-10 hydride generator. A selenium hollow cathode lamp (Perkin-Elmer Corp., Norwalk, CT) was operated under the conditions recommended by the manufacturer. A spectral slit width of 2.0 nm was selected to isolate the 196.0-nm line. All analyses were performed in peak height mode to calculate absorbance values.

Materials and Reagents. All glassware was washed with detergent solution, rinsed with tap water followed by distilled water, and placed in 20% (v/v) HNO_3 for at least 48 h before use. The glassware was then rinsed with distilled water and ultrapure water with a specific sensitivity of 18 MΩ cm and oven-dried at 60 °C.

All solutions were prepared from analytical reagent grade reagents: HNO_3 (65%), HCl (37%) (Carlo Erba, Italy). A commercially available 1000 μg/mL selenium standard solution (prepared from SeO_2 in 0.5 mmol/mL HNO_3) was used (Titrisol, Merck). Sodium tetrahydroborate solution was prepared by dissolving first 2.5 g of NaOH (Merck) and then 7.5 g of $NaBH_4$ (Merck) in 250 mL of ultrapure water. This solution was stirred for 10 min and filtered before use.

The Community Bureau of Reference–Commission of the European Communities (CBR–CEC) certified material reference (CRM) used was mussel tissue (CRM 278).

Samples. The muscular meat of 31 of the most common species of fresh fish were obtained at the port of Motril (southeastern Spain). The samples were lyophilized, pulverized, and, after homogenization, stored at −18 °C before analysis. Three 300-mg fractions were taken from each sample for analysis.

Procedure. Three hundred milligrams of lyophilized sample was placed in a 60-mL volumetric flask, and the sample was mineralized by addition of 4 mL of concentrated HNO_3 and heating at 80 °C for 1 h in a sand beaker. Another 3 mL of HNO_3 was added and heating continued for an additional 3 h until the sample was completely mineralized. The digest was cooled and the resulting solution diluted to 10 mL with ultrapure water.

Se^{6+} was reduced to Se^{4+} by taking a 1-mL aliquot in a test tube and adding 1 mL of concentrated HCl and heating at 100 °C for 10 min in a thermostated bath (Palacios et al., 1985; Peterson and Olin, 1991). When cool, this was diluted to volume with a 2.0% (w/v) HCl solution. A 10-mL aliquot was then transferred to a reaction vessel, which was placed in the MHS-10

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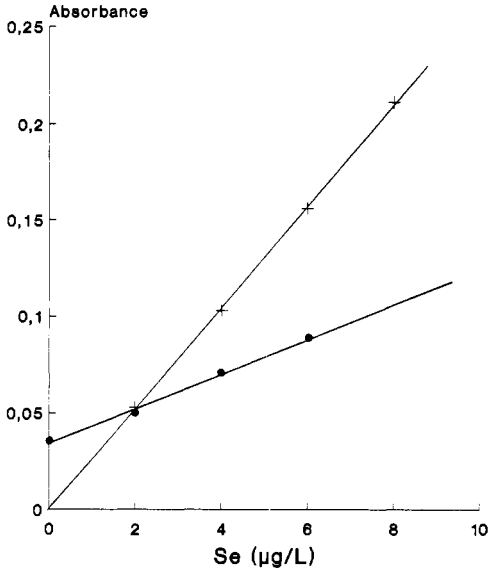

Figure 1. Comparison of the plotting of the calibration graph and the standard addition method in *Engraulis encrasicholus* L. (●).

system. Selenium determination was carried out using the HG-AAS technique. Hydride generation was carried out using a solution of 3% (w/v) $NaBH_4$ in 1% (w/v) NaOH.

The samples were analyzed by the standard addition method, by addition of 0.00–0.060 µg of selenium to four aliquots of the sample after reduction to Se^{4+} (Figure 1).

All of the samples and blanks were mineralized and diluted using the same procedure.

Reproducibility and Accuracy. The reproducibility of the Se measurements in the present study was determined by using the procedure described above to measure the Se content of each of a series of random fish samples seven times. The accuracy of the method was estimated by addition: for each of a series of random fish samples the concentration of Se was determined both without addition of Se standard and after various quantities of standard had been added. We also used CBR certified reference material (CRM 278 mussel tissue) with a certified Se content of 1.66 ± 0.04 µg/g (dry weight).

Method. The most popular methods for the routine determination of Se in fish are the fluorometric method (Moreno Domínguez, 1983) and atomic absorption spectrometry using either electrothermal atomization (Maage et al., 1991) or hydride generation (HG-AAS) (Egaas and Julshamn, 1978; Flanjak, 1978; Giaccio and Cichelli, 1985; Giaccio et al., 1987; Hansson et al., 1989; Brumbaugh and Walther, 1989). The fluorometric and hydride generation methods both require prior sample digestion to destroy organic matter and reduction to convert the selenium to the Se^{4+} state.

The hydride generation method was used here because by generating the selenium hydride by addition of a reducing agent (sodium tetrahydroborate) to an acidified sample solution, the analyte is separated from the bulk matrix, which leads to a substantial decrease in interferences, compared with flame or graphite furnace methods (Mayer et al., 1992). The methodology presented here has proved to be both precise and accurate, as well as procedurally routine, for trace selenium determinations in a large number of fish samples.

RESULTS AND DISCUSSION

The method used is suitable for determination of Se in fish. It can also be applied in routine control analyses of the nutritional quality and toxicological importance of this element in human food.

Comparison of the plotting of the calibration graph and the standard addition method indicated the presence of interference of the matrix (Figure 1). All determinations were carried out by the standard addition method.

Table 1. Recovery of Selenium from Spiked Fish Samples

sample	Se present, µg	Se added, µg	Se found, µg	recovery, %
S. sarda Bloch	0.059	0.000	0.060	101.69
	0.059	0.020	0.072	91.14
	0.059	0.030	0.089	96.63
	0.059	0.040	0.098	98.45
A. cuculus	0.047	0.000	0.044	93.62
	0.047	0.015	0.063	101.62
	0.047	0.030	0.070	90.91
	0.047	0.045	0.090	97.83

Table 2. Comparison of HG-AAS Selenium Measurements with CRM 278 Certified Concentration

	Se values, µg/g	
sample	certified[a]	determined[a]
CRM 278 (mussel tissue)	1.66 ± 0.04	1.62 ± 0.12

[a] Mean and content range with 95% certainty based on 10 replicate analyses of 300-mg samples.

Table 3. Precision Study

sample	mean Hg content,[a] µg/g	RSD, %
S. sarda Bloch	0.646 ± 0.0258	4.00
A. cuculus	0.350 ± 0.0165	4.71

[a] Mean and standard deviation based on seven replicate analyses of 300-mg samples.

The calculated analytical detection limit was 2.83 ng for the instrumental conditions used in the analyses of the samples. The Se concentrations in all of the samples analyzed were above the analytical detection limit.

The mean recoveries obtained for spiked samples were 96.97% in *Sarda sarda Bloch* and 95.99% in *Aspitrigla cuculus* (Table 1). The accuracy of the measured concentrations of Se in CRM 278 gave a result of 1.62 ± 0.17 µg/g (dry weight) (Table 2). The relative standard deviation was normally better than 5.00% in the range of concentrations analyzed in this paper (Table 3). These results confirm that the procedure is appropriate for determination of total Se in fish products.

The Se levels detected in the edible muscular part of the different species of fish analyzed are shown in Table 4. We can infer from the results obtained that the highest accumulations of Se are found in the samples of *Lophius piscatorius* L. (2.014 µg/g), *Sardina pilchardus* (0.666 µg/g), *S. sarda Bloch* (0.646 µg/g), and *Crangon vulgaris* (0.525 µg/g) and the lowest concentrations in *Galeorhinus galeus* L. (0.150 µg/g) and *Parepenaeus longinostris Leach* (0.173 µg/g) (fresh weight).

The high levels of Se in shrimps (0.525 µg/g) agree with the results of other authors, who mention the high capacity of shrimps to accumulate Se (Hammond and Beliles, 1980; Goyer, 1986; Hershey et al., 1988). Shrimps are therefore one of the most important food sources of selenium in the diet. Nonetheless, Mejuto Martí et al. (1987) detected a Se concentration of 0.078 µg/g (fresh weight) in shrimps from Coruña (northeastern Spain), which is considerably less than the concentration determined in this paper.

We can compare the results obtained by us (Table 4) with those of other authors in other parts of Spain. From the results obtained by Mejuto Martí et al. (1987) for 14 species of fish from Coruña (northeastern Spain) common to both studies, the average Se concentration was 0.213 µg/g in comparison to our average of 0.471 µg/g (fresh weight). Moreno Domínguez et al. (1983) determined the Se concentrations in different foodstuffs of unknown origin. The Se concentrations determined by these authors were considerably higher (0.617 µg/g) than those detected

Table 4. Selenium Content of Fish from Southeastern Spain (Micrograms per Gram, Fresh Weight)

sample[a]	Se, content	sample[a]	Se content
Gadus poutassou L.	0.303	*Trachinus araneus* L.	0.331
Aspitrigla cuculus	0.350	*Pagellus cantabricus Asso.*	0.368
Crangon vulgaris	0.525	*Engraulis encrasicholus* L.	0.308
Galeorhinus galeus L.	0.150	*Trachurus trachurus* L.	0.289
Illex coindetti	0.322	*Merluccius merluccius* L.	0.321
Escomber scombrus L.	0.353	*Luvarus imperialis Ver.*	0.338
Cepola rubescens	0.376	*Helicolenus dactylopterus*	0.228
Allotenthys media L.	0.269	*Seppia officinalis* L.	0.214
Mullus surmuletus	0.428	*Urophycis bleunioides*	0.269
Sarda sarda Bloch	0.646	*Squilla mantis*	0.205
Leander serratus	0.235	*Octopus vulgaris Lamarck.*	0.268
Belone belone L.	0.265	*Loligo vulgaris Lamarck.*	0.187
Boops boops L.	0.255	*Mugil* sp.	0.425
Aphya minuta Risso	0.382	*Brama raii Bloch*	0.260
Sardina pilchardus	0.666	*Lophius piscatorius* L.	2.014
Parepenaeus longirostris Leach.	0.173		

[a] Taken from Ministerio de Sanidad y Consumo (1991).

by us (0.327 μg/g) in the five fish products common to both studies. Outside Spain, Thorn et al. (1978) detected Se levels of 0.280–0.380 μg/g in fresh fish from the United Kingdom, which are very similar to the levels found in this study. However, during the process of determining the Se content in the daily diet in France, Simonoff and Simonoff (1991) detected levels of 0.200 μg/g in cooked fish and 0.250 in cooked seafood, both of which are slightly lower than the levels determined by us in this paper (0.426 μg/g in fresh fish and 0.284 μg/g in fresh seafood). On the other hand, Arthur (1972) determined Se levels of 0.900 μg/g in seafish in Canada, which is substantially higher than the levels reported in this study. All of the results show significant differences, probably due to the different geographic areas of fish capture.

Considerable differences were detected in the average Se content of each of the three subdivisions of fish products, i.e., fish (0.426 μg/g), shellfish (0.284 μg/g), and cephalopods (0.252 μg/g) (fresh weight). The highest Se contents determined for fish were mainly due to three species: *L. piscatorius* L. (2.014 μg/g), *S. pilchardus* (0.666 μg/g) and *S. sarda Bloch* (0.646 μg/g).

On the basis of the data in Table 4, the contributions of these kinds of fish to the mean intake of selenium in Andalusia per person per day have been estimated by multiplying the mean Se content of each fish by the mean consumption of that kind of fish in Andalusia (southern Spain) per person per day (Dirección General de Política Alimentaria, 1991). We were thus able to estimate the amount of selenium provided by this source at 15.70 μg per person per day. To calculate the total Se intake for all fish produce, we should also have to consider frozen, tinned, and cooked fish, apart from fresh fish. We did not consider these other products as they have a different geographical origin than those considered here.

In view of these results, and taking into account the fact that the recommended daily dietary intake of Se is established at 50–200 μg (National Research Council, 1989), there appear to be no problems regarding Se intake in the daily diet of the area under consideration. However, these results should be complemented by a study on the Se provided by meat products and vegetables, to determine the exact daily intake from all food products in the area.

On the other hand, since Se is an indispensable trace nutrient, its bioavailability in several foodstuffs is more important than the Se content itself (Cantor et al., 1975b; Yoshida et al., 1984). If we therefore consider that only a small fraction of the total Se ingested is absorbed and transformed into a biologically active form (Cantor et al., 1975a,b; Yoshida et al., 1984; Simonoff and Simonoff, 1991) and that the bioavailability of Se in foodstuffs differs according to their origin (in the case of animal foodstuffs, including fish, it is slightly less than 25%; Cantor et al., 1975a; Simonoff and Simonoff, 1991), the amount of bioavailable Se in fish in the area under consideration would be approximately 4.92 μg per person per day.

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