Determination of Selenium Levels in Vegetables and Fruits by Hydride Generation Atomic Absorption Spectrometry

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The selenium content in 87 vegetable and fruit samples was determined using hydride generation atomic absorption spectrometry. Sample recoveries, precision studies, and analyses of NIST reference materials demonstrate the reliability and accuracy of this technique. The selenium concentrations varied from not detectable in grape, pear, plum, and custard apple to 29.65 ng/g in cultivated mushroom (*Psalliota hortensis*). Considering the average daily individual consumption of these vegetables and fruits in Andalusia (southern Spain), the daily dietary intake of Se supplied by this source is estimated to be 1.21 μg per person per day.

Keywords: *Selenium in vegetables and fruits; daily dietary intake; HG-AAS*

INTRODUCTION

Selenium is at present the object of numerous studies because of its dual nature as a toxic element that is, nonetheless, essential to the human organism (Brätter et al., 1984; Simonoff and Simonoff, 1991).

The biological effects of selenium in both humans and animals were initially considered only with regard to its toxicity, which appears when the intake of the element is higher than the organism's capacity to eliminate it. Acute intoxication affects the central nervous system, and chronic intoxication has been shown to cause fetotoxicity and teratogenicity in humans (Albert, 1988).

On the other hand, selenium intake, as an essential ingredient in the daily diet, is of great interest and varies widely among various populations throughout the world (Bunker et al., 1988; Levander, 1991). Epidemiological studies carried out in several countries have shown the possible effects of selenium in the prevention and regression of cancer (Nomura et al., 1987; Thompson and Ronan, 1990).

Although the principal source of selenium for humans is food, the daily intake mainly depends on the origin of the food products, which present selenium contents directly related to the levels found in the soil (Ari et al., 1991). However, in most agricultural areas, soils contain so little available Se that cultivated crops do not absorb more than traces of this element (Banuelos and Meek, 1989). It also appears that vegetables grown in selenate-laden soil accumulate higher concentrations of Se than plants grown in selenite-laden soil (Banuelos and Meek, 1989).

In view of the lack of studies on the determination of Se ingested in the daily diet of southeastern Spain, the aim of this study was to determine the Se content in 87 samples from 30 different species of the most commonly grown crops in the Motril area (southeastern Spain) and also in the most commonly consumed vegetables and fruits produced in the same area, using hydride generation atomic absorption spectrometry. Moreover, by using the tables of food consumption in Spain, we have quantified the daily intake of Se from the vegetable and fruit products in the normal diet of the area concerned, as a previous study (Diaz-Alarcón et al., 1994) did for Se ingested in fresh fish.

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MATERIALS AND METHODS

Apparatus. An atomic absorption spectrophotometer (Perkin-Elmer 1100 B) was used, with a hydride generator (Perkin-Elmer MHS-10) and an 11-mA hollow cathode lamp, at a slit width of 2.0 nm. Atomization of the hydride generated after reduction of the sample with $NaBH_4$ solution was developed in a quartz cell heated over an air–acetylene flame. All measurements were performed in peak height mode. The samples were mineralized in an Invester sand bath and reduced to Se^{4+} by exothermic reaction in HCl solution in a Selecta thermostated bath.

Reagents. All solutions were prepared with ultrapure water with a specific resistivity of 18 MΩ/cm, obtained by filtering double-distilled water through a Milli-Q purifier (Millipore) immediately before use. The standard solution of Se (Tritisol, Merck) was used at a concentration of 100 ng/mL. HNO_3 (65%), HCl (37%), $HClO_4$ (65%) (Carlo Erba, Italy), $NaBH_4$, and NaOH (Merck analytical grade) were also used.

Samples. Part of the samples was obtained in the field, from farms located along the coast of the province of Granada (southeastern Spain) in an area where the predominant crops are sugarcane (*Saccharum officinarum*) and garden vegetables. The remainder was bought in commercial foodstores. The samples were thoroughly washed or peeled, dried under controlled temperature conditions (T = 60 °C), depending on their humidity, and then ground manually to a fine powder in a mortar. Three 300-mg subsamples of the comestible portions of each sample were taken for analyses.

Procedure. The method used for mineralization of the samples and subsequent determination of the Se content is similar to the method used for determination of this element in fish caught in the same area (Diaz-Alarcón et al., 1994). Amounts of 300-mg of dried, homogenized sample were mineralized with 5.0-mL concentrated HNO_3, heated at 80 °C for 1 h in a sand bath. Another 5.0 mL of a 4:1 mixture of HNO_3 and $HClO_4$ was added and heating continued for an additional 3 h until the sample was completely mineralized.

For reduction of Se^{6+} to Se^{4+}, 2 mL of concentrated HCl was added to the mineralized sample and kept heating at 100 °C for 10 min in a thermostated bath (Palacios et al., 1985). When cool, this was diluted to a 15-mL volume with a 1.9% HCl solution. An aliquot was transferred to a reaction vessel, which was placed in the MHS-10 system. After hydride

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Table 1. Recovery of Selenium from Spiked Vegetable Samples

sample	Se present, μg	Se added, μg	Se found, μg	recovery, %
lemon 1 (*Citrus limonum*)	0.0078	0.0000	0.0075	96.15
	0.0078	0.0060	0.0153	110.87
	0.0078	0.0105	0.0192	104.92
	0.0078	0.0150	0.0270	118.42
	0.0078	0.0195	0.0309	113.20
green beans 2 (*Phaseolus vulgaris*)	0.0120	0.0000	0.0121	100.83
	0.0120	0.0150	0.0260	96.30
	0.0120	0.0450	0.0550	96.50

Table 2. Precision Study

sample	mean Se content,[a] ng/g	RSD, %
lemon 1 (*Citrus limonum*)	2.79 ± 0.585	20.97
green beans 4 (*Phaseolus vulgaris*)	6.56 ± 0.569	8.67

[a] Mean and standard deviation based on seven replicate analyses of 300 mg samples (fresh weight).

generation using the $NaBH_4$ solution, Se determination was carried out in a quartz cuvette heated over an air–acetylene flame at 196.0 nm.

The samples were analyzed by the standard addition method. All the samples and blanks were mineralized and diluted using the same procedure. The data presented were corrected for blank values, which were usually very low for this method.

Reproducibility and Accuracy. The reproducibility of the Se measurements was obtained following the procedures indicated by Diaz-Alarcón et al. (1994). The reference standard consisted of citrus leaves (National Institute of Standards and Technology (NIST), standard reference material SRM 1572) with a noncertified Se content of 0.025 μg/g (not sufficiently homogeneous for certification).

Method. Due to the difficulties of mineralizing plants because of their high fiber content (Olson et al., 1983), in this study we used the classic method of HNO_3–$HClO_4$ acid digestion mixture, which can be used without loss of selenium even on prolonged heating (Olson et al., 1983; Moreno Domínguez et al., 1983).

The hydride generation method was used in this study, as there is a considerable elimination of interferences when the element is separated from the matrix, in comparison to flame or furnace methods (Hershey et al., 1988).

RESULTS AND DISCUSSION

For the instrumental conditions used in sample analysis, our calculated analytical detection limit, defined as the Se concentration corresponding to 3 times the standard deviation of the blank (Long and Winefordner, 1983) was 0.210 ng/mL. This value was comparable to those reported by other authors.

The accuracy of the method was evaluated by recovery tests. Mean recovery for the added samples was 104.6% (Table 1). The Se concentration determined in NIST SRM 1572 standard was 28.9 ng/g (n = 7) for a noncertified value of 25.0 ng/g.

The technique was accurate and reproducible. The results of seven determinations in two different species were analyzed statistically, following Steel (1982). The results of the precision test are shown in Table 2.

In view of the results obtained, the technique described above provided highly sensitive measurements with a low detection limit, which make it suitable for rapid, direct analyses in the vegetable samples tested.

Table 3 shows the ranges and mean concentrations of Se determined in the different species of vegetables analyzed. These results show that the highest levels of Se are found in the samples of cultivated mushroom (29.65 ng/g in *Psalliota hortensis*, and 28.11 ng/g in *Pleuorotus ostreatus*), olive (21.96 ng/g), peas (14.81 ng/g), garlic (13.66 ng/g), and sweetpotato (12.66 ng/g), whereas the lowest concentrations were found in grapes,

Table 3. Selenium Content of Vegetables and Fruits (Nanograms per Gram, Fresh Weight)

sample	no. of samples	Se, range	Se, mean
sugarcane (*Saccharum officinarum*)	24	0.24–5.85	1.45
potato (*Solanum tuberosum*)	8	0.44–7.73	1.30
green beans (*Phaseolus vulgaris*)	5	0.75–2.99	1.54
lettuce (*Lactuca* scariola)	1		2.39
tomato (*Lycopersicum sculentum*)	3	0.78–3.66	2.32
cauliflower (*Brassica oleracea* var. Botrytis)	2	1.22–2.40	1.81
courgette (*Cucurbita pepo*)	2	1.36–2.18	1.77
cucumber (*Cucumis sativus*)	2	1.35–1.51	1.43
aubergine (*Solanum melongena*)	2	3.06–4.35	3.70
radish (*Raphanus radicula*)	1		4.51
pepper (*Capsicum annuum*)	2	1.68–2.57	2.12
sweetpotato (*Ipomea batatas*)	2	10.92–14.40	12.66
onion (*Allium cepa*)	2	3.29–6.56	4.92
cultivated mushrooms (*Psalliota hortensis*)	2	24.89–34.41	29.65
cultivated mushrooms (*Pleuorotus ostreatus*)	2	26.52–29.71	28.11
peas (*Pisum sativum*)	1		14.81
apple (*Malus communis*)	2	1.39–12.39	
grape (*Vitis vitifera*)	2		ND[a]
plum (*Prunus domestica*)	1		ND[a]
custard apple (*Annona cherimola*)	2		ND[a]
pear (*Pyrus communis*)	2		ND[a]
avocado (*Persea gratgissima*)	2	0.267–27.91	
orange (*Citrus aurantium* var Dulcis)	2	1.53–2.36	1.94
lemon (*Citrus limonum*)	2	1.11–1.62	1.37
pomegranate (*Prunica granatum*)	1		0.85
banana (*Musa paradidiaca*)	2	ND[a]–2.4	1.20
prickly pear (*Ficus carica*)	1		1.77
garlic (*Allium sativum*)	3	5.75–24.69	13.66
carrot (*Dancus carota*)	2	ND[a]–0.615	0.308
olive (*Olea europaea*)	2	12.00–31.92	21.96

[a] Not detectable.

plum, custard apple, and pear, where no traces of Se were detected using the technique described.

Comparison of the results obtained in this study with those of other authors in different areas of Spain shows that, in Galicia (northwestern Spain), mean Se concentration found in fruit samples (1.9 ng/g fresh weight) (Mejuto-Martí et al., 1988) is lower than that detected by us (4.17 ng/g). However, the Se concentrations are higher in the case of potato (4.4 ng/g fresh weight) and vegetable (7.8 ng/g fresh weight) samples as against our values (1.8 and 4.1 ng/g, respectively). Moreno Domínguez et al. (1983) determined Se concentration in samples purchased in commercial establishments in Salamanca (central Spain), with results lower than those found by us in practically all the vegetable samples examined.

In other countries, Shacklette et al. (1978) determined the Se levels in fruit and vegetable samples purchased in summer (1974) from 11 retail markets in metropolitan areas of the United States, on the basis of wide geographic coverage and large size. Their results showed mean Se levels <1.6 ng/g in apples, 13 ng/g in potatoes, 2.4 ng/g in oranges, 9.6 ng/g in carrot, 1.9 ng/g in lettuce, 2.6 ng/g in tomatoes, 6.9 ng/g in onions, and 2.9 ng/g in cucumber. In general, all these concentrations are similar to those determined in this study (Table 3), except for potato, carrot, and apple, where we found a mean concentration of 1.80 ng/g, 0.308 ng/g and 6.89 ng/g respectively (Table 3). In the United Kingdom, Thorn et al. (1978) found Se levels of <0.010 ng/g (fresh weight) in fruit and from <10 to 20 ng/g in vegetables, which were also generally similar than those detected by us (Table 3). In France, Simonoff and Simonoff (1988) found Se levels in aubergines (<3 ng/g), peas (9 ± 3 ng/g), and apples (<2 ng/g) lower than those found in our study (Table 3). In bananas (<2 ng/g), lettuce (<2 ng/g), cauliflower (<3 ng/g), tomatoes (<3 ng/g), and cucumber (<3 ng/g) the levels detected by Simonoff and Simonoff (1988) were similar to those found by us (Table 3), whereas those of potatoes (6 ± 2 ng/g), green beans (19 ± 4 ng/g), grapes (12 ± 4 ng/g), plums (<2 ng/g), and courgettes (10 ± 3 ng/g) were higher (Table 3).

These results show the high degree of variability in Se levels detected in vegetable and fruit samples. These differences can be attributed to the fact that the Se content in plants is strongly conditioned by the amount of Se biologically available in the soil and, therefore, is related to geographic origin (Banuelos and Meek, 1989; Ari et al., 1991).

The high concentration of selenium (29.65 ng/g) detected by us in cultivated mushrooms (*Psalliota hortensis*), which are the type mainly consumed in the area, coincides with the results of other authors, who mention the mushrooms' high capacity for selenium accumulation, which in turn depends on the type of the species taken into consideration (Piepponen et al., 1984; Simonoff and Simonoff, 1991).

Considering the results obtained in the vegetable samples analyzed (Table 3), we have calculated the contributions to the mean selenium intake per person per day in Andalusia by multiplying the mean Se content in each vegetable species by the mean consumption of each species per person per day in Andalusia (Dirección General de Política Alimentaria, 1991). We also considered the comestible portion of each vegetable species, using the foodstuff comparison tables by Jiménez-Cruz et al. (1990). On the basis of the results obtained, we can estimate the amount of selenium provided by the vegetable species analyzed to be 1.21 μg per person per day. If we take into account the fact that the selenium intake from fish in the zone under consideration is 15.70 μg per person per day (Diaz-Alarcón et al., 1994), the total selenium intake from both food sources would be 16.91 μg per person per day. Since we know 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.

If we consider that only a small portion of the total amount of selenium ingested through food in the daily diet is absorbed and transformed into a biologically active form (Cantor et al., 1975a,b; Yoshida et al., 1984; Thomson and Robinson, 1986; Simonoff and Simonoff, 1991) and that the bioavailability in food products of vegetable origin is high (around 60% of the total content; Cantor et al., 1975a; Simonoff and Simonoff, 1991), the quantity of bioavailable selenium in the vegetables analyzed is approximately 0.726 μg per person per day.

LITERATURE CITED

Albert, L. A. In *Control Básico de Toxicología Ambiental.*; Limusa: México City, 1988; 311 pp.

Ari, Ü.; Volkan, M.; Aras, N. K. Determination of Selenium in diet by Zeeman Effect Graphite Furnace Atomic Absorption Spectrometry for Calculation of Daily Dietary Intakes. *J. Agric. Food Chem.* **1991,** *39,* 2180–2183.

Banuelos, G. S.; Meek, D. W. Selenium Accumulation in Selected Vegetables. *J. Plant Nutr.* **1989,** *12,* 1255–1272.

Brätter, P.; Negretti, V. E.; Rösick, V.; Jaffé, W. G.; Hernán Mendez, C.; Guillermo Tovar, E. Effects of Selenium Intake in Man at High Dietary Levels of Seleniferous Areas of Venezuela. *Trace Elem. Anal. Chem. Med. Biol.* **1984,** *3,* 29–46.

Bunker, V. W.; Lawson, M. S.; Stansfield, M. F.; Clayton, B. E. Selenium Balance Studies in Apparently Healthy and Housebound Elderly People Eating Self-Selected Diets. *Br. J. Nutr.* **1988,** *59,* 171–180.

Cantor, A. H.; Scott, M. L.; Noguchi, T. Biological Availability of Selenium in Feeds and Selenium Compounds for Prevention of Exudative Diathesis in Chicks. *J. Nutr.* **1975a,** *105,* 96–105.

Cantor, A. H.; Langevin, M. L.; Noguchi, T.; Scott, M. L. Efficacy of Selenium in Selenium Compounds and Feeds for Prevention of Pancreatic Fibrosis in Chicks. *J. Nutr.* **1975b,** *105,* 106–111.

Diaz-Alarcón, J. P.; Navarro-Alarcón, M.; López-Martínez, M. C.; López-García de la Serrana, H. Determination of Selenium in Fresh Fish from Southeastern Spain for Calculation of Daily Dietary Intake. *J. Agric. Food Chem.* **1994,** *42,* 334–337.

Direccíon General de Poltíca Alimentaria, Secretaría General de Alimentación, Ministerio de Agricultura, Pesca y Alimentación. *El Consumo Alimentario en España*; Gráficas Monterreina: Madrid, 1991.

Hershey, W.; Oostdyk, T. S.; Keliher, E. N. Determination of Arsenic and Selenium in Environmental and Agricultural Samples by Hydride Generation Atomic Absorption Spectrometry. *J. Assoc. Off. Anal. Chem.* **1988,** *71,* 1090–1093.

Jiménez-Cruz, A.; Cervera-Ral, P.; Bacardi-Gascón, H. *Tablas de Composición de Alimentos*; S. A. E. Wander: Barcelona, 1990.

Levander, O. A. Scientific Rationale for the 1989 Recommended Dietary Allowance for Selenium. *J. Am. Diet. Assoc.* **1991,** *91,* 1572–1576.

Long, G. L.; Winefordner, J. D. Limit of Detection: a Closer Look at the IUPAC Definition. *Anal. Chem.* **1983,** *55,* 713A–715A.

Mejuto-Martí, M. C.; Bollain-Rodríguez, M. H.; Lorenzo-Ferreira, R. A.; Bermejo-Martínez, F. Selenium Content of Vegetables, Fruits, and Cereals in Galicia (Northwest Spain). *J. Agric. Food Chem.* **1988,** *36,* 293–295.

Moreno Dominguez, T.; Mateos Notario, P.; Garcia Moreno, C. Selenium in Foods. *Anal. Bromatol.* **1983,** *35,* 1–8.

National Research Council (Food and Nutrition Board). *Recommended Dietary Allowance*, 10th ed.; National Academy of Science: Washington, DC, 1989.

Nomura, A.; Heilbrun, L. K.; Morris, J. S.; Stermmermann, G. N. Serum Selenium and the Risk of Cancer by Specific Sites: Case-Control Analysis of Prospective Data. *J. Natl. Cancer Inst.* **1987,** *79*, 103–107.

Olson, O. E.; Emerick, R. J.; Palmer, I. S. Measurements of Selenium in Plants of High Selenium Content by Flame Atomic Absorption Analysis. *At. Spectrosc.* **1983,** *4,* 55–58.

Palacios, M. A.; Arevalo, I.; Camara, C. Determination of Selenium in Biological Samples by Atomic Absorption with Hydride Generation. *Quim. Anal.* **1985,** *4,* 320–325.

Piepponen, S.; Linkkonen-Lilja, H.; Kuusi, T. The Selenium Content of Edible Mushrooms in Finland. *Z. Lebensm. Unters. Forsch.* **1984,** *177,* 257–260.

Shacklette, H. T.; Erdman, J. A.; Harms, T. F.; Papp, C. S. E. Trace Elements in Plants Foodstuffs. In *Toxicity of Heavy Metals in the Environment,* Dekker: New York, 1978; Part 1, 25 pp.

Simonoff, M.; Simonoff, G. *Le Sélénium et la Vie*; Masson: Paris, 1991.

Simonoff, M.; Hauron, C.; Moretto, P.; Llabador, Y.; Simonoff, G. N. Selenium in Foods in France. *J. Food Compos. Anal.* **1988,** *1,* 295–302.

Steel, R. G. *Principles and Procedures of Statistics*; McGraw-Hill: London, 1982.

Thompson, H. J.; Ronan, A. M. Differences in Selenium Concentrations in Target Tissues and Their Relevance to its Anticarcinogenicity. *Nutr. Res.* **1990,** *10,* 81–89.

Thomson, C. D.; Robinson, M. F. Urinary and Fecal Excretions of a Large Supplement of Selenium: Superiority of Selenate over Selenite. *Am. J. Clin. Nutr.* **1986,** *44,* 659–663.

Thorn, J.; Robertson, J.; Buss, D. H.; Bunton, N. G. Trace Nutrients. Selenium in British Food. *Br. J. Nutr.* **1978,** *39,* 391–396.

Yoshida, M.; Iwami, K.; Yasumoto, K. Determination of Nutritional Efficiency of Selenium Contained in Processed Skipjack Meat by Comparison with Selenite. *J. Nutr. Sci. Vitaminol.* **1984**, *30*, 395–400.

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