JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Determination of Selenium in Nuts by Cathodic Stripping Potentiometry (CSP)

GIACOMO DUGO* LARA LA PERA, VINCENZO LO TURCO, EKATERINI MAVROGENI, AND MARIA ALFA

Organic and Biological Department, University of Messina, Salita Sperone 31, 98166 Messina, Italy

The aim of this work was to determine the selenium content in nut samples by cathodic stripping potentiometry. Dry-powdered nuts were digested by HNO_3 and dissolved with concentrated hydrochloric acid. To avoid the interference of natural oxygen, the potentiometric determination of selenium was carried out in an electrolyte solution consisting of 2 M $CaCl_2$ and 4 M HCl. The analysis was executed applying an electrolysis potential of −150 mV for 60 s and a constant current of −30 μA. Under these conditions, detection limits lower than 1.0 ng g^{-1} were obtained for selenium analysis in nuts. The relative standard deviation of these measurements (expressed as rsd %) ranged from 0.44 to 0.88% while recoveries ranged from 90.2 to 95.3%. The results obtained with the proposed method were compared with those obtained via hydride vapor generation atomic absorption spectroscopy, a common method for determining selenium. The results of the two methods agreed within 5% for almond, hazelnut, and pistachio samples. The mean concentrations of selenium determined in Sicilian samples of almond, hazelnut, and pistachio were 531 ± 1, 865 ± 1, and 893 ± 4 μg/kg, respectively.

KEYWORDS: Cathodic stripping potentiometry; nuts; selenium

INTRODUCTION

Selenium has become one of the most discussed essential micronutrients in biology and medicine (*1*). Selenium compounds play an important role in protecting cells from oxidative damage and may reduce the risk of cardiovascular diseases and other pathologies brought on by oxidative stress. Recent studies indicate that supplemental Se in the human diet may also reduce cancer risk (*2*). Selenium enters the food chain through plants that take it up from the soil, so the availability of selenium depends heavily on geography (*3*). For an adult, the recommended daily allowance is 50−70 μg/day; however, concentrations higher than 400 μg/day may become toxic (*4*). Meat and seafood are good sources of selenium (400−1500 μg/kg), as are cereals and cereal products (100−800 μg/kg), fresh fruits, and vegetables (<100 μg/kg) (*5*). This work deals with the determination of selenium content in different types of nuts: almond (*Prunus amygdalus*), hazelnut (*Corylus avellanus*), and pistachio (*Pystacia vera*) (*6*). The kernels are a rich source of oil and contain oleic, linoleic, and linolenic fatty acids. In addition, the high levels of fiber, vitamins, proteins, and minerals such as K, Na, Ca, Mg, Fe, Zn, and Cu make them highly nutritious (*7*). Raw or roasted nuts are consumed worldwide and are important in the confectionery industry as an ingredient in cookies and candy. The fact that nuts are considered highly nutritious and are consumed worldwide indicates that the levels of a micronutrient as important as selenium should be determined in them reliably. Selenium quantification in food is very problematic because this micronutrient is present in very low concentrations in many biological forms (selenite, selenate, selenocysteine, selenomethionine, and selenoproteins) (*8*); moreover, most inorganic and organic selenium compounds volatilize at temperatures >200 °C (*9*, *10*). Therefore, the commonly used sample preparation procedures and analytical techniques that involve the use of high temperatures, such as dry ashing followed by atomic absorption spectroscopy (*11*), may cause a severe loss of selenium compounds through volatilization. Alternative techniques such as voltammetry (*12*), gas chromatography (*13*), and fluorimetry (*6*) have been successfully employed. In this work, cathodic stripping potentiometry (CSP) (*14*) is used to determine the selenium content of nuts that were studied. A glassy carbon mercury film electrode was employed as the working electrode. This electrode has been used previously in the determination of various trace food matrices (*15*−*17*). Selenium was extracted from nut samples by nitric acid digestion at 80 °C followed by hydrochloric acid treatment.

MATERIALS AND METHODS

Reagents. Almond (*P. amygdalus*), hazelnut (*C. avellanus*), and pistachio (*P. vera*) raw samples from the crop year 2002 produced in Sicily were studied. Storage was under ambient conditions (20−25 °C).

All of the reagents used were of analytical grade. Hydrochloric acid (34−37%) and Se(IV) (1000 μg/mL, 0.5 N HNO_3) standard solutions were purchased from Panreac (Barcelona, Spain). The Se(IV) standard solution was diluted with ultrapure water to obtain a 5 μg/mL Se(IV) solution. Anhydrous $CaCl_2$ (Baker J. T., Deventer, Holland) was used to prepare a 2 M aqueous solution of $CaCl_2$ solution, 4 M HCl. Nitric acid (Merk, Darmstadt, Germany) was used to digest the dried sample.

10.1021/jf021256m CCC: $25.00
Published on Web 05/20/2003

Table 1. Electrochemical Conditions for CSP Selenium Determination in Samples of Almond, Hazelnut, and Pistachio

		Se
integration range	mV	−680; −470
potential range	mV	−700; −400
conditioning potential	mV	−720 × 5 s
electrolysis potential	mV	−150
electrolysis time	s	60
acquisition final potential	mV	−750
cathodic current	μA	−30
sampling time	μs	300
discharge potential	mV	−560
agitation speed	turn/s	2
cycles	n	4
standard additions	n	2

The nut extracts were filtered on a Supelclean ENVI-Carb SPE carbon column (0.5 g, 6 mL, Supelco, Bellefonte, PA). The carbon columns were activated by methanol (Carlo Erba Reagenti, Milan, Italy). Ultrapure water (18.2 MΩ/cm) was prepared at the Department of Organic and Biological Chemistry, University of Messina.

Apparatus. Selenium analysis was carried out on a PSA ION 3 potentiometric stripping analyzer (Steroglass, S. Martino in Campo, Perugia, Italy), which was controlled by NEOTES 2.0.1 software (Steroglass) run on an IBM compatible personal computer. This software also generated the potentiometric data for Se(IV) during the analysis. The determination was executed in a conventional three-electrode cell. The working electrode was a glassy carbon electrode coated with a thin mercury film; the reference electrode was an Ag/AgCl electrode (3 M KCl), and a platinum wire auxiliary electrode was also used.

Sample Preparation. The whole nuts were crushed with a mortar and a pestle until powdery samples were obtained. Exactly 2.0 g of the powdered sample was placed into a crucible and dried in a 180 °C oven to remove water and volatile compounds overnight. The dried nuts were digested with 1.0 mL of 70% HNO_3 at 80 °C for 1 h. The digested sample turned from yellowish to dark brown, was transferred into a Teflon beaker, and dissolved in 10.0 mL of 37 % HCl with gentle heating for about 1 h. This treatment allowed the conversion of all selenium to the electropositive Se(IV) species. To remove any residue of organic matter, the dissolved sample was filtered on a carbon column previously activated by 2.0 mL of methanol followed by 2.0 mL of ultrapure water. The filtrates appeared colorless.

Electrode Preparation: Plating. The electrodes were stored in ultrapure water. Before each analysis was started, they were well-cleaned with pure absolute methanol and filter paper. Before each analysis, the working electrode was plated with Hg^0 by electrolyzing a 20.0 mL volume of 1000.0 μg/mL Hg(II) standard solution at −950 mV for 1 min.

CSP Analysis. For the determination of selenium in nuts, 1.0 mL of the hydrochloric acid extracts and 9.0 mL of the 2 M $CaCl_2$/4 M HCl solution were placed into the electrochemical cell. With this exchange medium, O_2 interferences were avoided without the need for time-consuming deoxygenation processes (*14*). In fact, O_2 solubility in acid solutions of electrolytes is lower than in water (*14*, *18*, *19*). All of the electrochemical parameters are specified in **Table 1**.

The quantitative analysis was done by the multiple point standard addition method (*20*). Optimum precision and accuracy were obtained with the addition of two 0.1 mL aliquots of a 5.0 μg/mL standard solution of Se(IV).

Precision and Reproducibility Tests. The precision and reproducibility of the analytical method were evaluated by executing the extraction procedure three times on each nut sample and quantifying selenium four times in each extract. The instrument precision is indicated as the mean rsd % for each extract, and the method reproducibility is represented by the total mean rsd % for all of the extracts (**Table 2**).

Recovery Test. The possibility of loss or gain of Se, due to the extraction procedure, was explored. A 1 g sample of each powdered nut was spiked with varying volumes of a 1.0 μg/mL Se(IV) standard solution. The obtained mixtures were homogenized under magnetic stirring overnight. The spiked samples were then dried, and the extraction procedure previously described was executed. The recovery results are given in **Table 3**. To verify weather the drying step may cause selenium loss, a separate spike and recovery test, in which the samples of nuts were spiked with a known volume of a 1.0 μg/mL Se(IV) standard solution, was done at the HNO_3 digestion state. The results are given in **Table 4**.

Table 2. Instrument Precision ($n = 4$), Method Reproducibility (Expressed as Total rsd % and Calculated from Three Extracts), and Detection Limits for CSP Determination of Se(IV) in Nuts

	almond	hazelnut	pistachio
first extraction			
mean ± SD (μg/kg)	529 ± 4	866 ± 4	894 ± 4
rsd %	0.83	0.41	0.46
second extraction			
mean ± SD (μg/kg)	531 ± 5	864 ± 4	891 ± 4
rsd %	0.96	0.46	0.45
third extraction			
mean ± SD (μg/kg)	532 ± 5	864 ± 4	894 ± 4
rsd %	0.86	0.46	0.46
total mean ± SD (μg/kg)	531 ± 1	865 ± 1	893 ± 2
total rsd %	0.88	0.44	0.46
detection limit (μg/kg)	0.44	0.49	0.63

Table 3. Selenium Recoveries from Nuts[a]

	Se (μg/kg)	added (μg/kg)	expected (μg/kg)	found (μg/kg)	recovery %
almond	530.86	500.0	1030. 86	985 ± 6	95.3 ± 0.5
hazelnut	864.61	800.0	1664.61	1497 ± 6	90.7 ± 0.7
pistachio	892.82	900.0	1792.82	1617 ± 7	90.2 ± 0.5

[a] The percent recovery values are the mean of four determinations.

Table 4. Spike and Recovery Test at HNO_3 Digestion Stage[a]

	Se (μg/kg)	added (μg/kg)	expected (μg/kg)	found (μg/kg)	recovery %
almond	530.86	500.0	1030. 86	998 ± 7	96.8 ± 0.9
hazelnut	864.61	800.0	1664.61	1548 ± 9	93.0 ± 0.7
pistachio	892.82	900.0	1792.82	1664 ± 9	92.8 ± 0.6

[a] The percent recovery values are the mean of four determinations.

RESULTS AND DISCUSSIONS

The low concentration of selenium in different kinds of nuts required a highly sensitive method for its determination; CSP was well-suited for this purpose. The low detection limits of this technique were mostly due to the preconcentration of the trace selenium onto the mercury film of the working electrode.

The preconcentration of selenium(IV) occurred at −150 mV vs the Ag/AgCl reference electrode; the electrolysis time was 60 s

$$SeO_3^{2-} + Hg + 6H^+ + 4e^- \rightarrow Se^0(Hg^0) + 3H_2O$$

The deposited Se^0 was further reduced during the stripping step by a −30 μA cathodic constant current:

$$Se^0(Hg^0) + 2H^+ + 2e^- \rightarrow H_2Se + Hg$$

The selenium(II) peak appeared at −560 mV on the potentiogram (**Figure 1**). The electrolysis potential > −150 mV and the dissolution current > −15 μA resulted in a decrease of the

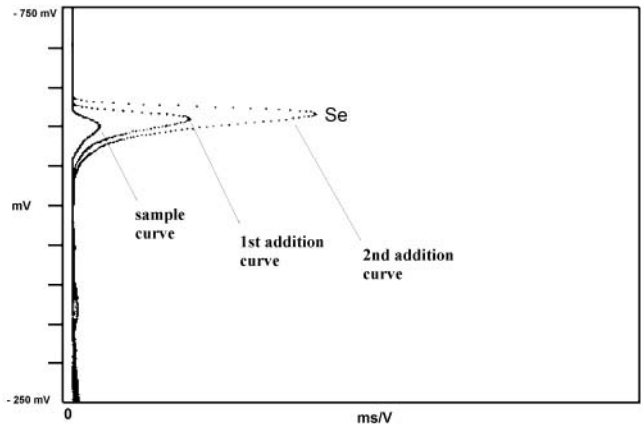

Figure 1. Selenium potentiogram as determined for a sample of nuts obtained under the experimental conditions specified in **Table 1**.

Table 5. Sensitivity and Detection Limits Values for CSP Determination of Selenium in Almond at Different Electrolysis Times[a]

t (s)	sensitivity (mV·kg/s·μg)	detection limit (μg/kg)
30	375	0.9
120	750	0.2
180	3375	0.1

[a] The electrolysis potential was –150 mV, the cathodic current was –30 μA, and the final acquisition potential was –750 mV.

peak resolution. The selenium peak area and the instrumental sensitivity increased with increasing electrolysis time; however, good resolution and sensitivity were obtained in the range of 30−180 s (**Table 5**). The applied electrochemical conditions are reported in **Table 1**. In these conditions, the linear concentration range was 0−1000 ng/g. Detection limits were evaluated using the expression $3\sigma/S$ (*22*, *23*): σ indicated the standard deviation of the response (set at 200 mV/s), and *S* was the sensitivity obtained from the slope of the calibration curve ($R^2 \geq 0.995$) (**Figure 2**); these values are given in **Table 6**. Instrumental reproducibility, calculated by four determinations on the same extract, was always <1.0 rsd % (**Table 2**). Sample preparation played an important role in ensuring the accuracy of determination, because selenium was present in very low concentrations in a complex matrix. A temperature higher than 190 °C could not be employed to destroy the organic matrix, because most of the inorganic and organic selenium compounds present in food matrixes (*8*) volatilitize in the temperature range

Table 6. Sensitivity, Detection Limits, and Correlation Coefficent Values of CSP Determination of Selenium in Nuts in the Electrochemical Conditions Described[a]

	almond	hazelnut	pistachio
sensitivity (mV kg/s μg)	1500	1200	1000
detection limit (μg/kg)	0.4	0.5	0.6
correlation coefficent	0.999	0.996	0.997

[a] The electrolysis potential was –150 mV for 60 s, the cathodic current was –30 μA, and the final acquisition potential was –750 mV.

Table 7. Percent Difference Calculated with Respect to HGAAS Selenium Determination

	HG-AAS (μg/kg) ($n = 4$)	CSP (μg/kg) ($n = 4$)	% difference
almond	520 ± 6	532 ± 1	+2.3
hazelnut	898 ± 8	865 ± 1	–3.7
pistachio	850 ± 7	893 ± 2	+5.0

of 190−315 °C (*9*, *10*). Nevertheless, chemical digestion at 80 °C of the dry, powdered nuts with 70% HNO_3, followed by HCl dissolution of the digested samples, successfully employed extracted selenium. A further purification of the acid extracts was achieved by the filtration on the carbon column. The extraction procedure reproducibility (**Table 2**), calculated by extracting in triplicate each nut sample, was <0.9% (expressed as total rsd %). The recovery tests confirmed that no significant loss occurred during the whole extraction procedure: the lowest recovery—90.2 ± 0.5%—was obtained from pistachio, followed by hazelnut (90.7 ± 0.7%) while the highest, 95.3 ± 0.5%, was from almond (**Table 3**). Moreover, the absolute percent differences in the results of the recoveries executed by spiking the samples at the HNO_3 digestion stage (**Table 4**) provided evidence that considering the standard deviation range, the drying step at 180 °C may cause a 1.0% selenium loss from almond and a 2.0% selenium loss from hazelnut and pistachio; this small fraction could be made up by highly volatile methylated selenium compounds, which are present in some food and environmental matrixes (*24*, *25*).

Table 7 shows that the results obtained by CSP determination of selenium in nuts, which favorably agreed with the results obtained by HGAAS analysis of the same samples; the absolute percent difference in the results ranged from −3.7 to 5.0%.

Moreover, the obtained results evidenced that the studied samples of Sicilian nuts are a good source of selenium (**Figure 3**): the highest content was found in pistachios and

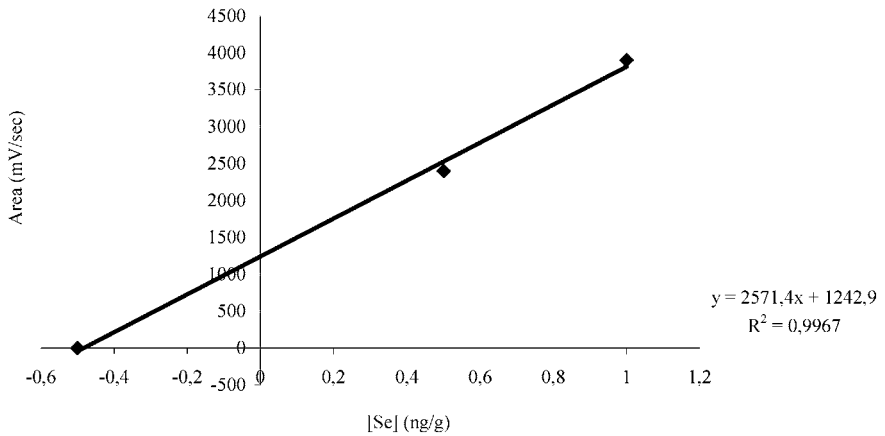

Figure 2. Calibration curve for selenium determination in nuts, obtained with the addition of two 0.1 mL aliquots of a 5.0 μg/mL standard solution of Se(IV).

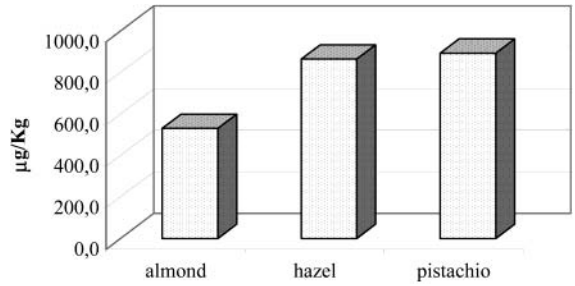

Figure 3. Mean selenium content of Sicilian samples of nuts.

hazelnuts (about 900 μg/kg), and the lowest was found in almonds (about 500 μg/kg). These levels are comparable to those in meat and seafood.

CONCLUSION

The proposed method has been used successfully to determine trace selenium levels in nuts. The method involves the use of relatively low sample preparation temperature and does not require a sample preconcentration step, which induces the selenium losses observed with other methods. CSP is an accurate, sensitive, and inexpensive technique and can be applied to other food and biological samples.

LITERATURE CITED

(1) Wendel, A. *Selenium in Biology and Medicine*, 1st ed.; Springer-Verlag: Berlin, 1989.
(2) Rayman, M. P. The importance of selenium to human health. *Lancet* **2000**, *356*, 233−241.
(3) McLaughlin, M. J.; Parker, D. R.; Clarke, J. M. Metals and micronutrients − food safety issues. *Field Crop Res.* **1999**, *60*, 143−163.
(4) Bayoumy, E. K. The protective role of Selenium on genetic damage and on cancer. *Mutat. Res*. **2001**, *475*, 123−129.
(5) WHO. *Selenium Environmental Health Criteria*; World Health Organization: Geneva, 1987; pp 58−65.
(6) Chang, J. C.; Gutenmann, W. H.; Raid, C. M.; Lisk D. J. Selenium content of Brasil nuts from two geografic location in Brasil. *Chemosphere* **1995**, *30*, 801−802.
(7) Cappelli, P.; Vannucchi, V. *Chimica Degli Alimenti. Conservazione e Trasformazione*, 3rd ed.; Zanichelli: Bologna, Italy, 1998; Chapter 26, p 549.
(8) Stadlober, M.; Sager, M.; Irgolic, K. J. effect of selenate supplemented fertilisation on the selenium level of cereals-identification and quantification of selenium compounds by HPLC−ICP-MS. *Food Chem*. **2001**, *73*, 357−366.
(9) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th ed.; Wiley & Sons: New York, 1988; Chapter 16, pp 513−515.
(10) *Aldrich Catalogue*; Aldrich, St. Louis, MO, 2003−2004; p 1638.
(11) Finely, J. W.; Matthys, L.; Shuler, T.; Korynta, E. Selenium content of food purchased in North Dakota. *Nutr. Res.* **1996**, *16*, 723−728.
(12) Stoica, A. I.; Babaua, J. R.; Iorgulescu, E.; Marinescu, D. Differential pulse cathodic stripping voltammetric determination of selenium in pharmaceutidcal products. *J. Pharm. Biomed. Anal*. **2002**, *30*, 1425−1429.
(13) McCarthy, T. P.; Brodie, B. B.; Milner, J. A.; Bevill, R. F. Improved method for selenium determination in biological samples by gas chromatography. *J. Chromatogr*. **1981**, *225*, 9−16.
(14) Adeloju, S. B.; Jagner, D.; Renman, L. Cathodic stripping potentiometric determination of selenium in biological and environmental materials on a combined electrode with a rotating sample platform. *Anal. Chim. Acta* **1997**, *338*, 199−207.
(15) La Pera, L.; Lo Curto, S.; Visco, A.; La Torre, L.; Dugo, G. Derivative potentiometric stripping analysis (dPSA) used for determination of cadmium, copper, lead and zinc in Sicilian olive oils. *J. Agric. Food. Chem*. **2002**, *50*, 3090−3093.
(16) Salvo, F.; La Pera, L.; Di Bella, G.; Nicotina, M.; Dugo, G. Influence of mineral and organic pesticides treatments on Cd (II), Cu (II) Pb (II) and Zn (II) content determined by Derivative Potentiometric Stripping Analysis in Italian Red and white wines. *J. Agric. Food Chem.* **2002**, *51*, 1090−1094.
(17) La Pera, L.; Saitta, M.; Di Bella, G.; Dugo, G. Simultaneous determination of Cd (II), Cu (II) Pb (II) and Zn (II) in citrus essential oils by derivative potentiometric stripping analysis. *J. Agric. Food Chem.* **2002**, *51*, 1125−1129.
(18) Silvestroni, P. *Fondamenti di Chimica*, 9th ed.; Masson: Milan, Italy, 1993; Chapter 8, pp 258−259.
(19) Atkins, P, W. *Chimica Fisica*, 2nd Italian ed.; Zanichelli: Bologna, Italy, 1997; Chapter 8, pp 177−178.
(20) Renman, R.; Jagner, D. Asymmetric distribution of results in calibration curve and standard addition evaluation. *Anal. Chim. Acta* **1997**, *357*, 157−166.
(21) Inam, R.; Somer, G. A direct method for the detrmination of selenium and lead in cow's milk by differential pulse cathodic stripping voltammetry. *Food Chem*. **2000**, *69*, 345−350.
(22) Lo Coco, F.; Monotti, P.; Rizzotti, S.; Ceccon, L. Determination of lead in oil products by derivative potentiometric stripping analysis. *Anal. Chim. Acta* **1999**, *386*, 41−46.
(23) Pharmeuropa. The European Pharmacopoeia Forum. *Technical Guide for the Elaboration of Monograph*, 3rd ed.; December 1999; p 66.
(24) Ganther, H. E.; Lawrence, J. R. Chemical tranformation of selenium in living organism. Improved forms of selenium for cancer prevention. *Tetrahedron* **1997**, *53*, 12299−12310.
(25) Pyrzynsca, K. Analysis of Selenium species by capillary electrophoresis. *Talanta* **2001**, *55*, 657−667.
(26) Amouroux, D.; Donard, O. F. X. Evasion of selenium volatile to the atmosphere via biomethylation process in the Gironde estuary, France. *Mar. Chem*. **1997**, *58*, 173−188.

Received for review December 30, 2002. Revised manuscript received March 19, 2003. Accepted March 27, 2003.

JF021256M