JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLES

Determination of Selenium Content in Different Types of Seed Oils by Cathodic Stripping Potentiometry (CSP)

GIACOMO DUGO,* LARA LA PERA, DONATELLA POLLICINO, AND MARCELLO SAITTA

Dipartimento di Chimica Organica e Biologica, University of Messina, Salita Sperone 31, 98166 Messina, Italy

Seed oils are consumed worldwide; moreover, they are used in the alimentary, cosmetic, pharmaceutical, and chemical industries. Due to their diffusion, it is interesting to investigate the presence of important micronutrients such as selenium in seed oils. The aim of this work was to develop a rapid, precise, and sensitive cathodic stripping potentiometry (CSP) method to determine the concentration of selenium in different types of seed oils. Selenium was extracted from the oily matrix by concentrated hydrochloric acid treatment at 90 °C. The analysis was executed by applying an electrolysis potential of −150 mV for 60 s and a constant current of −30 μA. Under these conditions, detection limits of <0.5 ng g^{-1} were obtained. The method reproducibility (expressed as total RSD %) spanned from 0.2 to 0.8%. Recoveries ranged from 92.1 to 97.5%, providing evidence that selenium quantification remained unaffected by the extraction procedure described. The results obtained with the proposed method were compared with those obtained via graphite furnace atomic absorption spectroscopy (GFAAS), a common method for determining selenium. The results of the two methods agreed within 93.5−107.7%. The mean amounts of selenium found were 313.0 ± 2.0, 458.3 ± 1.3, 224.6 ± 0.9, 99.5 ± 0.8, 332.2 ± 0.5, 144.0 ± 0.7, and 295.5 ± 1.2 ng g^{-1}, respectively, in peanut, soybean, sunflower, rice, corn, grapestone, and seed oils.

KEYWORDS: Cathodic stripping potentiometry; seed oils; selenium

INTRODUCTION

The trace mineral selenium is an essential nutrient of fundamental importance to human biology. It is a component of a large number of enzymes, in which it is present as the amino acid selenocysteine (*1*). The well-characterized selenium-containing enzyme glutathione peroxidase (*2*) inhibits the oxidation of lipid membranes by free radicals, thus preventing pathologies brought on by oxidative stress such as inflammation, atherosclerosis, and cardiovascular diseases (*3*). Moreover, recent research correlates the process of aging and uncontrolled tumor growth to a poor dietary selenium intake (*4*). Selenium proteins are also involved in the production of the active thyroid hormone, in muscle function, in the reproduction process (*5*), and in the immune response to some infections (*6*). The antioxidant and antitumorigenic activities of selenium have increased the interest in studying its presence in food. Selenium enters the food chain through plants that take it up from the soil, so the availability of selenium depends heavily on geography and season (*1*). The Recommended Dietary Allowance (RDA) for an adult is 0.05−0.07 mg/day (*7*); meanwhile, effects from poisoning may appear at concentrations >0.4 mg/day (*4*). Selenium is present in food matrices in organic (selenoamino acids, selenoproteins, and organoselenium) and inorganic (selenite and selenate) forms (*1*, *2*). Good sources of selenium are meat and seafood (0.4−1.5 mg/kg), cereals and cereal products (0.1−0.8 mg/kg), Brazil nuts (0.2−1.0 mg/kg), and garlic (0.3−0.5 mg/kg). The levels of selenium in fresh fruits, vegetables, and dairy products are much lower (<0.01 mg/kg) (*8*−*11*). The literature reports few data about the selenium content of vegetable oils (0.1−0.8 mg/kg) (*12*, *13*). Selenium quantification in oily matrices is very problematic because this micronutrient is present in very low concentrations; moreover, most inorganic and organic selenium compounds volatilize at temperatures >200 °C (*15*, *16*). Therefore, multistep sample preparation processes and analytical methods that involve the use of high temperature—such as dry ashing (*17*) followed by atomic adsorption spectroscopy (*10*, *18*) or gas chromatography (*19*)—may cause a severe selenium loss and are time-consuming. The aim of this paper was to develop a rapid and precise method comprising concentrated hydrochloric acid extraction followed by cathodic stripping potentiometric analysis (CSP) (*17*, *20*) to determine the selenium content in different

10.1021/jf0300792 CCC: $25.00
Published on Web 08/08/2003

Table 1. Instrument Precision ($n = 4$) and Method Reproducibility (Expressed as Total RSD Percent and Calculated from Three Extracts) for CSP Determination of Se(IV) in Seed Oils

	peanuts	soybean	sunflower	rice	corn	grapestone	seeds
first extraction							
mean ± SD (ng g^{-1})	311.9 ± 4.9	457.0 ± 4.2	224.2 ± 2.8	98.8 ± 3.9	332.7 ± 3.7	144.7 ± 3.9	294.1 ± 3.4
RSD %	1.6	0.9	1.2	4.0	1.1		1.2
second extraction							
mean ± SD (ng g^{-1})	311.7 ± 4.4	458.4 ± 4.0	225.6 ± 2.9	99.5 ± 3.2	331.7 ± 3.1	143.4 ± 3.1	296.0 ± 3.9
RSD %	1.4	0.9	1.3	3.3	0.9		1.3
third extraction							
mean ± SD (ng g^{-1})	315.2 ± 3.6	459.6 ± 4.8	223.9 ± 2.8	100.3 ± 3.0	332.2 ± 1.1	143.9 ± 3.2	296.3 ± 4.1
RSD %	1.1	1.1	1.3	3.0			1.4
mean RSD %	1.4	0.9	1.3	3.4	1.0	2.4	1.3
total mean ± SD (ng g^{-1})	313.0 ± 2.0	458.3 ± 1.3	224.6 ± 0.9	99.5 ± 0.8	332.2 ± 0.5	144.0 ± 0.7	295.5 ± 1.2
total mean RSD %	0.6	0.3	0.4	0.8	0.2	0.5	0.4

types of commercial seed oils: peanut, soybean, sunflower, corn, grapestone, seeds. A glassy carbon mercury film electrode (GCMFE) was employed as the working electrode; this electrode has been used previously in the determination of trace metals in various food matrices (*21*−*23*). At first, Se(IV) was electrolytically accumulated onto the mercury film as Se^0-Hg^0 at unvarying potential; then, the potential decreased and Se^0 was further reduced to Se(−II) and redissolved by a cathodic current (*17*, *20*). Potential and time data were digitally converted into d*t*/d*E* and plotted against *E*, obtaining a Gaussian curve.

MATERIALS AND METHODS

Reagents. Commercial peanuts, soybean, sunflower, corn, grapestone, and seed oils were sampled and stored in dark glass bottles at 4 °C until analysis.

All of the reagents used were of analytical grade. Hydrochloric acid (34−37%) and Se(IV) (1000 μg mL^{-1}, 0.5 N in HNO_3) standard solutions were purchased from Panreac (Barcelona, Spain). The Se(IV) standard solution was diluted with ultrapure water to obtain 5.0 and 10.0 μg mL^{-1} Se(IV) solutions. Anhydrous $CaCl_2$ (J. T. Baker, Deventer, The Netherlands) was used to prepare a 2 M aqueous solution of $CaCl_2$ solution, 4 M HCl. The oil extracts were filtered on a carbon column Supelclean ENVI-Carb SPE (0.5 g, 6 mL) from Supelco (Bellefonte, PA). The carbon columns were activated by methanol (Carlo Erba Reagenti, Milan, Italy). Ultrapure water (18.2 Mohm cm^{-1}) 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 (*19*−*21*). The determination was executed in a conventional three-electrodes cell: the working electrode was a glassy carbon one 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. To confirm the analytical results of CSP, the oily extracts were subjected to AAS, using a Shimadzu 800 series graphite furnace atomic absorption spectrometer, equipped with autosampler ASC-6100.

Sample Preparation. A 5.0 g aliquot of each seed oil sample, 2 mL of 35% H_2O_2 and 5.0 mL of 36% ultrapure hydrochloric acid were placed in a Teflon beaker. The extraction was carried out for ∼30 min under magnetic stirring at the temperature of 90 °C. The mixture was transferred in a separating funnel: the acid phase was taken apart in a 10.0 mL flask while the organic layer was extracted twice with 2.5 mL of concentrated hydrochloric acid for 10 min under the conditions described earlier. The treatment with concentrated HCl allowed the conversion of all selenium to the electropositive Se(IV) species (*17*, *20*, *24*). To remove any oily residue, the collected acid phases were filtered on a carbon column previously activated by 2.0 mL of methanol followed by 2.0 mL of ultrapure water. Sample preparation took ∼1 h.

Table 2. Selenium Recoveries from Seed Oils

oil	Se (ng g^{-1})	added (ng g^{-1})	found (ng g^{-1})	expected (ng g^{-1})	recovery[a] (%)
peanut	313.0	100.0	400.5	413.0	97.0
	313.0	300.0	605.0	613.0	98.7
	313.0	500.0	786.2	813.0	96.7
					97.5 ± 1.1
soybean	458.0	200.0	630.5	658.0	95.8
	458.0	450.0	868.6	908.0	95.6
	458.0	600.0	993.3	1058.0	93.9
					95.1 ± 1.0
sunflower	225.0	100.0	301.5	325.0	92.8
	225.0	200.0	399.0	425.0	93.8
	225.0	400.0	598.8	625.0	95.8
					94.1 ± 1.5
rice	99.5	50.0	140.7	149.5	94.1
	99.5	100.0	189.6	199.5	95.0
	99.5	200.0	285.4	299.5	95.3
					94.8 ± 0.6
corn	332.0	100.0	400.0	432.0	92.6
	332.0	300.0	595.1	632.0	94.1
	332.0	500.0	793.1	832.0	95.3
					94.0 ± 1.4
grapestone	144.0	50.0	180.5	194.0	95.0
	144.0	150.0	279.3	294.0	94.3
	144.0	300.0	418.8	444.0	94.4
					94.4 ± 0.5
seeds	296.0	100.0	375.5	396.0	94.8
	296.0	300.0	564.4	596.0	94.7
	296.0	500.0	769.1	796.0	96.6
					95.4 ± 1.1
blank	nd[b]	10.0	9.3	10.0	93.0
	nd	50.0	45.6	50.0	91.2
	nd	100.0	92.1	100.0	92.1
					92.1 ± 0.9

[a] Percent recovery values are the mean of four determinations. [b] Not detectable (<0.4 ng g^{-1}).

Electrode Preparation: Plating. The electrodes were stored in ultrapure water; before the start of each analysis, 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 mg/mL Hg(II) standard solution at −950 mV for 1 min.

Cathodic Stripping Potentiometric Analysis. For the determination of selenium in seed oils, 2.0 mL of the hydrochloric acid extracts and 10.0 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. In fact, O_2 solubility in acid solutions of electrolytes is lower than in water (*17*, *25*).

The preconcentration of Se(IV) occurred at −150 mV versus an Ag/AgCl reference electrode, and the electrolysis time was 60 s. The deposited Hg−Se was further reduced to Se(−II) during the stripping

Table 3. Sensitivity and Detection Limits for CSP Selenium Determination in Seed Oils

	peanuts	soybean	sunflower	rice	corn	grapestone	seeds
sensitivity (mV g s^{-1} ng^{-1})	9780.4	7516.6	8835.6	10400.0	11506.3	9010.5	11660.0
detection limit (ng g^{-1})	0.4	0.5	0.4	0.3	0.3	0.4	0.3
correlation coefficent	0.998	0.995	0.999	0.999	0.995	0.995	1.0

Table 4. Reliability Calculated with Respect to ZGFAAS Selenium Determination in Different Types of Seed Oil[a]

oil	CSP (ng g^{-1})	AAS (ng g^{-1})	reliability (%)
peanut	315.2 ± 3.6	310.5 ± 5.0	101.5
soybean	459.6 ± 4.8	465.7 ± 7.1	98.7
sunflower	223.9 ± 2.8	228.3 ± 3.7	98.1
rice	100.3 ± 3.0	93.1 ± 3.9	107.7
corn	332.2 ± 0.5	325.5 ± 6.0	102.2
grapestone	144.0 ± 0.7	153.9 ± 2.7	93.5
seeds	296.3 ± 4.1	295.2 ± 6.6	100.4

[a] Each value is the mean of four determination.

step by a −30 μA cathodic constant current while the potential decreased to −750 mV. The peak of selenium appeared at −580 mV, and the final acquisition potential was −780 mV. The quantitative analysis was executed by the multiple points standard additions method (*26*): optimum precision and accuracy were obtained by executing two 0.05 or 0.10 mL standard additions of 5.00 μg mL^{-1} of Se(IV) standard solution and performing each measurement four times. Each analysis lasted 20 min.

Precision and Reproducibility Tests. The precision and reproducibility of the analytical method were evaluated by executing the extraction procedure three times on each oil sample and quantifying selenium four times in each extract. The instrument precision is indicated as the mean relative standard deviation (RSD) percent for each extract, and the method reproducibility is represented by the total mean RSD percent for all of the extracts. The obtained results are reported in **Table 1**.

Recovery Test. The possibility of gain or loss of Se amount, due to the extraction procedure, was explored. Aliquots of 5.0 g of each seed oil sample were separately spiked at different levels with 10.0 mg mL^{-1} Se(IV) standard solution. To homogenize the obtained mixture, it was subjected to magnetic stirring overnight (*21*). Then the extraction procedure described earlier was executed. Both spiked and unspiked samples of each type of oil were analyzed. A recovery from a selenium-free oily matrix (blank) was performed by spiking the sample with 10.0, 50.0, and 100.0 ng g^{-1} of 10.0 μg mL^{-1} Se(IV) standard solution. Then the procedure stated above was executed and the recovery percent calculated; the obtained results are given in **Table 2.**

Detection Limits. Detection limits were evaluated using the expression $3\sigma/S$·cdr (*22*, *23*, *27*, *28*): σ indicated the standard deviation of the response (set at 200 mV/s), S was the sensitivity obtained from the slope of the calibration curve ($R^2 \geq 0.995$), and cdr indicated the cell dilution ratio **(Table 3)**.

ZGFAAS Confirmation Analysis. The seed oil samples were analyzed in triplicate by Zeeman graphite furnace atomic absorption spectroscopy for Se determination. The analysis was carried out by adding, for each 20.0 μL injection, 5.0 μL of a $Pd(NO_3)_2$ solution (Pd concentration = 100 μg mL^{-1}), as matrix modifier. The working wavelength was 196.0 nm. Obtained results are given in **Table 4**.

RESULTS AND DISCUSSION

Cathodic stripping potentiometry (CSP) is a precise, rapid, and inexpensive technique; moreover, its sensitivity allows trace selenium quantification in different types of seed oil. The high sensitivity of this method is mostly due to the preconcentration of selenium onto the mercury film of the working electrode.

The preconcentration of Se(IV) occurred at −150 mV versus the Ag/AgCl reference electrode, and the electrolysis time was

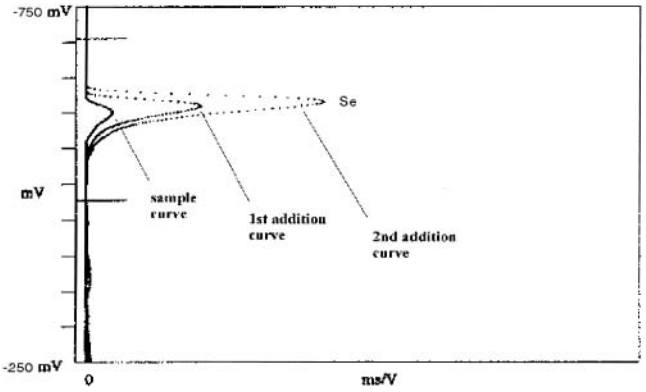

Figure 1. Selenium potentiometric curve.

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 Se(−II) peak appeared at −580 mV on the potentiogram (**Figure 1**). The sensitivity may be enhanced by increasing the electrodeposition time (*20*). Under the conditions described, the detection limits obtained were 0.3 ng g^{-1} for rice, corn, and seed oils, 0.4 ng g^{-1} for peanut, sunflower, and grapestone oils, and 0.5 ng g^{-1} for soybean oil (**Table 3**); the linear concentration range was 0−800.0 ng g^{-1}. The instrument precision, calculated by four determinations on the same extract, was always <2.0 RSD %; the method reproducibility parameters (expressed as the total mean RSD %) for the studied vegetable oils ranged from 0.2 to 0.6% (**Table 1**). Sample preparation was important in ensuring the accuracy of determination because selenium was present in very low concentrations in a complex matrix. Temperatures >190 °C could not be employed to destroy the organic matrix because most of the inorganic and organic selenium compounds present in food matrices (*14*) volatilize in the temperature range 190−315 °C (*15*, *16*). The simple extraction procedure described involved the use of relatively low temperatures, avoiding the selenium losses observed with other methods that require the use of high temperatures (*17*, *29*). Moreover, the hydrochloric acid extraction was less time-consuming than dry ashing and mixed acid digestion methods and required few reagents (*17*, *29*, *30*). The recovery test provided evidence that selenium quantification remained unaffected by hydrochloric acid treatments followed by the filtration on a carbon column: mean obtained recoveries spanned from 94.0 ± 1.4 to 97.5 ± 1.1%, and the recovery from the blank was 92.1 ± 0.9% (**Table 2**). **Table 4** shows that the results obtained by CSP determination of selenium in seed oils favorably agreed with those obtained by GFAAS analysis of the same samples; the reliability parameters ranged from 93.5 to 107.7%. The obtained result shows that soybean oil had the highest content of selenium (458.3 ± 1.3 ng g^{-1}), followed by

corn oil (332.2 ± 0.5 ng g^{-1}), peanut oil (313.2 ± 2.0 ng g^{-1}), and seed oil (295.5 ± 1.2 ng g^{-1}), which showed similar values. Sunflower (224.6 ± 0.9 ng g^{-1}) oil and grapestone oil (144.0 ± 0.7 ng g^{-1}) had lower selenium amounts; the lowest concentration was found in rice oil (99.5 ± 0.8 ng g^{-1}).

In conclusion, the proposed method enabled the determination of trace selenium in seed oil, avoiding the use of too high temperature, which is the main cause of selenium losses. CSP, besides being an accurate and sensitive technique, is rapid enough to allow routine analyses. Furthermore, the utilized technique is cheaper than atomic absorption spectroscopy methods, with regard to both lower equipment cost and easier instrument maintenance.

LITERATURE CITED

(1) McLaughlin, M. J.; Parker, D. R.; Clarke, J. M. Metals and micronutrients—food safety issues. *Field Crop Res.* **1999**, *60*, 143−163.

(2) Stadtman, T. C. Biosynthesis and functions of selenocysteine-containing enzymes. *J. Biol. Chem.* **1991**, *266*, 16257−16263.

(3) Combs, G. F.; Gray, W. P. Chemopreventive agents: Selenium. *Pharmacol. Ther.* **1998**, *79*, 179−192.

(4) El Bayoumy, K. The protective role of selenium on genetic damage and on cancer. *Mutat. Res.* **2001**, *475*, 123−129.

(5) Rayman, M. P. The importance of selenium to human health. *Lancet* **2000**, *356*, 233−241.

(6) Dhur, A.; Galan, P.; Hercberg, S. Relationship between selenium, immunity and resistance to infections. *Comp. Biochem. Physiol.* **1990**, *96C*, 271−280.

(7) Kaim, W.; Schwedersky B. *Bioinorganic Chemistry: Inorganic Elements in The Chemistry of Life*, 1st ed.; Wiley: Chichester, U.K., 1994; Chapter 2, p 13.

(8) WHO. *Selenium Environmental Health Criteria*; Geneva, Switzerland, 1987; pp 58−65.

(9) Chang, J. C.; Gutenmann, W. H.; Raid, C. M.; Lisk, D. J. Selenium content of Brasil nuts from two geographic location in Brasil. *Chemosphere* **1995**, *30*, 801−802.

(10) Finley, J. W.; Matthys, L.; Shuler, T.; Korynta, E. Selenium content of food purchased in North Dakota. *Nutr. Res.* **1996**, *16*, 723−728.

(11) Inam, R.; Somer, G. Determination of selenium in garlic by cathodic stripping voltammetry. *Food Chem.* **1999**, *66*, 381−385.

(12) Bratakos, M. S.; Zapiropoulos, T. F.; Siskos, P. A.; Ioannou, P. V. Selenium in food produced and consumed in Greece. *J. Food Sci.* **1987**, *52*, 817−822.

(13) Simonoff, M.; Hamon, C.; Moretto, P.; Labador, L.; Simonoff, G. Selenium in food in France. *J. Food Compos. Anal.* **1988**, *1*, 295−302.

(14) 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.

(15) Cotton, F. A.; Wilkinson G. *Advanced Inorganic Chemistry*, 5th ed.; Wiley: New York, 1988; Chapter 16, pp 513−515.

(16) Aldrich Catalogue 2003−2004, p 1638.

(17) 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.

(18) Kadrabova, J.; Madaric, A.; Ginter, E. The selenium content of selected food from Slovak Republic. *Food Chem.* **1997**, *58*, 29−32.

(19) 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.

(20) Dugo, G.; La Pera, L.; Lo Turco, V.; Mavrogeni, E.; Alfa, M. Determination of selenium in nuts by cathodic stripping potentiometry. *J. Agic. Food Chem.* **2003**, in press.

(21) 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.

(22) 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.* **2003**, *51*, 1090−1094.

(23) 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.* **2003**, *51*, 1125−1129.

(24) 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.

(25) Atkins, P. W. *Chimica Fisica*, 2nd Ital. ed.; Zanichelli: Bologna, Italy, 1997; Chapter 8, pp 177−178.

(26) Renman, R.; Jagner, D. Asymmetric distribution of results in calibration curve and standard addition evaluation. *Anal. Chim. Acta* **1997**, *357*, 157−166.

(27) Pharmeuropa. The European Pharmacopoeia Forum. *Technical Guide for the Elaboration of Monograph*, 3rd ed.; Dec 1999; p 66.

(28) 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.

(29) Zhang, M.; Yuan, L.; Tian, J. Determination of selenium in rice, the bulb of garlic and tea by flame AAS with a slotted quartz tube. *Spectrosc. Spectral Anal.* **1994**, *14*, 101−104.

(30) Guanghan, L.; Jianhua, L.; Yiping, H.; Fang, W. Polarographic determination of selenium in tea leaves using a dropping mercury electrode. *Food Chem.* **1996**, *56*, 177−180.

Received for review January 31, 2003. Revised manuscript received April 16, 2003. Accepted April 22, 2003.

JF0300792