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Determination of selenium in Italian rices by differential pulse cathodic stripping voltammetry

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Abstract

The total selenium content in white, black, red rice and white rice hull samples, grown in Northern Italy cultivars, has been determined using the differential pulse cathodic stripping voltammetry (DPCSV) on the hanging drop mercury electrode (HDME), in the presence of Cu(II). The digestion was performed in open vessel through a combination of wet acid/dry ashing with Mg(II) salts. The calibration curve was linear in the concentration range 0.15–8 ppb, the detection limit was estimated to be 0.07 ppb, and the recovery was in the range 85–102%. Reproducibility was from 1.9% to 9.0% (RSD, $n = 4$). Rice samples were analyzed by the standard addition method and the results were compared with those obtained by a spectroscopic technique (HG-ICP-AES). The proposed procedure, sensitive, inexpensive, easy-to-handle and precise can be successfully applied for the determination of selenium in nutritional products. The resulting selenium contents in different Italian rice varieties were: 20.1 ± 1.8 ppb (white), 53.0 ± 1.0 ppb (red), 26.7 ± 1.3 ppb (black), 45.3 ± 4.1 ppb (white rice hull).
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1. Introduction

Selenium is a trace element essential to human health since it is incorporated in the active center of antioxidant seleno-enzymes (glutathione peroxidase and thioredoxin reductase) (Szpunar, 2005). Owing to these antioxidant properties, an adequate alimentary selenium supply is claimed to delay the onset of aging, cardiovascular diseases and cancer, enabling an optimal immune response, and to guarantee an appropriate functionality of the endocrine system indispensable for the male reproductive system (Ip, 1998).

Geographical differences in the content and availability of selenium from soil to foods, (Kelly et al., 2002) combined with environmental conditions and cultivation practices, (Gupta, Gupta, & Gupta, 2000; Fordyce, Johnson, Navaratna, Appleton, & Dissanayake, 2000) have deep influence on the selenium dietary intake in human beings of different communities.

The selenium Recommended Dietary Allowance (RDA) in humans is 70 μg/day, although supra-nutritional supplementation of 100–150 μg/day in population at high risk of skin and breast carcinoma is considered to be chemo-preventive and strongly recommended. Anyway, a dietary intake around 350 μg/day has been found to be toxic (Reilly, 1998). Since the range between beneficial effect and toxicity of this trace element is narrow, and in many countries most of the dietary selenium comes from grain foods, such as meals (wheat or rice) and cereals, reliable and sensitive methods have to be used for the determination of selenium concentration in biological samples where

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this volatile element is present at low concentration level and embedded in a complex matrix. In fact, organic selenium is generally present in plants or in animals in the form of selenoamino acids, like selenomethionine or selenocysteine, and their derivatives or some methylated species (Suzuki, 2005).

Many papers concerning selenium determination in rices have been published, but most of them deal with white rice and its meals. In fact, white rice accounts for more than 85% of the consumed rice, but also coloured rices (red and black), are grown in South Asia and other countries, such as Italy, Greece and United States, for dietary purposes. Interest in coloured rice varieties has grown up since these products have been shown to possess remarkable properties in the prevention of cardiovascular (Ling, Cheng, Ma, & Wang, 2001; Ling, Wang, & Ma, 2002) and cancer diseases (Ip, 1998).

At present, only a few studies exist in the literature on the evaluation of selenium content in coloured rice; they are limited to products coming from Asian countries and barely accessible since written in native languages.

This prompted us to evaluate the level of selenium in different rice varieties grown in Northern Italy, and to compare their contents with those reported in literature for rices grown in other European and extra-European countries.

In general, the methods for the analysis of selenium in foods involve a step of acid digestion for the decomposition of the organic matter followed by selenium determination by an instrumental technique, such as hydride generation coupled with either atomic absorption spectroscopy HG-AAS (Bujdos, Kubova, & Stresko, 2000; Narasaki & Ikeda, 1984) or with inductively coupled plasma atomic emission spectroscopy ICP-AES (Hahn, Wolnik, & Fricke, 1982; Yang & Husain, 2006), graphite furnace atomic absorption spectroscopy (GF-AAS) (Deaker & Maher, 1997), and differential pulse polarography (DPP) combined with cathodic stripping voltammetry (CSV) or differential pulse cathodic stripping voltammetry (DPCSV) (İnam & Somer, 2000; Korolczuk & Grabarczyk, 2003; Ochsenkuhn-Peropoulou & Tsopelas, 2002; Van den Berg & Khan, 1990).

Actually, voltammetric methods, if compared with techniques involving atomic spectroscopy offer some important advantages: (i) the instrumentation is relatively inexpensive and easy to handle; (ii) the CSV response for Se(IV) is highly sensitive, allowing lower detection limits; (iii) the determination is given with good precision and accuracy (Ferri, De Luca, & Ticconi, 2001; Locatelli, 2004).

However, these methods are susceptible to matrix effects and other interferences (Adeloju, Bond, & Briggs, 1984), and frequently the digestion procedures that are suitable for atomic spectroscopy analysis may be inadequate for voltammetry, because of incomplete decomposition of the organic matrix, or incomplete reduction to the electroactive species Se(IV).

Actually, two groups of digestion procedures can be distinguished:

1. Wet digestion procedures: mineralization is performed by heating the sample in the presence of acids mixtures and oxidizing agents such as HNO_3, $HClO_4$, H_2SO_4 and H_2O_2, in variable amounts. Anyway these additives may decompose the organic matter only partially, and hamper the selenium determination by the voltammetric techniques (Lambert & Turoczy, 2000).
2. Dry oxidation or ashing: within this group, calcination at high temperature (450 °C in a muffle furnace) is frequently used. It ensures the quantitative removal of organic matter and allows the digestion of large sample amounts, very useful if trace elements must be detected. The ash is free of organic matter and therefore the digested solution is suitable for the electroanalytical methods. However, an important drawback of this kind of digestion is the possible analyte loss connected to the high volatility of selenium. To prevent this, an oxidative pre-digestion with nitric acid followed by addition of magnesium nitrate as ashing aid is commonly employed (Mindak & Dolan, 1999).

In this work, we have used the DPCSV as an analytic technique after a combined wet acid/dry ashing digestion of the sample (with $Mg(NO_3)_2 \cdot 6H_2O$ as ashing aid), (Higham & Tomkins, 1993; Lambert & Turoczy, 2000) to ensure the quantitative removal of organic matter. A successive dissolution step with HCl has been added, to ensure the complete reduction to Se(IV).

The DPCSV procedure was optimised for our rice samples, varying the main voltammetric parameters. The reliability of the method has been confirmed by comparing the voltammetric results with those obtained using inductively-coupled-plasma atomic-emission spectrometry, with hydride generation (HG-ICP-AES).

2. Materials and methods

2.1. Instrumentation

Samples were digested in Pyrex glassware using a sand bath (Gerhardt, containing sand washed with HCl/HNO_3) and a muffle furnace. DPCSV was performed using an EcoChemie Autolab PGSTAT 12 potentiostat/galvanostat, run by a PC with GPES software and equipped with a Metrohm 663 VA Stand, together with a hanging-drop mercury electrode (HDME, Metrohm). A platinum wire was used as counter electrode and a saturated calomel electrode (SCE) as reference electrode. The determinations were performed in a glass cell thermostated at 298 K, on solutions purged with nitrogen.

The analytical instrumentation used for the determination of selenium by HG-ICP-AES was an OPTIMA 3300 DV instrument (Perkin–Elmer, Milan, Italy). Hydrides were generated using a FIMS-100 apparatus (conventional

500 μL loop and plastic liquid separator, Perkin–Elmer, Milan, Italy) and nebulized using an adapted Scott's chamber. Instrumental parameters were set as follows: plasma flow 18 L/min, auxiliary gas flow 0.6 L/min, nebulizer gas flow 0.15 L/min, peristaltic pump flow 2.5 ml/min, emission wavelength 196 nm.

Automatic micropipettes [Gilson P20 (2–20 μL), Gilson P200 (50–200 μL) and Gilson P5000 (1–5 mL)] were used for the reagent additions.

2.1.1. Reagents

Suprapur® grade nitric acid (Merck, 65% w/w) and hydrochloric acid (Merck, 37% w/w) and analytical grade $Mg(NO_3)_2 \cdot 6H_2O$ (Fluka) were used in the digestion procedure and analytical grade MgO (Fluka) was used for the preparation of blank solutions. Hydrochloric acid (0.1 M, Merck) was used as supporting electrolyte in the voltammetric analysis. The Se(IV) working solution (1.0×10^{-3} M) was prepared diluting a commercial standard Se(IV) solution for atomic spectroscopy (Aldrich, 994 ppm) with 0.1 M HCl, and kept in polythene bottles at 277 K for no more than one month. The diluted selenium solutions (about 1.0×10^{-4} M and 1.0×10^{-5} M) were freshly prepared by diluting concentrated working solution with 0.1 M HCl. A standard solution of Cu(II) for atomic spectroscopy (1020 ppm, Aldrich) was used to make Cu(II) additions. Stock solutions of different trace metals, used to test metal interferences, were prepared by dilution of standard solutions for atomic spectroscopy (1000 ppm, Aldrich). Ultrapure water (resistivity 18 MΩ/cm^2, milli-Q, Millipore) was used for the preparation of all solutions. Mercury (99.9999%, Fluka) is used in the hanging-drop mercury electrode (HDME). Before use, glassware was treated with 7 M HNO_3 at 120 °C for at least 5 h, maintained in milli-Q water acidified with Suprapur® HNO_3 (4:1 ratio), and washed with Milli-Q water just before use.

2.2. Experimental procedure

2.2.1. Sample digestion

The digestion method used in this study consists of a combination of wet acid and dry ashing techniques, in which $Mg(NO_3)_2 \cdot 6H_2O$ was used as an ashing aid. The concentrated HNO_3 (10 mL, 65% w/w) and 4.0 g of $Mg(NO_3)_2 \cdot 6H_2O$ were added to 1.0 g of dried rice/rice product sample (the flour was left for 1 day in oven at 60 °C to remove water content and obtain a constant weight) into a 50 mL tall-form beaker. The beaker was then covered with a watch-glass and the sample was allowed to predigest at room temperature overnight. The pre-digested material was progressively heated at about 373 K in a sand bath and slowly evaporated to dryness within a period of 5 h. The temperature of the sand bath was then raised to 473 K until no more fumes evolved. The beaker was finally placed in a muffle furnace at the temperature of 723 K for 30 min, then removed and allowed to cool. The white residue was dissolved in 7 mL of 6 M HCl and the beaker was then heated at 373 K in a sand bath for about ten minutes in order to reduce Se(VI) to Se(IV). After the reduction step the solution was transferred from the beaker to a 25 mL volumetric flask, the beaker was thoroughly washed with 5 ml milli-Q water aliquots for 3 times. The washings were added to the volumetric flask and the solution then diluted to 25 ml.

2.2.2. Voltammetric determination

To evaluate the signal intensity of the reagent and solvent background, we tested the voltammetric response of the supporting electrolyte constituted by a solution prepared by adding 60 μL of a Cu(II) solution (1020 mg/L) to 20 mL of HCl (0.1 M) and 5 mL of $MgCl_2$ 0.6 M (dissolved in HCl 0.5 M). The solution was purged with high purity nitrogen (99.9999%, SIAD) for 5 min and analyzed by DPCSV, using the experimental setup summarized in Table 1.

The calibration curve was obtained by adding known amounts of Se(IV) (0.15–8.0 ppb) to blank solutions obtained as above described. Linearity was observed up to 8.5 ppb with a slope of 17.4 ± 0.1 nA ppb^{-1} ($r^2 = 0.9983$).

The selenium concentration in the sample solutions was determined by the standard addition method, adding known amounts of Se(IV) (0.8–2.6 ppb) to the cell, and repurging the solution in the cell with nitrogen for 100 before each measurement (generally three additions, each data point being the average of four distinct measurements). All data reported in the paper were the average of $n = 3$–5 separate analyses.

2.2.3. HG-ICP-AES measurements

Sample solutions coming from the digestion procedure were diluted 1:1 (v/v) with concentrated HCl (37%, w/w) and directly injected into the HG-ICP-AES apparatus. The gaseous hydride was formed after mixing and reaction of the sample solution with $NaBH_4$ by using the FIMS-100 apparatus. The total selenium concentration in the samples was determined on a calibration curve in the range of

Table 1
Instrumental parameters for the determination of Se(IV) by differential pulse cathodic stripping voltammetry

	Setup for Se(IV) concentration range 0.15–8.0 μg L^{-1}
Deposition potential E_d (versus SCE) (mV)	−350
Deposition time t_d (s)	180
Sweep potential range (mV)	−400 to −900
Potential scan rate (mV/s)	50
Drop surface × 10^2 (cm^2)	0.74
Equilibration time (s)	10
Purge time (s)	300
Step amplitude (mV)	50
Modulation time (ms)	10
Stirring speed (r.p.m.)	400

1–50 ppb ($r^2 > 0.97$) starting from a 1000 ± 2 g/L Se(IV) solution in HNO_3 0.5 M (NIST STM 3149 from Merck, Milan, Italy). Stock solution of 1 mL and 3 mL of concentrated HCl (37% w/w) were added in a volumetric flask and the resulting solution was diluted to 100 mL with ultra-pure water (solution B, 10 ppm); (2) 3 ml of concentrated HCl were added to 10 mL of solution B and the solution diluted to 100 mL (solution C, 1 ppm); 1 mL of concentrated HCl were added to 0.050, 0.100, 0.250, 0.500, 1.000 and 2.500 mL of solution C, and the solutions were diluted to 100 mL with ultra-water to obtain working standard solutions in 1, 2, 5, 10, 20 and 50 ppb concentrations (LOD = 0.1 ppb, LOQ = 0.5 ppb under our experimental conditions).

3. Results and discussion

3.1. Effects of the Cu(II) concentration

The accumulation of Se(IV) at the hanging drop mercury electrode (HDME) has been widely studied, (Mattsson, Nyholm, & Olin, 1994a; Zelic & Branica, 1990; Zuman & Somer, 2000) and it has been shown that Se(IV) can be accumulated on the electrode surface at −0.2 V as mercury-selenide HgSe (Eq. (1)).

$$H_2SeO_3 + 4H^+ + Hg + 4e^- \rightarrow HgSe + 3H_2O \quad (1)$$

Further reduction in acidic medium of Se(II) to Se(−II) (Eq. (2)), results in a well-shaped stripping peak at −0.56 V (SCE), well suited for the determination of selenium concentration through the application of the standard addition technique.

$$HgSe + 2H^+ + 2e^- \rightarrow H_2Se + Hg \quad (2)$$

Therefore, the formation of HgSe on mercury electrode has been exploited for the determination of Se(IV) by cathodic stripping voltammetry (CSV) in aqueous solution. Unfortunately the limit of detection found was unsatisfactory for our purposes.

Some authors have enhanced the performance of the DPCSV method by adding Cu^{2+} ions (Korolczuk & Grabarczyk, 2003; Mattsson, Nyholm, & Olin, 1994b; Sladkov & Fourest, 2003; Van den Berg & Khan, 1990).

Metallic Cu dissolved in the mercury enhances the peak current and shifts the peak position at a more negative potential, due to the change of the composition of the electrode material. The most commonly accepted hypothesis is that during the deposition step a metal selenide Cu_2Se (or Cu_xSe) is formed and accumulated on the electrode surface. Two reactions may take place, a first one during the deposition step (Eq. (3))

$$Se(IV) + 2Cu(Hg) + 4e^- \rightarrow Cu_2Se(Hg) \quad (3)$$

and a second one during the stripping step (Eq. (4))

$$Cu_2Se(Hg) + 2H^+ + 2e^- \rightarrow H_2Se + 2Cu(Hg) \quad (4)$$

The reduction of the Cu(I) in this step causes a peak at −0.7 V. The reduced Cu is then again amalgamated and the released selenide becomes protonated and diffuses away causing a drop in the capacitive current at potential more negative than the reduction peak at −0.7 V.

The increase of the area of the Se(IV) stripping peak in the presence of Cu(II) depends on many factors, first of all on the initial concentration of Se(IV) and on the ratio between the Se(IV) and Cu(II) concentrations. In the presence of adequate amount of Cu(II), the reduction peak current of selenium is a linear function of Se(IV) concentration, with no effect deriving from small variations in Cu(II) concentration (Zuman & Somer, 2000). We have carried out a detailed study for the optimization of the total selenium determination by DPCSV technique in the presence of Cu(II).

In order to determine the optimal Cu(II) concentration, we added increasing amounts of Cu(II) to reference solutions containing Se(IV) (6.5 nM), HCl (20 mL, 0.1 M) and $MgCl_2$ (5 mL, 0.6 M in HCl 0.5 M) and we have measured the Cu_2Se reduction peak area after 3 min of deposition time at −0.35 V (SCE) (Fig. 1, each data point being the average of three distinct measurements). A sharp single peak was obtained at a deposition potential shifting toward negative values with the increase of Cu(II) concentration (from −0.609 V (SCE) for 1.0 μM Cu(II), to −0.687 V (SCE) for 64 μM Cu(II), reaching a plateau afterwards) (Fig. 2).

A concentration of Cu(II) lower than 30 μM (Fig. 1, section A) resulted in a highly dispersed peak current between the peak current and Se(IV) concentration. In this range even small variations in the amount of Cu(II) added to the voltammetric cell can markedly affect the determination of the peak area, with the consequent loss of linear correlation between the peak area and the Se(IV) concentration in the same set of measurements. By contrast, concentra-

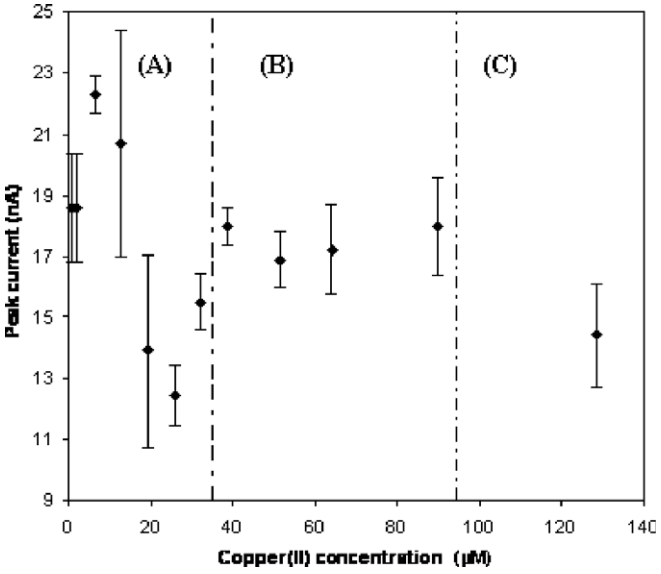

Fig. 1. Effect of Cu(II) concentration on the selenium (IV) peak current in a blank solution. Se(IV) concentration = 6.5 nM; deposition time = 3 min; deposition potential = −0.35 V(SCE).

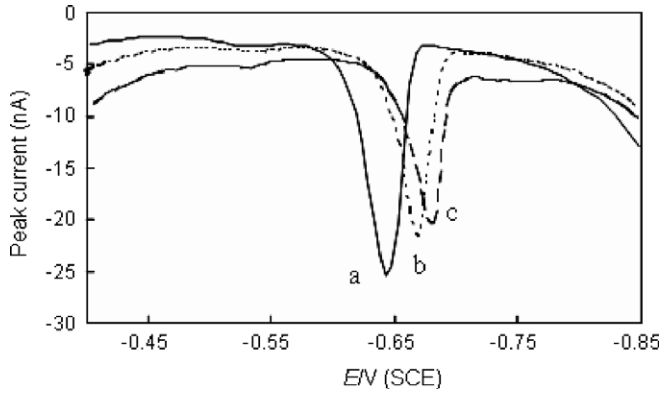

Fig. 2. Effect of Cu(II) concentration on the Se(IV) peak position in a blank solution. Se(IV) concentration = 6.5 nM. (a) 6.4 μM Cu(II); (b) 19 μM Cu(II); (c) 38.5 μM Cu(II).

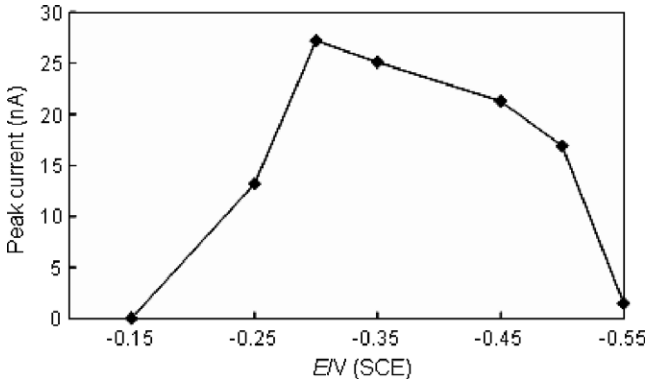

Fig. 3. Effect of the deposition potential on the DPCSV peak current from a blank solution containing 5 nM Se(IV) (deposition time = 3 min).

tions of Cu(II) higher than 90 μM (Fig. 1, section C) resulted in the decrease of the peak current, since the formation of an insoluble Cu_2Se on the electrode surface (Van den Berg & Khan, 1990). The optimum concentration interval was in the range of 40 μM $< c_{Cu(II)} <$ 90 μM (Fig. 1 section B). In the present work, we have set the concentration of Cu(II) at 40 μM since for this value we have found the best reproducibility (see Fig. 1) and a satisfactory linearity between peak current and selenium concentration (see below, paragraph 3.4.1).

3.2. Effect of the deposition potential

As already observed by Mattsson (1994) and Korolczuk and Grabarczyk, 2003, the presence of a high concentration of chloride anions significantly affects the determination of Se(IV), because in the Cu(II) reduction step they interfere in the formation of the amalgamated Cu(Hg) film, this last formed before the conversion to Cu_2Se. Probably, at a deposition potential between +0.15 V and −0.2 V Cu(II) was reduced to $CuCl_2^-$ rather than to Cu(Hg). In our experimental conditions we had to face high concentration of chloride anions ($[Cl^-]$ 0.4 M in the voltammetric cell), arising from the HCl required for the dissolution of MgO formed in the ashing step and for the reduction of Se(VI) to Se(IV).

Hence, we have evaluated the effect of the deposition potential in the interval between −0.150 V and −0.550 V (SCE) (Fig. 3) on the selenium peak current using blank solutions spiked with a standard solution of Se(IV) (5 nM final concentration). In the range between −0.30 V and −0.45 V (SCE), the peak current was fairly constant (2.45 ± 0.3 nA) and showed a satisfactory linear correlation with Se(IV) concentration. In particular, the sensitivity parameters, obtained from the slope of the calibration curve were: $E_d = -0.35$ V, $S = 17.2$ nA/ppb, $r^2 = 0.998$; $E_d = -0.40$ V, $S = 23.2$ nA/ppb, $r^2 = 0.994$; $E_d = -0.45$ V, $S = 22.4$ nA/ppb, $r^2 = 0.994$. The absence of peak current when the deposition potential was higher than −0.15 V may be due to the incomplete reduction of Se(IV) to Se(−II) (Kolthoff & Lingane, 1952). On the basis of these results we set the working deposition potential at −0.35 V (SCE).

3.3. Heavy metal interferences

The possible interferences deriving from the following heavy metals: Ni^{2+} (180 nM), Cd^{2+} (8 nM), Zn^{2+} (23 μM), Fe^{3+} (27 μM), Pb^{2+} (150 nM) and Mn^{2+} (14 μM) were evaluated on blank sample solutions containing 1.6 ppb of Se. These are the most abundant metal species contained in the certified rice flour 1568a (NIST, USA) and for the evaluation of the possible interference we have used concentrations of the contaminants up to four times greater than those present in the certified rice. No significant interference on the selenium peak currents or peak potentials has been observed at these concentrations of contaminants; indeed, changes lower than 2σ have been observed in all cases.

3.4. Analytical characteristics of the method

3.4.1. Sensitivity and detection limit

Standard additions of Se(IV) were made to blank solutions obtained as described. The selenium peak area of the voltammogram at −0.67 V (SCE) was found to be linearly correlated ($r^2 = 0.9983$) to the selenium concentration in the range 0.15–8.0 μg/L. The limit of detection (LOD) was evaluated using the expression 3σ/S, (Boumans, 1994; Miller & Miller, 1984) where σ indicated the standard deviation of the response (at −0.67 V), S was the sensitivity obtained from the slope of the analytical calibration curve. The LOD found for the standard reference solution was 0.07 ppb Se(IV) (i.e., 0.84 nM). This limit can be further lowered using a longer deposition time, since no saturation effect on HDME was observed in the working concentration range.

3.4.2. Recovery and accuracy

To investigate the reliability of the digestion procedure (possible loss in total selenium or incomplete reduction of

Table 2
Mean total selenium content determined by DPCSV and ICP-HG-AES in white rice ($n = 4$), black rice ($n = 5$), red rice ($n = 3$) and in white rice hull ($n = 4$) from Northern Italian cultivars

Rice sample	Se(IV) content (ng/g) DPCSV	RSD (%)	Recovery (%)	Se(IV) content (ng/g) ICP-HG-AES
White rice ($n = 4$)	20.1 ± 1.8	9.0	99 ± 14	22 ± 1
Black rice ($n = 5$)	26.7 ± 1.3	4.7	100 ± 23	31 ± 2
Red rice ($n = 3$)	53.0 ± 1.0	1.9	76 ± 5	n.d.
White rice hull ($n = 4$)	45.3 ± 4.1	9.0	85 ± 14	48 ± 5

Values are expressed as mean value ± standard deviation. n.d.: not determined. Relative standard deviations (RSD) are reported in brackets.

Se(VI) to Se(IV) during the different digestion steps) we have added known amounts of Se(VI) (typically 8.0 ppb) to the blank solutions constituted by all the reagents except the matrix. The resulting solutions were submitted to the same digestion procedure used for the rice-containing samples. The amount of selenium was determined using the standard addition method. The determination was carried out in quadruplicate. Recovery was 92% and the reproducibility of the results in terms of relative standard deviation (RSD) was 4.5%. The recovery values obtained for the blank solution indicate the absence of analyte loss during the digestion procedure and the completeness of Se reduction.

In order to evaluate the accuracy of the digestion method in our samples, i.e., to verify that the complete destruction of the organic matter does not promote Se sequestering or loss and that all Se was actually detected, several rice and hull samples were spiked with a known amount of Se(IV) standard solution (20 ng/g) just before the digestion process. Recovery experiments were carried out on several rice (see Table 2) and hull samples (1 g). The analyses were made in quadruplicate and the average recovery value for selenium was $97 \pm 8\%$ (see Table 2). This indicates the absence of analyte losses or contamination during the digestion of samples.

3.5. Determination of the Se(IV) content in rice samples with DPCSV

Once optimized, DPCSV method was applied for determining the total selenium content in three different rice varieties and in a sample of white-rice hull. All foodstuff was from Northern Italy cultivars. A typical DPCSV analysis pattern (red rice sample) was shown in Fig. 4. Table 2 reports the mean selenium content obtained after repeated measurements of the rice samples.

3.6. Spectroscopic measurements

The HG-ICP-AES method was a well established technique for the determination of selenium in foodstuffs, beverages, soils, etc owing to its high reproducibility and specificity. For this reason, in order to evaluate the consistency/reliability of the proposed DPCSV and digestion method, the solutions obtained from the white and black rice and the white rice hull samples digestion, were subjected to the HG-ICP-AES analysis. The results (Table 2) show a satisfactory agreement between the data determined by DPSCV and HG-ICP-AES techniques. For white rice, spiked samples were also analyzed obtained very satisfactory recovery (about $98 \pm 3\%$). The satisfactory recoveries obtained by both methods provide a mutual validation.

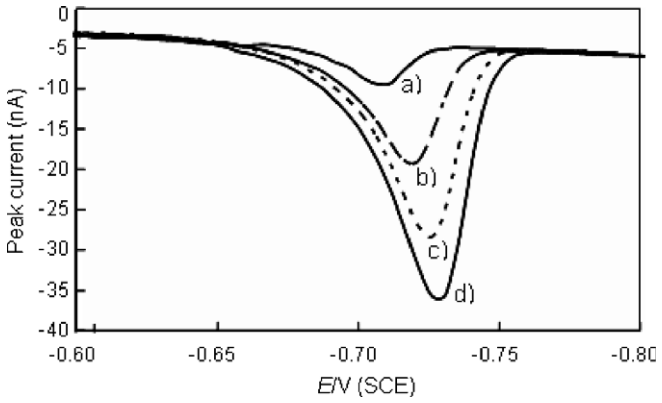

Fig. 4. Representative example of DPCSV voltammograms obtained by the standard addition method to a digested red rice sample (spiked with Se(IV) (a) 0 ppb, (b) 0.86 ppb, (c) 1.73 ppb, (d) 2.59 ppb).

4. Conclusions

The results of this study, although limited to a small number of rice varieties, provide, to our knowledge, the first information on the selenium content colored rice grown in Northern Italy. Moreover they allow some interesting consideration: (1) the white rice hull appears significantly richer in total selenium than its flour, and this implies that the removal of the outer layer (rice hull) involves a loss of this important antioxidant element, a fact that can well justify the use of whole grain (integral) rice by the consumer. This is in agreement with what found in studies performed on Chinese cultivations, in which the selenium content in the rice bran was 2.58 times higher than in the milled rice. (Jingui, Jinying, Jiahuan, & Aihua, 2005). (2) Among all the varieties, red rice is the richest in total selenium (53.0 ± 1.0 ppb), while the difference in Se content between black and white rice is small. The higher Se concentration in colored rice is in agreement with what already known for rice samples coming from Asiatic regions (Mingwei et al., 2000), and it could be ascribed to the variety or to cultivar conditions.

The selenium content in all the three species is largely below to the level of 110 ppb, which has been indicated by some authors as the threshold value allowing to distinguish an European rice sample from one produced in the USA (Kelly et al., 2002). This confirms that the level of this trace element can constitute a key marker of geographic origin.

The analytical procedure used in this study provides an accurate, cheap and easy-to-handle method, that can be successfully used for the determination of the total selenium, at trace and ultra-trace levels, in nutritional/dietary products. Compared to other non-voltammetric methods, DPCSV provides a higher degree of sensitivity, allowing very low limits of detection (0.07 versus 0.5 ppb) (Chen et al., 2002; Hu, Chen, Xu, Zhang, & Pan, 2002), especially when the peak area is employed as instrumental datum (Locatelli, 2004). Moreover the methodology here proposed sounds reliable, since it exibits precision and specificity. In particular:

(1) the Se(IV) recoveries from blanks and spiked samples before the digestion procedure are satisfactory, since the digestion procedure ensures complete decomposition of organic matter without loss of this volatile trace element;
(2) it is not sensitive to the presence of the most common interfering metal contaminants;
(3) the DPCSV results match satisfactorily with those coming from HG-ICP-AES analysis.

Moreover this analytical procedure requires an inexpensive and easy-to-handle equipment. The possibility to use an inexpensive equipment is of paramount importance not only in western countries, but in particular in less developed countries, where rice is the main food for inhabitants, and its concentration must be strictly controlled for reducing the risk of colon and breast cancer.

References

Adeloju, S. B., Bond, A. M., & Briggs, M. H. (1984). Critical evaluation of some wet digestion methods for the stripping voltammetric determination of selenium in biological materials. *Analytical Chemistry, 56*, 2397–2401.

Bujdos, M., Kubova, J., & Stresko, V. (2000). Problems of selenium fractionation in soils rich of organic matter. *Analytica Chimica Acta, 408*, 103–109.

Boumans, P. W. J. M. (1994). Detection limits and spectral interferences in atomic emission spectrometry. *Analytical Chemistry, 66*, A459–A467.

Chen, L., Yang, F., Xu, J., Hu, Y., Hu, Q., Zhang, Y., et al. (2002). Determination of selenium concentration of rice in China and effect of fertilization of selenite and selenate on selenium content of rice. *Journal of Agricultural and Food Chemistry, 50*, 5128–5130.

Deaker, M., & Maher, W. (1997). Low-volume microwave digestion for the determination of selenium in marine biological tissues by graphite furnace atomic absorption. *Analytica Chimica Acta, 350*, 287–295.

Ferri, T., De Luca, C., & Ticconi, L. (2001). Selenium speciation in environmental matrices: a preliminary study on plants. *Analytical Letters, 34*, 975–988.

Fordyce, F. M., Johnson, C. C., Navaratna, U. R. B., Appleton, J. D., & Dissanayake, C. B. (2000). Selenium and iodine in soil, rice and drinking water in relation to endemic goitre in Sri Lanka. *The Science of the Total Enviroment, 263*, 127–141.

Gupta, U. G., Gupta, S. C., & Gupta, M. D. (2000). Selenium in soils and crops, its deficiencies in livestock and human implications for management. *Communications in Soil Science and Plant Analysis, 31*, 11–14.

Hahn, M. H., Wolnik, K. A., & Fricke, F. L. (1982). Hydride Generation/condensation system with an inductively coupled argon plasma polychromator for determination of arsenic, bismuth, germanium; antimony, selenium and tin in foods. *Analytical Chemistry, 54*, 1048–1052.

Higham, A. M., & Tomkins, R. P. T. (1993). Determination of trace quantities of selenium and arsenic in canned tune fish by using electroanalytical techniques. *Food Chemistry, 48*, 85–93.

Hu, Q., Chen, L., Xu, J., Zhang, Y., & Pan, G. (2002). Determination of selenium concentration of rice and the effect of foliar application of Se-enriched fertiliser or sodium selenite on the selenium content of rice. *Journal of the Science of Food and Agriculture, 82*, 869–872.

İnam, R., & Somer, G. (2000). A direct method for the determination of selenium and lead in cow's milk by differential pulse stripping voltammetry. *Food Chemistry, 69*, 345–350.

Ip, C. (1998). Lessons from basic research in selenium and cancer prevention. *Journal of Nutrition, 128*, 1845–1854.

Jingui, Z., Jinying, W., Jiahuan, J. & Aihua, L. (2005). (Studies on six mineral element contents of rice bran and milled rice from three colour rice cultivars) CAN 143:438932 AN 2005:373400.

Kelly, S., Baxter, M., Chapman, S., Rhodes, C., Dennis, J., & Brereton, P. (2002). The application of isotopic and elemental analysis to determine the geographical origin of premium long grain rice. *European Food Research and Technology, 214*, 72–78.

Kolthoff, I. M., & Lingane, J. J. (1952). *Polarography*. New York: Interscience.

Korolczuk, M., & Grabarczyk, M. (2003). Determination of Se(IV) in on-line system by cathodic stripping voltammetry. *Electroanalysis, 15*, 821–826.

Lambert, D. F., & Turoczy, N. J. (2000). Comparison of digestion methods for the determination of selenium in fish tissue by cathodic stripping voltammetry. *Analytica Chimica Acta, 408*, 97–102.

Ling, W. H., Cheng, Q. X., Ma, J., & Wang, T. (2001). Red and black rice decrease atherosclerotic paque formation and increase antioxidant status in rabbits. *Journal of Nutrition, 131*, 1421–1426.

Ling, W. H., Wang, L. L., & Ma, J. (2002). Supplementation of the black rice outer layer fraction to rabbits decreases atherosclerotic paque formation and increases antioxidant status. *Journal of Nutrition, 132*, 20–26.

Locatelli, C. (2004). Heavy metals in matrices of food interest: sequential voltammetric determination at trace and ultratrace level of copper, lead, cadmium, zinc, arsenic. *Electroanalysis, 16*, 1478–1486.

Mattsson, G., Nyholm, L., & Olin, Å. (1994a). Cathodic stripping voltammetry of HgSe. *Journal of Electroanalytical Chemistry, 377*, 149–162.

Mattsson, G., Nyholm, L., & Olin, Å. J. (1994b). Cathodic stripping voltammetry of Cu_2Se at mercury electrodes. *Journal of Electroanalytical Chemistry, 379*, 49–61.

Miller, J. C., & Miller, J. N. (1984). *Statistics for Analytical Chemistry*. Chichester: Ellis Horwood Limited Publishers.

Mindak, W. R., & Dolan, S. P. (1999). Determinationof arsenic and selenium in food using a microwave digestion-dry ash preparation and flow injection hydride generation atomic absorption spectrometry. *Journal of Food Composition and Analysis, 12*, 111–122, and Refs. therein.

Mingwei, Z., Jianwei, C., Lin, S., Xiaoqi, Z., Laizhan, L., Zhihong, X. et al., (2000). (Nutritional evaluation of some black cereals and oil food resources) CAN 133:11 9311 AN 2000:378762.

Narasaki, H., & Ikeda, M. (1984). Automated determination of arsenic and selenium by atomic absorbtion spectrometry with hydride generation. *Analytical Chemistry, 56*, 2059–2063.

Ochsenkuhn-Peropoulou, M., & Tsopelas, F. (2002). Speciation analysis of selenium using voltammetric techniques. *Analytica Chimica Acta, 476*, 167–178.

Reilly, C. (1998). Selenium: a new entrant into the functional food arena. *Trends in Food Science and Technology, 9*, 114–118.

Sladkov, V., & Fourest, F. D. B. (2003). Copper-mercury film electrode for cathodic stripping voltammetric determination of Se(IV). *Analytical and Bioanalytical Chemistry, 375*, 300–305, and Refs. therein.

Suzuki, K. T. (2005). Metabolomics of selenium: Se metabolites based on speciation studies. *Journal of Health Science, 51*, 107–114.

Szpunar, J. (2005). Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics. *Analyst, 130*, 442–465.

Van den Berg, C. M. G., & Khan, S. H. (1990). Determination of selenium in sea water by adsorptive cathodic stripping voltammetry. *Analytica Chimica Acta, 231*, 221–229.

Yang, K. X., & Husain, L. (2006). Ultratrace determination of selenium by hydride generation-inductively coupled plasma mass spectrometry operated under no robust plasma conditions. *Spectroscopy Letters, 39*, 187–201.

Zelic, M., & Branica, M. (1990). Study of electrochemical reduction of selenium(+4) using square wave voltammetry. *Electroanalysis, 2*, 455–462.

Zuman, P., & Somer, G. (2000). Polarographic and voltammetric behaviour of selenious acid and its use in analysis. *Talanta, 51*, 645–665.