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Effect of modifiers on thermal behaviour of Se in acid digestates and slurries of vegetables by graphite furnace atomic absorption spectrometry

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Abstract

The influence of sample preparation strategy of vegetables on the electrothermal behaviour of Se without and with chemical modifiers such as $Pd(NO_3)_2$, $Pd(NO_3)_2$ + $Mg(NO_3)_2$, $Pd(NO_3)_2$ + $Cd(NO_3)_2$, pre-reduced Pd, $Mg(NO_3)_2$, and $Ni(NO_3)_2$ was investigated. Acid digestates and slurries of vegetables (0.1% m/v in 1% v/v HNO_3 + 0.005% v/v of Triton X-100) were used to prepare reference solutions or slurries. For 10 µl of each modifier tested, pyrolysis and atomization temperatures were evaluated using pyrolysis and atomization curves, respectively. Best conditions, such as thermal stability, signal profile, repeatability and sensitivity were attained using $Pd(NO_3)_2$ as chemical modifier. The following heating program (temperature, ramp/hold time) of the graphite tube of the Varian SpectrAA-800Z atomic absorption spectrometer was used: dry step (85 °C, 5/0 s; 95 °C, 40/0 s; 120 °C, 10/5 s); pyrolysis step (1400 °C, 10/3s); atomization step (2200 °C, 1/2 s); clean step (2600 °C, 2/0 s). This pyrolysis temperature is 800 °C higher than when measuring without any modifier. For 20 µL sample volume and 10 µg $Pd(NO_3)_2$, analytical curves in the 3.0–30 µg Se l^{-1} range were obtained. The method was applied for Se determination in acid digestates and slurries of 10 vegetable samples and one standard reference material (rice flower) and results were in agreement at 95% confidence level. Recoveries varied from 89 to 95% for spiked samples. The lifetime of the graphite tube was ca. 250 firings and the relative standard deviations ($n = 12$) for a typical acid digestate and slurry containing 20 µg Se l^{-1} were 3.8% and 8.3%, respectively. The limits of detection were 2.0 µg Se l^{-1} and 0.6 µg Se l^{-1} Se for digestates and slurries, respectively.

1. Introduction

Selenium is an important element to be investigated in analytical chemistry in view of its antagonistic characteristics: it may be classified either as essential or toxic to human and animals depending on the ingested amount of its compounds (Alejos, Romero, & Díaz, 2000; Kim, Du, Otwell, Marshall, & Wei, 1998). The daily amount of selenium recommended to human beings depends on sex and age, but it is close to 75 µg Se for man and 55 µg Se for woman (Haygarth, 1994).

Some plants have the ability to accumulate high amounts of Se, such as cruciferous vegetables (e.g. cabbage, cauliflower, kale, broccoli), gramineous, legume, etc. (Terry & Zayed, 1994). Due to the narrow concentration interval between toxic and essential function (Mayland, 1994), the accurate determination of selenium in foodstuffs is important.

Among analytical techniques available for Se determination, graphite furnace atomic absorption spectrometry (GFAAS) is a suitable and widely used technique for the determination of this element at trace levels due to its selectivity, simplicity, high sensitivity and capability for direct analysis with minimal sample preparation (Butcher & Sneddon, 1998; Chen, Yen, Wei, Hu,

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Yu, Chung, & Kuo, 1998; Liu, Gong, Li, Xu, & Lin, 1996). The capability to analyze trace elements in different samples with or without extensive pretreatment is noteworthy for laboratories dealing with large-scale analyses (Knowles & Brodie, 1988). As the atomizer is considered a chemical reactor, decomposition of vegetable samples can be carried out inside the graphite tube (Aller & Robles, 1998, Lynch & Littlejohn, 1989). As Se-compounds present in workable samples are relatively volatile, selenium can be lost in the ashing step during the heating program of atomizer at temperatures higher than 200 °C (Welz & Sperling, 1999). This shortcoming can be circumvented using an appropriate chemical modifier to thermally stabilize Se at high temperatures and minimize matrix effects during the atomization step.

Nickel was the first modifier used for Se determination in real samples (Styris, 1986). Styris has demonstrated in studies involving Se determination in blood that the stabilization of Se by Ni was attributed to the formation of Se-C-Ni alloy. This compound was first decomposed in SeC_2, and after in Se. In excess of Ni, the dissociation of the nickel–selenide compound is delayed, which improved the stabilization of Se at high temperatures.

In 1991, Styris, Prell, Redfield, Holcombe, Bass, and Majidi studied the influence of palladium nitrate as a chemical modifier on the stabilization of Se in aqueous solution. The authors evaluated the electrothermal behaviour of Se by adding the modifier into the graphite tube together or prior to the sample. With the last procedure, Pd(II) was thermally reduced to Pd^0 before sample injection. In both modification approaches, the authors observed stabilization of Se at temperatures up to 1200 °C, which was attributed to the formation of compound [Pd,Se,O]. On the other hand, without using any modifier, losts of Se occurred at lower temperatures, mainly in the form of dimers, oxides and hydroxides.

Studies dealing with electrothermal behaviour of Se in slurry using different modifiers are important parameters to be considered in analytical methodologies. Qiao and Jackson suggested that stabilization of Se during the atomization stage of the furnace heating cycle may be due to the release of analyte physically entrapped in droplets of molten palladium (Qiao & Jackson, 1991, 1992). Chen and Jackson (1996), showed that the interaction between selenium (also Pb and Tl) and palladium is highly effective when they are in close contact through premixing prior to drying and on the graphite surface.

Because selenium is usually present in vegetables at trace levels (Alloway, 1995), the use of direct analysis is relevant from the point-of-view of quality control. Little attention has been given to the influence of different modifiers on the electrothermal behaviour of Se in plant slurries. Likewise, procedures involving solid sampling

for direct determination of Se in plant materials are also scarce in the literature (Chen & Marshall, 1999).

This study reports the electrothermal behaviour of Se in acid digestates and slurries of vegetables in different conditions: without or with $Pd(NO_3)_2$, $Pd(NO_3)_2$ plus $Mg(NO_3)_2$, $Pd(NO_3)_2$ plus $Cd(NO_3)_2$, pre-reduced Pd, $Mg(NO_3)_2$, and $Ni(NO_3)_2$. The performance of a simple and fast method for the direct determination of trace Se in plant materials using electrothermal atomic absorption spectrometry with Zeeman-effect background corrector was checked after analysis of acid digestates and slurries of vegetables and one standard reference material by GFAAS.

2. Materials and methods

2.1. Reagents, reference solutions and samples

All solutions were prepared with analytical grade chemicals. The water was distilled and deionized employing a Milli-Q system, (Millipore, Bedford, MA, USA). Argon (White Martins, Brazil) was 99.996% purity and Suprapur® nitric acid was used (Merck, Darmsdat, Germany). The autosampler washing solution was 0.005% v/v of Triton X-100 in 1% v/v HNO_3.

Selenium stock solution (1000 mg l^{-1}) was prepared by dissolving 1.404 g SeO_2 (Merck) in water and the volume was completed to 1000 ml with water.

Modifier solutions containing 0.1% m/v of Pd, Mg, Cd or Ni were prepared by diluting each stock solution (10 g l^{-1}, Merck) in water.

The reference solutions (3.0–30 μg Se l^{-1}) were prepared by appropriate dilution of stock solution in vegetable digestate containing 10% v/v HNO_3. Reference slurries (3.0–30 μg Se l^{-1}) containing 0.1% m/v of dried and powdered vegetables in 1% v/v HNO_3 + 0.005% v/v of Triton X-100 were also made up. After harvest, all samples were dried at 50 °C for 48 h in a forced air oven and ground in a cutting mill fitted with a 20-mesh screen at the bottom of the cutting chamber and thereafter powdered again in a cryogenic mill (Spex-Freezer Mill 6750, Metuchen, NJ, USA). An amount of 1.000 g of vegetable sample was inserted in a grinding vial, a polycarbonate cylinder supplied with two end plugs, immersed in liquid nitrogen and ground with a magnetically driven impactor. A time of 8 min was required to pulverize each sample. The grinding procedure was implemented with a first step of 3 min for sample freezing followed by two cycles with two stages of pulverization and cooling, with a total time of 8 min, in order to obtain particles with diameter smaller than 60 μm (Miller-Ihli, 1993). The vegetable samples and one standard reference material (1568a rice flower, from National Institute of Standards and Technology, Gaithersburg, MD, USA) were mineralized in triplicate with nitric

acid in a closed-vessel microwave-assisted acid-digestion system (Milestone Ethos 1600, Sorisole, Italy). A volume of 2.5 ml HNO$_3$ conc. and 0.5 ml of 30% v/v H$_2$O$_2$ were added to 250 mg of dried powdered materials placed into PTFE tubes, and the tubes were closed and placed on the microwave oven cavity. Thereafter, the following power/time program was run: step 1, 250 W/2 min; step 2, 0 W/2 min; step 3, 250 W/5 min; step 4, 400 W/4 min; step 5, 500 W/4 min; step 6 (ventilation) 0 W/4 min. After cooling, the resulting acid digestates were diluted to 25 ml with water. The samples were also cryogenically powdered in order to prepare slurries. A mass of 0.100 g of dried and powdered samples were dispersed in 100 ml of 1% v/v HNO$_3$ + 0.005% v/v Triton X-100 and the flasks containing slurries were placed on an ultrasonic bath during 15 min for homogenization. Thereafter, ca. 1.5 ml slurry was transferred to the autosampler cup. Immediately before sampling, the slurries were carefully homogenized by bubbling air using a Pasteur pipette. To avoid contamination problems, the pipette was rinsed between samples.

2.2. Instrumentation

A Varian SpectrAA-800Z atomic absorption spectrometer with Zeeman background corrector (Mulgrave, Victoria, Australia) equipped with autosampler, pyrolytic graphite coated graphite tubes (Varian Part No 63-100023-00) was used throughout. The monochromator was adjusted to 196.0 nm and the slit to 1.0 nm. Absorbance signals were measured in peak area mode. A hollow cathode lamp (Varian, Ultra-lamp) was used at an applied current of 15 mA. All measurements were carried out using Zeeman-effect background correction. Argon gas flow-rate was maintained at 3 l per minute during all temperature steps, except during the atomization in which the gas flow was stopped. The heating program is shown in Table 1.

Table 1
Heating program for the determination of Se in plant materials by GFAAS[a]

Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Ar (l/min^{-1})
1	85	5	0	3
2	95	40	0	3
3	120	10	5	3
4	Tp[c]	10	3	3
5[b]	Ta[c]	1	2	0
6	2600	2	0	3

[a] Conditions of pipetting sequence and running program: (1) Pipetting modifier; (2) Extra wash; (3) Pipetting sample; (4) Run step one to end.
[b] Read in this step.
[c] Variable temperature. Tp, Ta: pyrolysis and atomization temperatures, respectively.

2.3. Procedure

The influence of matrix on the electrothermal behaviour of Se was studied using pyrolysis and atomization curves in the presence of two different media, digestates and slurries of the following plant materials: beet, cauliflower, cucumber, radish, garlic, broccoli, carrot, kale, tomato, cabbage, lettuce, turnip, and spinach. For each medium, the following amounts of chemical modifiers were tested: 10 µg Pd; 5 µg Pd + 5 µg Mg; 5 µg Pd + 5 µg Cd; 5 µg of pre-reduced Pd; 10 µg Mg; and 10 µg Ni. The electrothermal behaviour of Se was studied using pyrolysis and atomization curves adopting the heating program showed in Table 1. This study employed the following solutions: acid digests of plants spiked with 20 µg Se l^{-1}, and 0.1% m/v plant slurries in 1% v/v HNO$_3$ plus 0.005% v/v Triton X-100 and spiked with 20 µg Se l^{-1}.

Matrix-matched slurries containing from 3.0 to 30 µg Se l^{-1} were prepared with cauliflower, radish, cucumber, kale, lettuce, cabbage, turnip, in order to investigate matrix effects.

A volume of 20 µl of sample was dispensed into the graphite tube and five replicates of each reference solution or suspension and sample were carried out.

3. Results and discussion

Pyrolysis and atomization curves were carried out in order to determine the optimum pyrolysis and atomization temperatures for each medium with and without modifiers. All results were based on peak area measurements and they are summarized in Table 2. For this study, acid digestates and slurries of plants were spiked with 0.4 ng Se. The effect of chemical modifier on both sensitivity and stabilization of Se in the presence of a sample matrix was relevant. Typical absorbance and background signals in aqueous solution, acid digestates and plant slurries in each modifier studied are shown in Figs. 1–6. Atomic absorption (AA) of Se and background (BG) transient signals in aqueous solution, plant digestates and plant slurries varied significantly for each modifier tested. Best AA signals were obtained for plant slurries in presence of palladium nitrate (Fig. 1C), palladium plus magnesium nitrates (Fig. 2C) or palladium plus cadmium nitrates (Fig. 3C) for which the total time of the transient signals were lower than ca. 2 s. Similar times were obtained when the mixture CdCl$_2$/PdCl$_2$ was used for Se determination in coal-fly-ash (Garcia-Ollara, Robles, Alemany, & Aller, 1991). Others modifiers, such as palladium nitrate, palladium plus magnesium nitrates, palladium plus molybdenum nitrates, palladium plus ascorbic acid and palladium plus C have also been studied (Qiao & Jackson, 1991) and the total time of transience was in the 1.5–4.5 s range. It should

Table 2
Optimized pyrolysis/atomization temperatures* (°C) and characteristic masses* (pg) for Se in 0.2% v/v HNO_3, acid-digestate (10% v/v HNO_3) and slurry using different modifiers

Modifier	HNO_3	Acid digestate	Slurry
None	200/1000/180	400/1600/220	800/1200/180
Pd	1200/2000/20	1200/2000/27	1400/2200/47
Pd + Mg	1200/2200/41	1400/2400/32	1200/2400/46
Pd + Cd	1200/1800/29	1400/1800/28	1200/2200/40
Pd^0	1400/2200/22	1000/2000/30	1400/2400/57
Mg	800/2000/53	1200/2200/39	800/2200/139
Ni	1000/2000/21	1200/2000/53	1000/2200/49

* Mean values for 10 plant materials.

Fig. 2. Atomic absorption (AA) and background (BG) transient signals of Se using palladium nitrate plus magnesium nitrate as modifiers; dotted and continuous lines correspond to the background and atomic signals for 0.4 ng Se in 0.2% v/v HNO_3 (a), digestate of lettuce containing 10% v/v HNO_3 (b) and slurry of lettuce containing 0.1% m/v plant material + 0.005% v/v Triton X-100 + 1% v/v HNO_3 (c).

modifiers, and that free selenium and palladium result from thermal dissociation of this solid compound (Jackson, 1999). It should be commented that the levels of magnesium usually found in plant materials are around 0.75% m/v and probably helped Se stabilization so that only Pd was efficient as chemical modifier. In some situations the use of a sample matrix as a modifier is possible (Quináia & Nobrega, 1999).
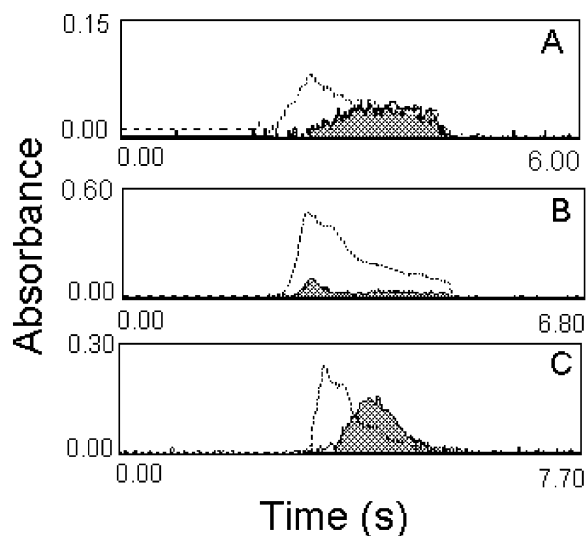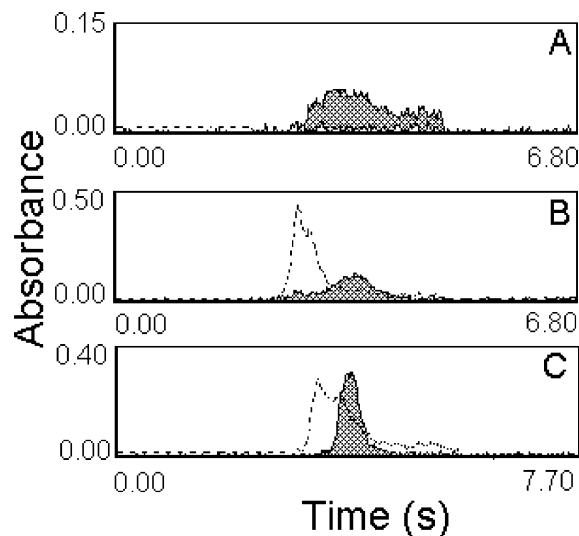

Fig. 1. Atomic absorption (AA) and background (BG) transient signals of Se using palladium nitrate as modifier; dotted and continuous lines correspond to the background and atomic signals for 0.4 ng Se in 0.2% v/v HNO_3 (a), digestate of lettuce containing 10% v/v HNO_3 (b) and slurry of lettuce containing 0.1% m/v plant material + 0.005% v/v Triton X-100 + 1% v/v HNO_3 (c).

be stressed that the use of cadmium nitrate as a modifier for Se may impair the routine analysis of laboratories when Cd is the analyte to be determined at trace levels, due to the possibility of atomizer contamination. Although best peak profile of Se was observed for $Pd(NO_3)_2$ + $Mg(NO_3)_2$, the pyrolysis temperature was 200 °C lower than that obtained for palladium nitrate alone (Table 2), while the atomization temperature was 200 °C higher.

Pre-reduced (Pd^0) palladium (Styris, Prell, Redfield, Holcombe, Bass, & Majidi, 1991) stabilized Se in plant slurries up to 1400 °C, however $Pd(NO_3)_2$ gave better characteristic mass than Pd^0 (Table 2) and background signal attenuation was more pronounced (Figs. 1 and 4). This may be associated to the reduced performance of Pd^0 as modifier in temperatures close to 1500 °C (Fisher & Rademeyer, 1998). It was reasonable that same [Pd,Se,O] compound was produced for both
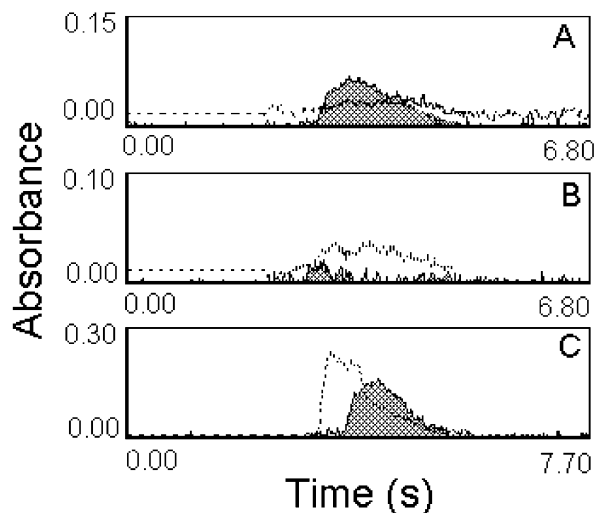

Fig. 3. Atomic absorption (AA) and background (BG) transient signals of Se using palladium nitrate plus cadmium nitrate as modifier; dotted and continuous lines correspond to the background and atomic signals for 0.4 ng Se in 0.2% v/v HNO_3 (a), digestate of lettuce containing 10% v/v HNO_3 (b) and slurry of lettuce containing 0.1% m/v plant material + 0.005% v/v Triton X-100 + 1% v/v HNO_3 (c).
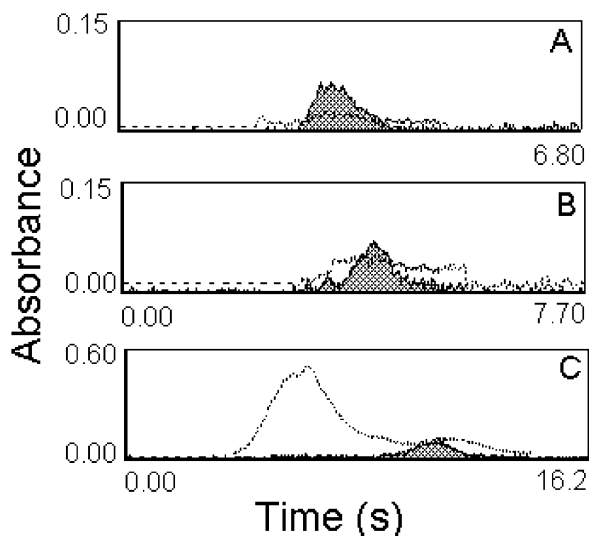
Fig. 4. Atomic absorption (AA) and background (BG) transient signals of Se using pre-reduced Pd as modifier; dotted and continuous lines correspond to the background and atomic signals for 0.4 ng Se in 0.2% v/v HNO₃ (a), digestate of lettuce containing 10% v/v HNO₃ (b) and slurry of lettuce containing 0.1% m/v plant material + 0.005% v/v Triton X-100 + 1% v/v HNO₃ (c).

Fig. 6. Atomic absorption (AA) and background (BG) transient signals of Se using nickel nitrate as modifier; dotted and continuous lines correspond to the background and atomic signals for 0.4 ng Se in 0.2% v/v HNO₃ (a), digestate of lettuce containing 10% v/v HNO₃ (b) and slurry of lettuce containing 0.1% m/v plant material + 0.005% v/v Triton X-100 + 1% v/v HNO₃ (c).

Typical peak profiles for Se in slurries using either nickel nitrate or magnesium nitrate are shown in Figs. 5 and 6. These modifiers gave the worst performance for Se resulting in poor sensitivity, low pyrolysis temperatures or high background signals.

Regarding the studies involving the mixture $Pd(NO_3)_2$ + $Mg(NO_3)_2$ as a modifier, the pyrolysis temperature was 200 °C lower than that obtained for palladium
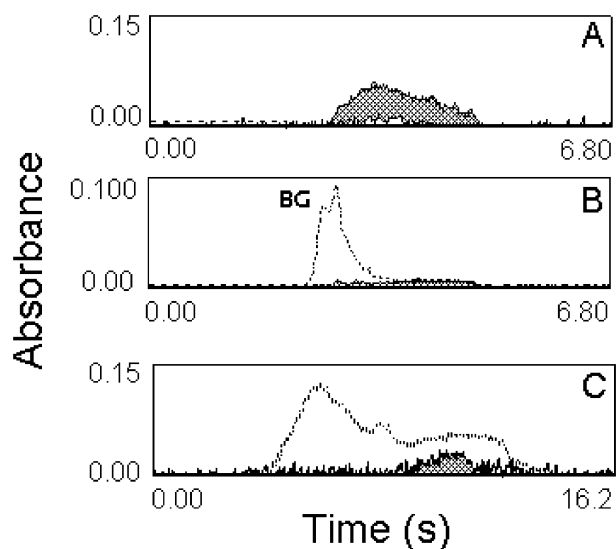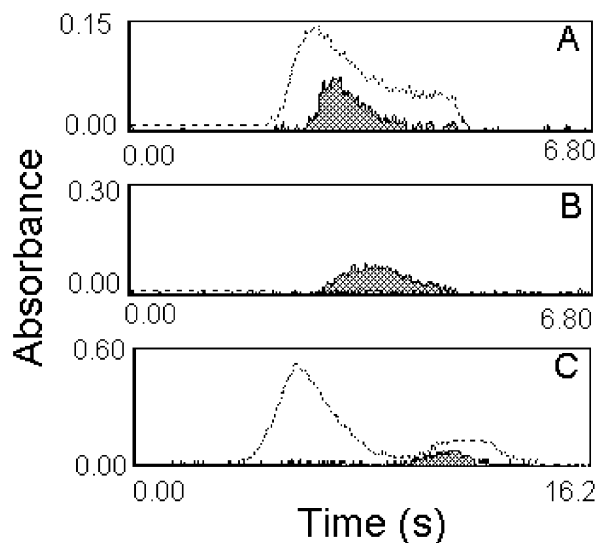

Fig. 5. Atomic absorption (AA) and background (BG) transient signals of Se using magnesium nitrate as modifier; dotted and continuous lines correspond to the background and atomic signals for 0.4 ng Se in 0.2% v/v HNO₃ (a), digestate of lettuce containing 10% v/v HNO₃ (b) and slurry of lettuce containing 0.1% m/v plant material + 0.005% v/v Triton X-100 + 1% v/v HNO₃ (c).

nitrate alone, but a similar atomization temperature and characteristic mass were attained.

So, palladium nitrate was chosen as the optimum modifier taking into account compromise parameters such as higher pyrolysis temperature, lower atomization temperature, sensitivity and AA and BG peak profile.

When palladium alone was used as a chemical modifier, the influence of a matrix of vegetables on atomic signals of Se was investigated by calibrating the system with solutions prepared using acid digestates obtained from different plants. For this study, reference solutions within the 3.0–30 $\mu g\ l^{-1}$ range of Se concentration were prepared in digestates and slurries of lettuce, cabbage, cauliflower, cucumber, kale, lettuce, radish, and turnip.

Matrix effects were evaluated by the average slope ratios (Oliveira, Moraes, Gomes Neto, & Lima, 2002) between analytical curves obtained from reference solutions prepared in 1% v/v HNO₃ and analytical curves obtained from Se additions in acid digestates [10% v/v HNO₃] or slurries. The calculated ratios were 1.1 and 1.3 for plant acid digestates and plant slurries, respectively.

Concerning plant acid digestates, the maximum difference obtained in slope for all analytical curves were ca. 20% and most of the curves presented similar slopes. On the other hand, the average slopes of these curves were 25% lower than the slope of the curve prepared in 1% v/v nitric acid. In the determination of Se in plant acid digestates, calibration with reference solutions prepared in diluted nitric acid was not suitable. However, when reference solutions were prepared in 10% v/v HNO₃, no significant difference in absorbance signals

was observed, so this strategy for calibration is advisable taking into account the acidity of plant digestates. In the calibration using plant slurries, the slopes of all analytical curves were close and characteristic masses for all samples varied within 43–50 pg Se. In addition, the average slopes of these analytical curves were 40% lower than the slope of analytical curves in 1% v/v nitric acid. For direct analysis, calibration with reference solutions was not suitable and any of the samples employed could be used to prepare matrix-matched reference suspensions. Liang, Mi, and Rao (1996) have observed that urine matrix influenced Se absorbance, and circumvented the drawback by performing calibration by the standard addition method. Despite the study carried out with addition of selenium in suspension, a loss of sensitivity for some vegetable samples was observed. The possible causes related to this depression can be the high background signal, in addition to the formation of carbonaceous residues inside the graphite tube. Backlog of carbonaceous residue may obstruct part of the analytical wavelength from the radiation source, impairing measurements. This was observed during method development for selenium determination in coconut milk (Aleixo, Nóbrega, Santos, & Muller, 2000). The loss of sensitivity for selenium determination in slurries of plant materials may also be attributed to the P, Fe, Ca (Houba & Uittenbogaard, 1996) and S (Welz, Bozsai, Sperling, & Radziuk, 1992) present in the matrices.

After optimization, the applicability and accuracy of the proposed procedure for Se determination were investigated in slurries of 10 plant samples and 1 standard reference material (Table 3). The accuracy of the results obtained were checked adopting a conventional microwave-assisted acid digestion procedure and they were not statistically different at a 95% confidence level (paired t-test). Recoveries within 89% and 95% range

were attained and the relative standard deviations were 3.8 and 8.3% for an acid digestate and a slurry containing 20 µg Se l^{-1} ($n = 12$). Detection limits (3σ blank/slope) were 0.6 µg l^{-1} and 2 µg l^{-1} for acid digestates and slurries, respectively. For 10 µg Pd and 20 µl sample, analytical curves in the 3.0–30 µg l^{-1} concentration range were always attained with good linearity ($r = 0.9994$) and the lifetime of the graphite tube was ca. 250 firings. The use of a 0.1% m/v slurries and 1% v/v HNO$_3$ usually reduce the lifetime of atomizer when compared to aqueous solutions containing 0.2% v/v HNO$_3$. According to a work developed for Se determination in slurries of coconut milk (Aleixo, Nóbrega, Santos & Muller, 2000), the number of firings of the graphite tube was ca. 270. If we consider the sampling of vegetable slurries directly into the atomizer, a very satisfactory lifetime was obtained in the present work.

In conclusion, palladium modifier can be successfully used for the determination of total selenium in plant slurries at trace levels. Release of Se involves its migration from palladium droplets, which is the same rate-limiting step occurring when palladium is added to the analyte in solution. Palladium does not need to be in direct contact with the analyte when introduced into the graphite tube as Se releases from slurry particles and migrates to a palladium during the pretreatment stages (Jackson, 1999). The developed procedure is simple and alternative to procedures involving hydride generation atomic absorption spectrometry, in which trace levels of Se can also be determined. The method of analysis described here, using only palladium as a chemical modifier, allowed direct calibration with matrix-matched reference suspensions for precise and accurate determination of selenium in plant slurries.

Table 3
Results ($n = 5$) in µg/g obtained for Se in enriched plant samples using the developed procedure with slurry sampling and with the microwave-assisted acid-digestion procedure

Sample	Slurry sampling	Microwave-assisted digestion
Beet	3.8±0.2	3.5±0.1
Garlic	0.17±0.01	0.15±0.01
Broccoli	1.9±0.1	2.1±0.1
Carrot	0.38±0.02	0.39±0.02
Kale	3.4±0.1	3.6±0.1
Tomato	0.30±0.01	0.34±0.02
Cabbage	3.2±0.2	3.0±0.1
Radish	5.1±0.4	4.7±0.2
Lettuce	0.63±0.03	0.55±0.05
Spinach	2.4±0.2	2.6±0.1
Rice flower[a]	0.25±0.02	0.27±0.01

[a] 1568a, Certified reference material (0.30±0.04 µg g^{-1}) from National Institute of Standards and Technology.

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