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Determination of ultratrace levels of selenium in fruit and vegetable samples grown and consumed in Portugal

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ABSTRACT

The selenium content in fruit and vegetable samples from two regions in Portugal were analysed using hydride generation atomic fluorescence spectrometry (HG-AFS) and radiochemical nuclear activation analysis (RNAA) – two analytical methods with very low limits of detection. The lower detection limits of HG-AFS, 3 μg kg^{-1} and 8 μg kg^{-1} (according to conditions used for digestion), and for RNAA, 10 μg kg^{-1}, meant that it was possible to determine selenium in samples previously analysed using the replicate sample instrumental nuclear activation analysis (RSINAA) with a higher detection limit associated.

The results obtained with the HG-AFS method were similar to those obtained using either RNAA or RSINAA, although in the case of RSINAA significant differences were found in three samples. The good accuracy and increased sample throughput, together with the relatively lower equipment and operating costs make HG-AFS the optimum of the three methods for determining trace amounts of selenium. Values obtained by HG-AFS were from 0.03 μg in tomato to 3.1 μg in cabbage (100 g fresh weight). Based on our results, the contribution of the analysed vegetables and fruits to the daily selenium intake was 1.80 μg per person per day for the Portuguese population.

1. Introduction

The main source of selenium for humans is the diet. Plants are the primary distributors of selenium, which they obtain from the soil and which is then consumed by animals and spread further on up through the food chain. The amount of selenium in plants is highly dependent upon both the amount and the availability of selenium in the soil and this can vary geographically, which in turn can affect the selenium status of entire communities (Combs & Combs, 1986; Reilly, 1996). Low amounts of selenium in fruits and vegetables (less than 2 μg/100 g) have been linked to serious health problems. For example, two diseases associated with severe selenium deficiency in humans are Keshan and Kaschin-Beck disease: the first is a cardiomyopathy and the second is an osteoarthropathy. Each occurs in rural areas of China and Russia in food systems that have extremely low selenium supplies (Abrahams, 2002, 2006; Tan et al., 2002). In these cases, where the diet consisted mainly of locally-produced cereals, dietary intake was as low as 7 μg day^{-1} (Combs & Combs, 1986). A plant's ability to absorb selenium from the soil also varies (Terry, Zayed, Souza, & Tarun, 2000). Since the transport mechanism of selenium is thought to resemble that of sulphur, plants that normally are rich in sulphur, such as members of the *Liliaceae* family (onions and garlic) and members of the *Cruciferae* family (cabbage and broccoli) are richer in selenium (Brown & Shrift, 1982), whereas plants rich in starch and sugar are poorer in the element. Plants that have elevated protein contents, such as cereals, where selenium is able to substitute for sulphur in protein amino acids (cysteine and cystine) are also considered an important source. In North America, where the selenium content of locally-produced plant foods is often higher, due to the high contents and availability of the element in the soil, cereal foods are the major source of selenium (Combs & Combs, 1986; Sager, 2006).

Generally, fruits and vegetables, in most human diets, provide only a small amount of the element. According to the literature, the contribution of vegetables to the daily intake of selenium is less then 8% of the total (Combs, 2001).

The determination of selenium in plant samples is challenging because of its low concentration and the losses that occur owing to volatilisation during the sample decomposition. Despite this, several analytical methods with low detection limits have been applied, the most common of which are spectrofluorimetry

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(Sirichakwal, Puwastien, Polngam, & Kongkachuichai, 2005; Tinggi, Reilly, & Patterson, 1992) inductively coupled plasma-mass spectrometry (ICP-MS) (McNaughton & Marks, 2002; Menegario & Gine, 2000), hydride generation atomic absorption spectrometry (HG-AAS) (Díaz-Alarcón, Navarro-Alarcón, Ga, de la Serrana, & López-Martínez, 1994; Kadrabova, Madaric, & Ginter, 1997; Klapec et al., 2004; Tinggi et al., 1992) and hydride generation atomic fluorescence spectrometry (HG-AFS) (Pappa, Pappas, & Peter, 2006; Smrkolj & Stibilj, 2004). Hydride generation coupled with atomic fluorescence spectrometry (HG-AFS) is one of the most convenient methods for determining low concentrations of selenium, owing to fewer interferences and because of its good sensitivity and low detection limits (Moreno, Pérez-Conde, & Câmara, 2003). Additionally, the instrumentation, when compared to alternative instruments, is less expensive.

Neutron activation analysis (NAA) is also capable of determining selenium in biological samples. For analysing trace levels, NAA based on the principles of cyclic activation analysis is most suitable. In this method, the signal-to-background ratio can be improved significantly by lowering the detection limits (Parry, 1982; Spyrou, 1981). In the radiochemical version (RNAA), a higher sensitivity is achieved by separating the desired radionuclide according to experimental conditions (Heydorn, 1984). The main disadvantage is that it is time-consuming, making it unsuitable for routine analysis (Stibilj, Dermelj, & Byrne, 1996).

The main objective of this work is to determine the amount of selenium in fruit and vegetable samples produced locally in two regions of mainland Portugal: Évora and Coimbra. Initial attempts to study selenium involved using NAA in its instrumental version (INAA); (Pacheco, Freitas, Ventura, Dionísio, & Ermakova, 2006), and in its replicate sample version (RSINAA); (Ventura, Freitas, Pacheco, Van Meerten, & Wolterbeek, 2007), but because of the low levels of selenium it was not possible to obtain accurate values for all the samples. For this reason the more sensitive methods, such as HG-AFS and RNAA were chosen. The second aim of this work was to determine the actual contribution that consuming fruits and vegetables make to selenium intake of the Portuguese population.

2. Experimental

2.1. Samples

Fruit and vegetable samples (orange, apple, tomato, potato, lettuce, cabbage, cauliflower, broccoli, and carrot) were purchased in the winter of 2003 and in the summer of 2004 from large commercial areas or municipal markets in Évora and Coimbra. The Coimbra region is in the west of the Centro region, central Portugal, while the Évora is situated in the Alentejo region in the centre of the southern part of the country. All fruit and vegetables were cultivated in horticultural plots in western Portugal and were chosen on the basis of the level of consumption (INE, 2003). Carrot, potato and fruit were peeled. All samples were chopped into small pieces, weighed and then washed with distilled water. These were then frozen and lyophilised (Heto-DW 8) at 45 °C and 0.2 mbar. After lyophilisation the samples were again weighed to determine their water content before being milled in Teflon capsules and stored in polyethylene vials. To prevent sample degradation, samples were kept frozen at −20 °C prior to digestion.

2.2. Selenium determination

2.2.1. HG-AFS

Thirty two samples were analysed by the HG-AFS method. For each sample, a small portion of material was accurately weighed (0.18–0.22 g) into a 50 ml polyfluoroethylene (PTFE) tube to which 0.5 ml of concentrated H_2SO_4 and 1.5 ml of HNO_3 were added. The closed tube was heated at 130 °C for 60 min in an aluminium block. After a period of cooling, 2 ml of H_2O_2 were added and the solution re-heated for 10 min at 115 °C. Then 0.1 ml of 40% HF were added, followed by a further period of heating at 115 °C for 10 min, followed by a second addition of H_2O_2 (2 ml). The solution was then heated for a final time under the same conditions. Hydrofluoric acid was added to the lettuce, cabbage, cauliflower and broccoli samples, i.e., vegetables with a high silica content. Hydrofluoric acid was not added: tomato, carrot and potato, which have a lower silica content.

After digestion, the solution was allowed to cool to room temperature before adding 0.1 ml of V_2O_5 in H_2SO_4. The sample was then heated at 115 °C for 20 min until the solution turned blue. Reduction of Se(VI) to Se(IV) was performed by adding 2.5 ml of concentrated HCl and heating at 100 °C for 10 min. The sample was diluted to 10 g or 15 g with Milli-Q water. The amount of selenium in the samples was then determined using HG-AFS, for which a series of matrix-matched standard solutions were prepared daily. The actual analytical procedure was followed as described in Smrkolj and Stibilj (2004).

Certified reference materials, Spinach Leaves (NIST-SRM 1570a), Peach Leaves (NIST-SRM 1547) and Mixed Polish Herbs (INCT-MPH-2) were used to check the accuracy of the analysis.

2.2.2. RNAA

Twelve samples, including four samples of lettuce and two samples of orange, apple, tomato and potato, were analysed. Samples were prepared in duplicate in plastic ampoules, while selenium standards were prepared by depositing approximately 0.15 g of a selenium solution (37.57 mg kg^{-1}) into separate ampoules. To ensure identical geometric conditions between standards and sample, the height of the material in the ampoules was kept constant. The mass of the samples varied from 0.200 to 0.400 g depending on material density.

The same standard reference materials used for the HG-AFS analysis were also used to check the accuracy of the RNAA analysis. Samples were irradiated during 40 h at a neutron flux of 1.1×10^{16} n m^{-2} s^{-1} and allowed to cool for a period of 2 weeks.

The radiochemical NAA procedure for selenium determination based on the destruction of the irradiated sample is described in Dermelj, Hancman, Gosar, Byrne, and Kosta (1985) and in Stibilj et al. (1996).

For sample digestion, 0.1 g of a carrier, Na_2SeO_4 (1.0005 g Se kg^{-1}), and 2–4 g of $Mg(NO_3)$ (10:1 w/w relative to sample mass) were placed together with the sample in a quartz crucible. A small amount of H_2O (1 ml) was then added to the crucible and the mixture heated on a hot plate at 250 °C until the formation of a residue. Before the residue was completely dry, small amounts of H_2O were added until the residue acquired a light yellow colour. The crucible was then transferred to an oven, where the temperature was raised in three steps to 520 °C to mineralise the sample. Reduction of Se(VI) was made at 100 °C with 20 ml of HCl (6 M)). After reduction, 1.5 ml of 4-nitro-1,2-diaminobenzene (1% in HCl (1M)) was added, and the obtained chelate, 5-nitro-2,1,3-benzoselenodiazole, extracted with 6 ml of toluene. The blank samples were prepared under the same conditions but without the carrier.

The γ-ray spectra of the separated ^{75}Se radionuclide in organic phase (5 ml) were measured using a well-type HPGe detector. After measuring the activity, the samples were diluted to 25 ml with toluene, and the yield determined spectrophotometrically from the absorption of the selenium chelate at 443 nm. A standard solution of the chelate in toluene (2.18 μg Se ml^{-1}) was prepared for this purpose. Absorbance was measured using a Perkin–Elmer Lambda 17 UV–Vis spectrophotometer.

2.3. Estimation of the fruit and vegetables' contribution to the selenium daily intake

An estimation of the contribution of the analysed samples to the selenium daily intake was obtained by taking the sum of the daily consumed weight of food and multiplying it by its mean selenium content, for each type of fruit and vegetable, according to Cameron and Van Staveren (1988):

$$Intake = \sum_{j=1}^{n} c_j w_j,$$

where c_j is the average selenium concentration determined in the fruit or vegetable j (ng g^{-1}), and w_j is the weight of fruit or vegetable j consumed per day (edible portion in g per person).

3. Results and discussion

3.1. Quality assessment

Table 1 shows a comparison between the selenium content, as determined by both HG-AFS and RNAA in the reference materials, compared with their certified values. With the exception of the HG-AFS value obtained for the peach leaves, the overall results are in good agreement and show that both methods are capable of providing accurate results with a precision lower than 8%.

The HG-AFS method detection limit is 0.11 μg kg^{-1} when using HF in the digestion procedure and as 0.06 μg kg^{-1} without HF. For green vegetables the samples were diluted to 15 g, while for fruit, tomato, potato and carrot samples they were 10 g. The detection limit was calculated as three times the base line noise divided by the slope of the regression curve. A similar value for the detection limit (0.14 μg kg^{-1} of solution and 10.5 μg kg^{-1} of sample) was obtained by Smrkolj and Stibilj (2004), for the determination of selenium in vegetable samples using HF during the digestion procedure. A higher sensitivity is achieved when HF is not used in the digestion procedure. For the RNAA method, the detection limit was 10 μg kg^{-1}. This value corresponds to three times the square root of the background adjacent to the peak. A higher detection limit was obtained for the replicate sample INAA method (RSINAA), used to determine selenium in the same set of samples as Ventura et al. (2007).

3.2. Selenium in vegetable and fruit samples

Table 2 shows the amount of selenium determined in the fruit and vegetable samples using both analytical methods. The values are presented on a wet weight basis and calculated using an average dry matter value for each vegetable. We found the following results for dry matter: 25% for potato, 7% for tomato, 15% for carrot, 13% for broccoli, 11% for cauliflower, 16% for cabbage, 17% for orange and 19% for apple. In general, there is a good agreement between values obtained by both methods, although, despite the low detection limit, the sensitivity of RNAA was insufficient to determine the selenium content in those samples (tomato and fruit samples) where the element concentration was very low.

The lower detection limits achieved by HG-AFS and RNAA means that it is possible to determine selenium in samples that were previously analysed by RSINAA but whose values were below the detection limit. Fig. 1 shows a comparison of the results obtained by HG-AFS in this work and by RSINAA in Ventura et al. (2007).

Generally, there exists a good agreement between the values obtained by RSINAA and HG-AFS, despite the actual levels being close to the detection limit. The values obtained using RSINAA are lower than those obtained by HG-AFS for the majority of samples but statistically significant differences at the 95% confidence level are only observed for three samples. According to a Student's t-test, p-values were from 0.1 to 0.8, except for two cabbage samples, Cab_E2 and Cab_C2, with p-values of 0.04 and 0.01, respectively, and one cauliflower sample, Cau_C2, with a p-value of 0.03.

When using HG-AFS, the sample must undergo decomposition prior to making a measurement. This, although a critical step, can be challenging because of loss of analyte can occur. In this study, samples were wet digested in a closed system, using an acidic mixture. The hydrofluoric step was performed for certain samples to assure silicate destruction. Since plant samples usually contain high amounts of siliceous compounds, this step was necessary to liberate the selenium. Furthermore, the entire procedure,

Table 1
Selenium concentrations in standard reference materials by HG-AFS and RNAA.

Sample	Selenium content (μg/kg dry weight)[a]		Certified value
	RNAA	HG-AFS	
Spinach leaves (NIST 1570a)	118 ± 6 (8)	107 ± 8 (8)	117 ± 9
Peach leaves (NIST 1547)	120 ± 7 (8)	96; 99 (2)	120 ± 9
Mixed polish herbs (INCT-MPH-2)	37 ± 3 (7)	34.3; 38.6 (2)	37.3[b]

[a] Average ± standard deviation (number of determinations).
[b] Information value.

Table 2
Selenium concentrations in vegetable and fruit samples (fresh weight) determined by HG-AFS and RNAA.

Vegetables/fruits	Se content (mg/100 g fresh weight)[a]	
	HG-AFS	RNAA
Potato	0.39 ± 0.32 (3) [0.12–0.74]	0.33(1)
Tomato	0.03; 0.03 (2)	ND
Carrot	0.29; 0.30 (2)	NA
Broccoli	1.15 ± 0.70 (4) [0.37–1.7]	NA
Cauliflower	1.9; 0.44 (2)	NA
Cabbage	1.26 ± 0.93 (4) [0.46–3.1]	NA
Lettuce	0.40 ± 0.04 (4) [0.36–0.44]	0.39 ± 0.05 (3) [0.32–0.42]
Orange	0.06; 0.06 (2)	0.13(1)
Apple	0.07(1)	ND

NA not analysed; ND not detected.
[a] Average ± standard deviation (number of samples) [range].

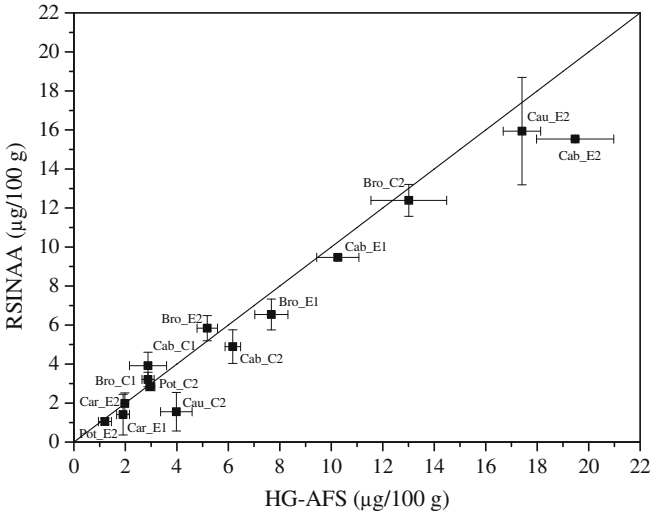

Fig. 1. Comparison of selenium data (in dry weight) for vegetable samples analysed by HG-AFS and RSINAA (Bro: broccoli; Cab: cabbage; Car: carrot; Cau: cauliflower; Pot: potato; C: Coimbra; E: Évora; 1 and 2 1st and 2nd collection, respectively).

Table 3
Comparison of selenium content (fresh weight) in vegetables produced and consumed in Portugal with other literature data.

Country	Method	Sample								
		Lettuce	Potato	Tomato	Cabbage	Cauliflower	Broccoli	Carrot	Apple	Orange
Selenium content (μg/100 g)[A]										
This study	HG-AFS	0.40 ± 0.04 (4) [0.36–0.44]	0.39 ± 0.32 (3) [0.12–0.74]	0.03; 0.03 (2)	1.26 ± 0.93 (4) [0.46–3.1]	1.9; 0.44 (2)	1.15 ± 0.70 (4) [0.37–1.7]	0.29; 0.30 (2)	0.07 (1)	0.06; 0.06 (2)
	RNAA	0.39 ± 0.05 (3) [0.33–0.42]	0.33 (1)	ND	NA	NA	NA	NA	ND	0.13 (1)
	RSINAA[E]	ND	0.26; 0.71 (2)	ND	1.35 ± 0.85 (4) [0.70–2.5]	0.17; 1.8 (2)	0.91 ± 0.50 (4) [0.42–1.6]	0.33 ± 0.14 (3) [0.21–0.49]	ND	ND
Spain[a]	HG-AFS	0.239 (1)	0.130(8) [0.044–0.773]	0.232 (3) [0.078–0.366]		0.181 (2) [0.122–0.240]		0.0308 (2) [nd-0.0615]	0.139; 1.239 (2)	0.153; 0.236 (2)
Greece[b]	HG-AFS	0.09; 0.38 (2)	0.31; 0.60 (2)	0.22; 0.24 (2)		0.12; 0.47 (2)	0.61; 1.18 (2)	0.36; 0.85 (2)	0.14 ± 0.02 (3) [0.11–0.19]	0.34; 0.51 (2)2
UK[c]	HG-AAS		1.6 ± 0.79 (18) [0.7-2.8]							
Slovenia[d]	HG-AFS	0.70 ± 0.81 (6) [0.03–2.0]	0.15 ± 0.03 (4) [0.11–0.17]	0.11–2.91 (4)	2.03 ± 2.70 (7) [0.11–7.67]			0.42 ± 0.37 (10) [0.06–1.16]		
Slovakia[e]	HG-AAS	0.09 ± 0.04 (3) [0.05–0.13]	0.35 ± 0.22 (8) [0.05–0.57]	0.05 ± 0.02 (3) [0.03–0.07]	0.20; 0.40; 1.66 (3)	0.22 ± 0.12 (4) [0.12–0.47]		0.13 ± 0.07 (6) [0.07–0.26]	0.14 ± 0.06 (6) [0.08–0.25]	0.13; 0.08 (2)
Croatia[f]	HG-AAS		0.95 ± 0.13 (3)[F] 0.72 ± 0.08 (2)[F]	0.79 ± 0.32 (2)[F] 1.02 ± 0.01 (2)[G]	6.61 ± 0.56 (2)[F] 0.83 ± 0.05 (2)[G]	2.44 ± 0.56 (2)[F] 2.49 ± 0.72 (2)[G]	2.50; 2.58 (2)	1.96 ± 0.02 (2) 0.81 ± 0.12 (2)	0.88 ± 0.16 (2)[F] 0.78 ± 0.02 (2)[G]	
USA[g]	ns	0.09–1.0 (7)		0.59 (1)	0.86 (1)	0.60; 0.77 (2)	2.34 ± 0.98	0.09; 0.14 (2)		0.50; 0.53 (2)
Canada[h]	PCINAA		2.9 ± 0.37							
Egypt[i]	ETAAS[B]/HG-AAS	<0.001		<0.001						<0.001
Pakistan[j,D]	INAA		4.8 ± 0.6	3.9 ± 0.4	3.1 ± 0.4	7.6 ± 0.8			3.8 ± 0.4	
Thailand[k]	F[C]	0.3 (1)		0.12 (1)	0.2–0.14 (4)	0.7 (1)	0.6 (1)	3.9 (1)		
Australia	ICP-MS/HG-AFS[l]	1.79 [0.3–2.28]		0.05	1.6				0.45 [0.30–0.50]	0.6 [0.3–0.7]
	HG-AAS[m]	0.3 ± 0.1 (3)		[<0.1–0.2]					0.3 ± 0.1 (4)	0.3 ± 0.1 (4)

ND not detected; NA not analysed.
[A] Average ± standard deviation (number of samples) [range];
[B] ETAAS: Electrothermal AAS.
[C] Fluorimetric method.
[D] Dry basis.
[E] Ventura, Freitas, Pacheco, Van Meerten and Wolterbeek (2007).
[F] Sava basin.
[G] Drava basin.
[a] Díaz-Alarcón, Navarro-Alarcón, López Ga de la Serrana and López-Martínez (1994).
[b] Pappa et al. (2006).
[c] Barclay, MacPherson, and Dixon (1995).
[d] Smrkolj and Stibilj (2004).
[e] Kadrabova et al. (1997).
[f] Klapec et al. (2004).
[g] USDA (2004).
[h] Shi, Sullivan, Holzbecher, and Chatt (1999).
[i] Hussein and Bruggeman (1999).
[j] Waheed, Zaidi, and Ahmad (2003).
[k] Sirichakwal, Puwastien, Polngam, & Kongkachuichai (2005).
[l] McNaugthton and Marks (2002).
[m] Tinggi, Reilly, and Patterson (1992).

from weighing to measuring, was performed in the same Teflon tube, to avoid handling errors. The optimisation of the used wet digestion method is described by Smrkolj and Stibilj (2004), average selenium recoveries of 90% are obtained when the procedure is followed. Values for repeatability and reproducibility were also calculated by the same authors and were 10 and 9%, respectively. In the case of RSINAA, since it is an instrumental method, no prior treatment of the samples was performed and no analyte losses are expected. From the analysis of the spectra, no spectral interferences that could have influenced the results were observed.

A possible explanation for the apparent discrepancy in the results is existence of some heterogeneity in the sample materials. Considering the number of replicates used for RSINAA ($n = 15$), deviations due to the heterogeneity of the sample material should be less relevant than in the case of HG-AFS, since approximately 2 g ($15 \times \sim 0.15$ g) of sample was analysed in the first method and only 0.2 g in the second.

From the above, HG-AFS is the most suitable of the methods tested for selenium determination in samples having a low selenium content. HG-AFS with its relatively low equipment and operating costs, and shorter analysis times is the most economical option. In addition the lower amount of reagents consumed for the analysis of a sample makes it the most environmentally friendly of the three methods discussed in this report.

Table 3, shows a comparison between the selenium concentrations obtained in this study by using HG-AFS and RNAA, and those obtained by RSINAA in Ventura et al. (2007) and concentrations obtained in other countries. The results reflect the high degree of variability in selenium levels that characterise these types of samples. These differences are usually attributed to the fact that selenium contents in plants are strongly related to the selenium that is available in the soil in which they are grown, which in turn depends on cultivation and geographical location. In the case of the Portuguese samples, the highest selenium contents are in cabbage samples rich in sulphur, whereas the lowest values are present in fruit samples rich in starch. Selenium contents were from 0.03 μg in tomato to 3.1 μg in cabbage (all per 100 g wet weight; values obtained from HG-AFS). The results are within the range of selenium concentrations reported in the literature. The amount of selenium in fruits and vegetables from Portugal are in the same order as those found in regions of the world that have soils containing low selenium concentrations. These regions include Finland before 1984 (since then, soils have been treated with fertilisers containing selenium, in order to overcome selenium deficiencies), New Zealand and north-east and south-central parts of mainland China (Combs, 2001).

Typical selenium contents in fruits and vegetables in these regions are 1–2 μg per 100 g (Combs, 2001). Information about selenium levels in Portuguese soils is scarce. Mean values of top soils according to different geological areas are presented in Ventura, Freitas, and Pacheco (2005). A lower level of 0.28 mg kg^{-1} was found in the central-littoral area, which stretches along the Atlantic coast south of Aveiro down to the Sado river estuary. North, south and central-inland areas have mean levels of 0.51, 0.54 and 0.66 mg kg^{-1}, respectively. The mean levels for the five areas are typical for most soils (0.1–2 mg kg^{-1}) (Combs, 2001), while according to the rating system of selenium in topsoils developed in New Zealand, the levels are classed as low (0.3–0.5 mg kg) to moderate (0.5–0.9 mg kg^{-1}) (Reilly, 1996). The vegetables sampled during this work were cultivated in the western areas of Portugal, which are mainly low selenium areas, however, with regards to the availability of selenium, other factors are more important than the total selenium concentration. For example the mobilisation of selenium from soils is influenced by its chemical form, soil pH, oxidation–reduction conditions, moisture levels, and the degree of aeration (Selim & Sparks, 2001). Both elemental selenium and the selenides are stable and largely insoluble, and thus not readily available to plants (Selim & Sparks, 2001). However, under acidic conditions, selenite tends to be associated with clay particles and iron complexes, whereas alkaline conditions favour the conversion of selenite to selenate, which is highly soluble in water. In regions where there is abundant rainfall, the soluble selenate is then leached, resulting in selenium deficiency, as is the case for New Zealand, Tasmania and in parts of Australia (Reilly, 1996). Johnson, Ge, Green, and Liu (2000) have undertaken a systematic study based on epidemiological data, that links the amount of selenium in grain directly to the element status of the soil in which the grain was grown, as well as to the selenium status of the people who consume it. The authors selected for study, villages of the Keshan disease belt in China. Keshan disease is present even in areas with high selenium content. It occurs when the element bioavailability is reduced owing to the organic matter content and pH value of the soil. According to the authors, the organic content is the most important factor in restricting the availability of selenium in the food chain. More studies will be necessary to determine the selenium availability in plants in the cultivation areas of the Portuguese mainland. Information on the geochemistry of the soils as well as on the solubility of selenium will be necessary, if a more thorough characterisation of the situation is to be made.

Fig. 2 shows a comparison of the selenium contents in fruit and vegetable samples collected in Coimbra and Évora. A comparison was made to see if differences exist between the two regions, and between seasonal samplings. The actual values shown are those obtained using HG-AFS, with the exception of the carrot sample from Évora's winter collection, which was analysed by RSINAA.

The results show a general trend, characterised by higher amounts of selenium in the samples collected during the summer in both locations. Seasonal variations resulting in different soil characteristics are one possible explanation for this observation. The selenium availability to plants is affected by soil moisture:

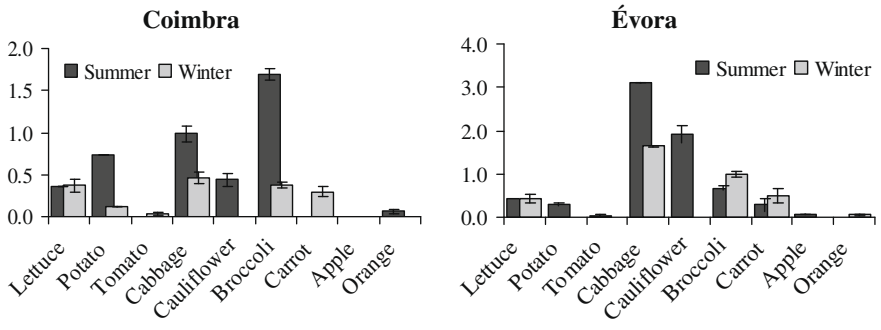

Fig. 2. Selenium levels (μg/100 g in fresh weight) in fruits and vegetables for Coimbra and Évora, considering the summer and winter sampling.

the element is most available under conditions of low precipitation and low soil leaching (Combs, 2001; Selim & Sparks, 2001). Temperature is also a factor influencing selenium uptake by plants; in a soil with a low selenium content, plants absorb a much higher amount when the temperature is above 20 °C than during the cooler seasons when temperatures fall below 15 °C (Kabata-Pendias, 2001). These facts agree with the high selenium levels found in samples collected during the summertime with higher temperatures and lower precipitation.

Table 4 gives the mean selenium contents in μg per 100 g fresh weight obtained, for the two regions. Except for cabbage and cauliflower, which have slightly different mean values, there is good agreement between the mean values determined for both regions; this fact was expected since the samples were cultivated in the same region of the Portuguese territory (Pacheco et al., 2006).

Based on the presented selenium levels in vegetables, we made an estimation of the contribution that such fruits and vegetables make to the selenium intake per person per day. Selenium composition data for vegetables were combined with published food consumption data for the Portuguese population (FAO Food Balance Sheet, 2004). The daily intake was calculated by multiplying the mean selenium content in each fruit and vegetable species by the mean consumption of each species per person per day. The edible conversion factors are described in the food composition tables (INSA, 2006). Table 5 presents the contribution of the analysed fruits and vegetables; the mean selenium daily intake is estimated at 1.80 μg per person. The analysed fruits and vegetables represent 40% of the total consumed in these two food categories, a fact that might lead to an underestimation of the determined value. The contribution to the daily selenium intake of other samples of vegetable origin, namely rice and beans, which are included in the cereals and legumes food groups, was determined by Ventura (2008), as 0.89 and 2.91 μg per day, respectively. Since the selenium concentrations obtained for both sampling sites were similar, and no regional food consumption data was available, no differences were obtained on the total contribution of vegetable and fruit samples to the selenium intake of Évora and Coimbra. The contributions were 1.71 and 1.69 μg per day, respectively.

The recommended value for the selenium intake, as established by the American Food and Nutrition Board of the Institute of Medicine, is 55 μg per day (DRI, 2000). According to the Portuguese Food Balance for 2003 (INE, 2006), the consumption of vegetables by the Portuguese population is low, representing 12% of the total food items consumed, which is half of the 23% recommended by the Food Wheel (Rodrigues, Franchini, Graça, & de Almeida, 2006). The recommended value for fruit is 20%, which is closer to the consumption by the Portuguese population of 15% (INE, 2006). Given the low selenium contents found in these kind of samples, even if we assumed that the recommended amounts for fruits and vegetables were consumed by the Portuguese population, fruit and vegetables would make only a small contribution to the element intake.

Table 4
Mean selenium concentrations found in the two studied regions of the Portuguese territory.

Vegetables/fruits	Se content (μg/100 g fresh weight)[a]	
	Coimbra	Évora
Potato	0.43 (2) [0.12,0.74]	0.30 (1)
Tomato	0.03 (1)	0.03 (1)
Carrot	0.30 (1)	0.39 (2) [0.29,0.49[b]]
Broccoli	1.0 (2) [0.37, 1.7]	0.84 (2) [0.68, 1.0]
Cauliflower	0.44 (1)	1.9 (1)
Cabbage	0.72 (2) [0.46, 0.99]	2.4 (2) [1.6, 3.1]
Lettuce	0.36 (2) [0.36, 0.37]	0.43 (2) [0.43, 0.44]
Orange	0.06 (1)	0.06 (1)
Apple	–	0.07 (1)

[a] Mean value (number of samples) [range].
[b] Value obtained by RSINAA.

Table 5
Contribution of analysed vegetables and fruits to mean daily selenium intake.

Vegetables	Daily consumption (g/person)	Daily selenium intake (Hg/person)
Potato	306	1.18
Tomato	41	0.01
Carrot	35	0.13
Cauliflower and broccoli	4	0.04
Cabbage	19	0.29
Lettuce	16	0.06
Orange	47	0.03
Apple	70	0.05
Total		*1.80*

4. Conclusions

In this work, HG-AFS and RNAA were applied for the determination of selenium in fruits and vegetables. Both methods provided accurate values and were considered reliable for measuring low concentrations of selenium in these kinds of matrices. An improvement in the detection limits using these two methods is apparent when compared with RSINAA, a fact which makes it possible to measure selenium in a larger number of samples. Economic, environmentally friendly and with a short analysis time, the HG-AFS method was considered the best analytical method for routine analysis, especially when compared with RNAA.

The levels determined in fruits and vegetables were of the same order of magnitude as those in areas where the selenium content of the soil is low. In addition, no significant difference exists between the mean values found for Évora and Coimbra regions but there is a difference in the amount of selenium present in both regions in samples collected in the summer and winter, This is believed to be a result of the increased availability of the element under conditions of low precipitation and high temperatures in the summertime. The selenium determined in the fruit and vegetables analysed makes only a small contribution to the dietary intake of selenium.

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