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Determination of selenium in nutritionally relevant foods by graphite furnace atomic absorption spectrometry using arsenic as internal standard

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Abstract

A method has been developed for the direct determination of Se in nutritionally relevant foods by graphite furnace atomic absorption spectrometry. Tungsten/rhodium carbide coating on the integrated platform of a transversely heated graphite atomizer or W coating with co-injection of $Pd(NO_3)_2$ were used as a permanent modifiers. Samples and reference solutions were spiked with 500 μg L^{-1} As and absorbance variations due to changes in experimental conditions were minimized. For 20 μL aqueous analytical solutions delivered into the graphite tube, analytical curves in the 5.0–40 μg L^{-1} with good linear correlation were established. Pyrolysis and atomization temperatures were evaluated using pyrolysis and atomization curves, respectively. The optimized heating program (temperature, ramp time, hold time) of the graphite tube of the Perkin–Elmer SIMAA 6000 atomic absorption spectrometer was: dry steps (110 °C, 5 s, 10 s; 130 °C, 15 s, 15 s); air-assisted pyrolysis step (600 °C, 20 s, 40 s; 20 °C, 1 s, 40 s); pyrolysis step (1300 °C, 10 s, 20 s); atomization step (2100 °C, 0 s, 4 s); clean step (2550 °C, 1 s, 5 s). The method was applied for Se determination in coconut water, coconut milk, soybean milk, cow milk, tomato juice, mango juice, grape juice and drinking water samples and four standard reference materials and results were in agreement at 95% confidence level. The lifetime of the tube was 500 firings and the relative standard deviations of measurements of typical samples containing 25 μgL^{-1} Se were 3.0% and 6.0% ($n = 12$) with and without internal standardization, respectively. The limits of detection were in the 0.35 μg L^{-1}–0.7 μg Se L^{-1} range. The accuracy of the proposed method was evaluated by an addition-recovery experiment and all recovered values were in the 98–109% range.

Keywords: Selenium; Nutritionally relevant food; Graphite furnace atomic absorption spectrometry; Internal standardization; Simultaneous determination

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1. Introduction

Human health problems may be caused by deficiency or excess of selenium in human diets (Alejos, Romero, & Dias, 2000; Kim, Du, Otwell, Marshall, & Wei, 1998). In large excess, selenium compounds may induce adverse effects. On the other hand, the lack of this element may cause cardiovascular, muscular dystrophy, fertility diseases, and others (Cowgill, 1998).

Foodstuffs are good sources of Se to humans. Among foodstuffs considered as a good source of Se are cruciferous vegetables, legumes, cereals, nuts, fruits, milks (Arruda, Gallego, & Valcárcel, 1994; Haygarth, 1994; Terry & Zayed, 1994). Besides relatively cheap and

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abundant in tropical areas, these foodstuffs are also a good source of other essential minerals such as Na, K, Fe, and Mg (Santoso, Kubo, Ota, Takadoro, & Mackawa, 1996; Seow & Gwee, 1997; Underwood, 1977). The concentration of Se in foodstuffs depends on conditions influencing its translocation from soil to plants (Mayland, 1994; Soltanpour & Workman, 1980). Since the range between essential and toxic levels of Se in human nutrition is short (Rayman, 2002), the accurate determination of this element in samples of human feeding and agribusiness commodities is important (Mayland, 1994).

In spite of the fact that graphite furnace atomic absorption spectrometry (GFAAS) is a suitable and widely used technique for trace element determination due to its, selectivity, sensitivity and capability for direct analysis with minimal sample preparation (Butcher & Sneddon, 1998; Welz & Sperling, 1999), matrices of coconut water, coconut milk, fruit juices, soybean milk and cow milk are rather complexes and may introduce errors in the analytical results (Aleixo, Nóbrega, Santos, & Muller, 2000; Casey, Smith, & Zhang, 1995; Hua, Kay, & Indyk, 2000). Heterogeneous matrices may affect the quality of sampling by changing the amount of analyte inside the pipette of auto sampler of spectrometer. As a consequence, the mass of analyte inside the atomizer can vary randomly, and so the repeatability and/or accuracy of measurements.

Internal standardization (IS) could be adopted as a simple strategy to improve the analyses of complex samples by atomic spectrometry (Feldman, 1970; Gerlach & Schweitzer, 1929). The basic principle of IS in chemical analysis is based on the addition of a known and fixed amount of one or more elements used as internal standards to all blanks, reference solutions, and samples. For elements similarly affected by changes in experimental conditions, the use of the ratio between the analyte and internal standard signals may compensate the fluctuation of analytical results, improving their precision (Fernandes, Moraes, Neto, Nóbrega, & Oliveira, 2002; Radziuk, Romanova, & Thomassen, 1999).

This paper reports on the evaluation and application of arsenic as internal standard for direct determination of selenium in nutritionally relevant foods by simultaneous multi-element atomic absorption spectrometry using W/Rh or W with co-injection of $Pd(NO_3)_2$ as permanent modifier. The performance of this coating on the platform of graphite tube was appraised in order to improve the lifetime of the atomizer. The performance of the proposed method was checked by the analysis of a sort of 4 standard reference materials and 20 commercial samples of coconut water, coconut milk, soybean milk, cow milk, tomato juice, mango juice, grape juice and sparkling drinking water samples.

2. Materials and methods

2.1. Instrumentation

A Perkin-Elmer SIMAA-6000 simultaneous multi-element atomic absorption spectrometer with a longitudinal Zeeman-effect background correction system was used. The standard PerkinElmer THGA™ Transversely Heated Graphite Furnace Atomizer (Part No. B050-4033) with integrated platforms was used after pre-treatment with W and Rh or W with co-injection of $Pd(NO_3)_2$ (Lima, Krug, & Jackson, 1998). Electrodeless discharge lamps (EDL) were used for As (193.7 nm and slit 0.7 nm; Perkin–Elmer Part No. N305-0605) and Se (196.0 nm and slit 0.7 nm; Perkin–Elmer Part No. N305-0572), respectively, and they were operated according to the conditions recommended by the manufacturer. The EDL lamps were operated applying 380 and 290 mA, respectively. Atomic signals were measured in peak area mode. High-purity argon (99.999%, White Martins, Brazil) was used as the purge gas. It should be pointed out that the experiments were carried out using the stabilized temperature platform furnace, the so-called STPF conditions (Welz & Sperling, 1999) including Zeeman-effect background correction.

Measurements of each analytical solution and samples were carried out in three replicates.

2.2. Reagents, analytical solutions and samples

High purity de-ionized water (resistivity 18.2 MΩ cm) obtained using a Milli-Q system (Millipore) and nitric acid Suprapur® (Merck, Darmstadt, Germany) were used throughout to prepare all solutions.

Analytical reference solutions containing 0.0, 5.0, 10, 20, 40 $\mu g\ L^{-1}$ Se plus 500 $\mu g\ L^{-1}$ As were daily prepared by dilution of the 1000 $mg\ L^{-1}$ Se and As stock solutions (Normex®, Carlo Erba) and acidified with nitric acid. The autosampler washing solution was 0.1% (v/v) of Triton® X-100 in 0.2% (v/v) HNO_3.

The tungsten chemical modifier (1.0 $g\ L^{-1}$ W) was prepared by dissolving 0.1794 g of $Na_2WO_4.2\,H_2O$ (Merck) in 100 mL of water. The rhodium solutions (1.0 $g\ L^{-1}$ Rh) was obtained dissolving 0.0386 g of $(NH_4)_3RhCl_6$. 1-1/2 H_2O (Johnson and Matthey) in 10 mL of 0.7 mol L^{-1} HNO_3. The palladium solution containing 0.05% (m/v) Pd was prepared by appropriate dilution of a 10 $g\ L^{-1}$ Pd stock solution (Perkin–Elmer Part No. BO19-0635). Five coconut water samples (2 samples "*in natura*", obtained directly from the coconut fruit; 2 samples in 200-ml tetra pack container; 1 frozen sample in 200-ml polyethylene bottle), 1 whole coconut milk and 1 low sugar and fat coconut milk samples in 250-ml glass bottle, 2 whole soybean milk in 1000-ml tetra pack container, 6 cow milk samples (2 whole milk, 2 low-fat milk and non-fat milk) in 1000-ml tetra pack container, 3 concentrated tomato, mango and grape juice samples in 500-ml glass bottle and 2 sparkling drinking water samples in 250-ml polyethylene bottle were purchased at a local market in Araraquara city, São Paulo State, Brazil. Water and milk standard reference materials from the National Institute of Standards & Technology (NIST) were also used for the method validation: 8435 Whole Milk Powder; SRM2: 1549 Non-Fat Milk Powder; SRM3: 1643d Trace elements in Water; SRM4: 1640 Trace Elements in Natural Water. For the direct determination, water samples was diluted (1 + 1 v/v) with 0.2% (v/v) HNO_3; coconut water, coconut milk, and soybean milk were diluted (1 + 4 v/v) with 1.0% (v/v) HNO_3; cow milk was diluted (1 + 9 v/v) with 1.0% (v/v) HNO_3 and fruit juices were diluted (1 + 1 v/v) with 1.0% (v/v) HNO_3. For all samples were spiked with 500 μg L^{-1} As. This dilution factor was selected as a compromise between required sensibility and minimum organic matter dispensed inside the graphite tube.

2.3. Selected parameters to illustrate internal standardization performance

The electrothermal behavior of Se and As were investigated in presence of W/Rh or W with co-injection of $Pd(NO_3)_2$ as permanent modifier. This study employed the following solutions: 25 μg L^{-1} Se plus 25 μg L^{-1} As in diluted foodstuffs samples. The integrated platform of the THGA atomizer was pre-treated with W and Rh, forming a coating of 250 μg W + 200 μg Rh (Lima et al., 1998) and the integrated platform of the THGA atomizer was pre-treated with W with co-injection of $Pd(NO_3)_2$ forming a coating of 250 μg W with co-injection for 5 μL of $Pd(NO_3)_2$ in all replicates. Although GFAAS with Zeeman-effect background corrector is a potentially efficient technique for the determination of Se at trace levels with a simple dilution of the sample prior analysis, the large amount of carbonaceous residue from organic matrices generated after a few firings impaired the adequate performance of the spectrometer as previously observed by Aleixo et al. (2000). This drawback could be circumvented using an additional air-assisted pyrolysis step at 600 °C for 40 s. The optimized heating program used for simultaneous measurements of As and Se is shown in Table 1. Employing this program, the electrothermal behaviors of Se and As were studied by means of pyrolysis and atomization curves.

The influence of variation of temperature of atomizer on absorbance was evaluated employing pyrolysis and atomization curves, from which the ratio of Se to As absorbance was plotted as a function of the pyrolysis or atomization temperatures in the 600–1800 and 2000–2400 °C ranges, respectively.

The electrothermal behavior of the analyte and internal standard were studied in presence of different samples. The influence of the sample composition on Se and As absorbance signals were investigated by evaluating the accuracy and the precision from addition-recovery tests and analysis of samples with and without IS. All samples were spiked with 25 μg L^{-1} Se and 500 μg L^{-1} As and three replicates were performed for each sample. The absorbance signal corresponding to each original sample was adopted as the blank.

Table 1
Heating program of the graphite tube atomizer

Step	Temperature (°C)	Time (s) (Ramp, Hold)	Gas flow (mL min^{-1})
1	110	5, 10	250 (Ar)
2	130	15, 15	250 (Ar)
3	600	20, 40	250 (air)
4	20	1, 40	250 (Ar)
5	1300	10, 20	250 (Ar)
6	2200	0, 4	0 (read)
7	2550	1, 5	250 (Ar)

The efficiency of arsenic as internal standard for Se determination in nutritionally relevant foods was also evaluated by means of correlation graphs (Mermet & Ivaldi, 1993). As example, twenty consecutive measurements of a solution containing 25 μg L^{-1} Se plus 500 μg L^{-1} As in a coconut water sample in the presence of W/Rh as permanent chemical modifier were tested. The absorbance values were normalized with respect to these consecutive measurements. The correlation graph was plotted from the normalized absorbance signals ($n = 20$) of internal standard (axis y) vs. analyte (axis x). Parameters such as correlation coefficient, intercept and slope were evaluated in order to verify the resemblance between simultaneous measurements obtained for the analyte and internal standard.

3. Results and discussion

3.1. Electrothermal behaviors of selenium and arsenic

Using the heating program of atomizer shown in Table 1, the behavior of Se and As in diluted nitric acid and samples were studied in presence of W/Rh or W + $Pd(NO_3)_2$ as permanent chemical modifier. Pyrolysis and atomization curves were employed to determine the optimal pyrolysis and atomization temperatures. All results were based on peak area measurements. The permanent modifiers stabilized As and Se at temperatures up to 1400 °C. It should be stressed that absorbance abruptly drops for temperatures greater than 1400 °C. Therefore, the selected pyrolysis temperature may be lower than 1400 °C in order to avoid Se losses if small positive variations in the pyrolysis temperature occur. Hence, 1300 °C was the pyrolysis temperature selected for the further experiments. The optimum atomization temperatures for As and Se in diluted water sample were within 2200–2300 °C range, which resulted in best signal profile and good repeatability of measurements. Atomization temperatures higher than 2200 °C can provoke Rh volatilization, compromising the lifetime of the chemical modifier. So, the atomization temperature was fixed at 2200 °C.

3.2. Effect of sample matrix on absorbance of Se and As

The correlation between absorbance for Se and absorbance for As associated to different nutritionally relevant foods samples is shown in Fig. 1. Coconut water samples were selected as example. Signals correspond to spikes of 25 μg L^{-1} Se and 25 μg L^{-1} As and simultaneous detection of both elements. It was observed that the absorbance for Se varied similarly to the absorbance for As for selected samples. This suggests the potential application of arsenic as internal standard for Se. It should be pointed out that the effect was also observed for other type of samples analyzed in this work. A plot of normalized absorbance of arsenic vs. normalized absorbance of Se was built up in order to obtain the correlation graph (Fig. 2). The linear coefficient between normalized absorbances for As and Se was $A^{As} = -0.035 \pm 0.02 + 1.03 \pm 0.02 \quad A^{Se}$, $r = 0.9950 \pm 0.007$ (Fig. 2). The performance of the IS can be observed by comparing the obtained correlation coefficient to the ideal (perfect matching between the analyte and IS) correlation straight ($A^{As} = A^{Se}$, $r = 1$). The obtained correlation graph suggests As as good IS for Se determination in coconut water using W/Rh as permanent modifier. For others samples of foodstuffs the same performance was observed by comparing the obtained correlation coefficient to the ideal.

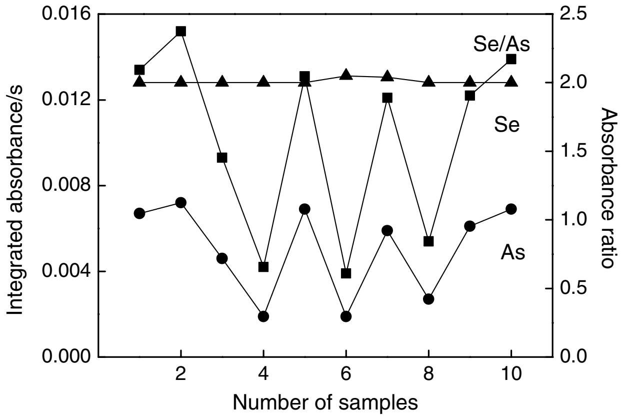

Fig. 1. Effect of the sample matrix on absorbance for Se and As, and corrected curve by internal standardization (Se/As). Spike signals in coconut water diluted in 1.0% (v/v) nitric acid refer to 25 μg L^{-1} Se and 25 μg L^{-1} As.

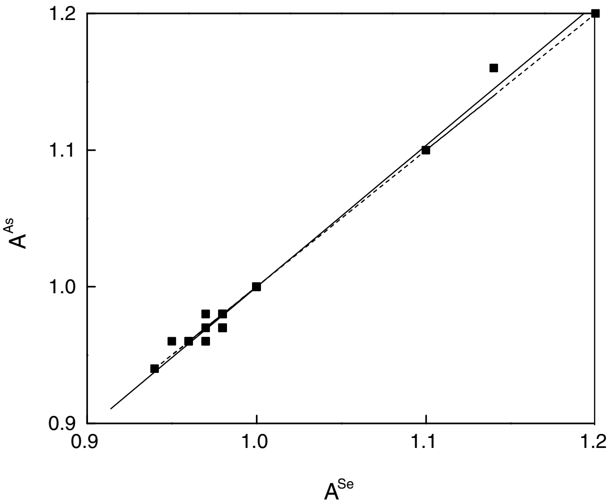

Fig. 2. Correlation graph between the internal standard (500 μg L^{-1} As) and analyte (25 μg L^{-1} Se). The dotted line indicates the ideal correlation straight.

3.3. Method validation

Precision and accuracy studies were carried out using addition-recovery tests for nutritionally relevant food samples. The results obtained with and without IS are shown in Table 2. It is interesting to stress that relative standard deviations were up to 3.0% and 6.0% with and without IS, respectively ($n = 12$, sample spiked with 25 μg L^{-1} Se). Good recoveries (98–109%) for Se spikes were obtained by using IS. The limits of detection were in the 0.35 μg L^{-1}–0.70 μg Se L^{-1} range. Characteristic mass obtained from calibration with Se in aqueous reference solutions or Se spiked in foodstuffs samples was close to 42 pg. Accuracy was also checked for selenium determination in milk and water standard reference materials and 5 coconut water samples, 2 coconut milk samples, 2 soybean milk samples, 6 cow milk samples, 3 concentrated tomato, mango and grape juice samples and 2 sparkling drinking water samples (Table 2). A paired *t*-test showed that results of SRMs were in agreement at 95% confidence level with certified values. Comparative results for other samples revealed the reliability of the proposed procedure involving IS to improving the analytical results. The Se levels found in this work for most selected samples were close to those obtained for coconut water and milk (Aleixo et al., 2000), fruit juice (Arruda et al., 1994; Haygarth, 1994; Terry & Zayed, 1994), sparkling water (Oliveira, Neto, Nóbrega, & Oliveira, 2004) and cow milk samples (Aleixo & Nóbrega, 2003). The long-term stability curves for untreated and W/Rh treated platform or W plus co-injection of $Pd(NO_3)_2$ were investigated for 25 μg L^{-1} Se in samples. Using permanent modifier, the lifetime of the graphite tube was equivalent to ca. 500 firings, which was ca. two times

Table 2
Results for selenium ($n = 3$) in coconut water; coconut, soybean and cow milks; tomato, mango and grape; drinking water samples and SRMs and comparative recoveries (in %) for samples without correction and using As as internal standard

Sample	Comparative and certified values	Se (μg L^{-1})	Recoveries (in %)	
			As	
			With IS	Without IS
Drinking water 1		–	109 (0.8)	83 (3.0)
Drinking water 2	<3.0 (L.O.D.)[a]	–	106 (1.0)	79 (4.0)
Coconut water 1	21.0 ± 1.3[b]	22.3 ± 0.6	100 (1.0)	75 (3.0)
Coconut water 2		20.0 ± 0.5	102 (1.5)	79 (3.5)
Coconut water 3		20.2 ± 0.4	100 (3.0)	70 (6.0)
Coconut water 4		7.0 ± 0.2	99 (1.0)	80 (3.5)
Coconut water 5		15.4 ± 0.4	101 (2.0)	72 (3.5)
Coconut milk 1		7.5 ± 1.5	104 (1.5)	85 (4.0)
Coconut milk 2	24.2 ± 1.2[b]	4.0 ± 2.2	98 (1.0)	86 (3.2)
Whole milk 1		10.6 ± 0.4	100 (1.2)	80 (4.1)
Whole milk 2	54.0 ± 6.0[c]	20.0 ± 0.8	99 (1.0)	70 (5.5)
Non fat milk 1		18.5 ± 0.2	100 (2.0)	70 (6.5)
Non fat milk 2	39.0 ± 4.0[c]	5.2 ± 0.1	100 (1.5)	75 (4.0)
Low fat milk 1		14.5 ± 0.6	99 (2.0)	84 (5.0)
Low fat milk 2	46.0 ± 7.0[c]	12.0 ± 0.5	100 (2.0)	89 (4.2)
Mango juice	12.0 ± 3.0[d]	6.6 ± 1.0	102 (2.0)	77 (3.0)
Tomato juice	20.4 ± 4.5[e]	4.4 ± 2.0	98 (1.4)	82 (3.5)
Grape juice	18.7 ± 3.4[f]	4.0 ± 1.5	105 (2.0)	78 (5.0)
Soybean milk 1		3.0 ± 1.0	99 (0.8)	75 (3.0)
Soy bean milk 2		6.0 ± 1.8	98 (1.0)	70 (2.5)
SRM1[*]	0.13 ± 0.01	0.13 ± 0.01		
SRM2[*]	0.11 ± 0.01	0.10 ± 0.008		
SRM3[**]	11.43 ± 0.17	11.39 ± 0.01		
SRM4[**]	21.96 ± 0.51	21.68 ± 0.60		

Numbers in parentheses represent % r.s.d.

[*] μg g^{-1}, [**] μg L^{-1} SRM1: 8435 Whole milk powder; SRM2: 1549 Non-fat milk powder; SRM3: 1643d Trace elements in water; SRM4: 1640 Trace elements in natural water.

[a] Oliveira et al. (2004).

[b] Aleixo et al. (2000).

[c] Aleixo and Nóbrega (2003).

[d] Haygarth (1994).

[e] Arruda et al. (1994).

[f] Terry and Zayed (1994).

the lifetime obtained for untreated platform using only Pd + Mg$(NO_3)_2$. Recoating of the platform with W/Rh or W plus co-injection of Pd$(NO_3)_2$ permanent modifier should be made after 200–250 consecutive firings. However, it is a good practice to set an acceptable relative standard deviation threshold before renewing platform coating.

4. Conclusions

Internal standardization may be an interesting tool to minimize problems associated to heterogeneous solutions (in this case, solutions containing high concentrations of gases, organic matter, fat and dissolved total solids). The use of an internal standard helps to improve analytical figures of merit and it is feasible in multi-element equipment. The IS is not limited to the selected analyte, internal standard or samples employed in this work. The permanent modifiers improved the THGA lifetime even working with air-assisted pyrolysis at 600 °C, leading to a substantial decrease in analytical costs.

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References

Aleixo, P. C., Nóbrega, J. A., Santos, D. J., & Muller, R. C. S. (2000). Determinação direta de selênio em água de coco e em leite de coco utilizando espectrometria de absorção atômica em forno de grafite. *Química Nova, 23*, 310–312.

Aleixo, P. C., & Nóbrega, J. A. (2003). Direct determination of iron and selenium in bovine milk by graphite furnace atomic absorption spectrometry. *Food Chemistry, 83*, 457–462.

Alejos, M. S., Romero, F. J., & Dias, R. C. (2000). Selenium and cancer: some nutritional aspects. *Nutrition, 16*, 376–383.

Arruda, M. A. Z., Gallego, M., & Valcárcel, M. (1994). Determination of selenium in fruit juices by flow injection electrothermal atomization atomic spectrometry. *Journal of Analytical Atomic Spectrometry, 9*(5), 657–662.

Butcher, D. J., & Sneddon, J. (1998). Sample preparation: an introduction. In *A practical guide to graphite furnace atomic absorption spectrometry* (pp. 119–173). New York: John Wiley & Sons.

Casey, C. E., Smith, A., & Zhang, P. (1995). *Handbook of milk composition*. San Diego: Academic Press.

Cowgill, U. M. (1998). The distribution of selenium and mortality owing to acquired immune deficiency syndrome in the continental United States. *Biological Trace Element Research, 56*(1), 43–51.

Feldman, F. J. (1970). Internal standardization in atomic emission and absorption spectrometry. *Analytical Chemistry, 42*, 719–724.

Fernandes, K. G., Moraes, M., Neto, J. A. G., Nóbrega, J. A., & Oliveira, P. V. (2002). Evaluation and application of bismuth as an internal standard for the determination of lead in wines by simultaneous electrothermal atomic absorption spectrometry. *Analyst, 127*, 157–162.

Gerlach, W., & Schweitzer, E. (1929). *Foundations and methods of chemical analysis by emission spectrometry* (p. 675). London: Adam Hilger.

Haygarth, P. M. (1994). Global importance and global cycling of selenium. In W. T. Frankenberger & S. Berson (Eds.), *Selenium in the environment* (pp. 1–27). New York: Marcel Dekker.

Hua, K. M., Kay, M., & Indyk, H. E. (2000). Nutritional element analysis in infant formulas by direct dispersion and inductively coupled plasma-optical emission spectrometry. *Food Chemistry, 68*, 463–470.

Kim, J., Du, W. X., Otwell, W. S., Marshall, M. R., & Wei, C. I. (1998). Nutrients in salmon and red grouper fillets as affected by chlorine dioxide (ClO_2) treatment. *Journal of Food Science, 63*, 629–633.

Lima, E. C., Krug, F. J., & Jackson, K. W. (1998). Evaluation of tugsten–rhodium coating on an integrated platform as a permanent chemical modifier of cadmium lead, and selenium determination by electrothermal atomic absorption spectrometry. *Spectrochimica Acta Part B, 53*, 1791–1804.

Mayland, H. F. (1994). Selenium in plant and animal nutrition. In W. T. Frankenberger & S. Berson (Eds.), *Selenium in the environment* (pp. 29–45). New York: Marcel Dekker.

Mermet, J. M., & Ivaldi, J. C. (1993). Real-time internal standardization for inductively-coupled plasma-atomic emission-spectrometry using a custom segmented-array charge-coupled-device detector. *Journal of Analytical Atomic Spectroscopy, 8*(6), 795–801.

Oliveira, A. P., Neto, J. A. G., Nóbrega, J. A., & Oliveira, P. V. (2004). Use of internal standardization for difficult sampling by graphite furnace atomic absorption spectrometry. *Talanta, 64*, 334–337.

Radziuk, B., Romanova, N. P., & Thomassen, Y. (1999). Evaluation of internal standardization in electrothermal atomic absorption spectrometry. *Analytical Communications, 36*, 13–16.

Rayman, M. (2002). Se brought to earth. *Chemistry in Britain, 10*, 28–31.

Santoso, U., Kubo, K., Ota, K., Takadoro, T., & Mackawa, A. (1996). Nutrients composition of kopyor coconuts (*Cocos nucifera* L.). *Food Chemistry, 57*(2), 299–304.

Seow, C. C., & Gwee, C. N. (1997). Coconut milk: chemistry and technology. *International Journal of Food Science and Technology, 32*(3), 189–201.

Soltanpour, P. N., & Workman, S. M. (1980). Use of NH_4HCO_3-DTPA soil test to assess availability and toxicity of selenium to alfafa plants. *Communications Soil Science and Plant Analyses, 11*, 1147–1156.

Terry, N., & Zayed, A. M. (1994). Selenium volatilization by plants. In W. T. Frankenberger & S. Berson (Eds.), *Selenium in the environment* (pp. 343–365). New York: Marcel Dekker.

Underwood, E. J. (1977). *Trace elements in human and animal nutrition* (4th ed.). New York: Academic Press.

Welz, B., & Sperling, M. (1999). The techniques of atomic absorption spectrometry. In *Atomic absorption spectrometry* (3rd ed., pp. 406–408). Überlingen: Wiley-VCH.