POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2004, Vol. 13/54, No 4, pp. 415–420

ASSESSMENT OF SELENIUM CONTENT IN DIETS USING TWO ANALYTICAL METHODS – A COMPARATIVE STUDY

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Key words: selenium, selenium content in Polish diets, fluorimetric analysis, radiochemical neutron activation analysis, comparison of methods

The selenium content in the five lyophilised reconstructed Polish diets was determined with two analytical methods: fluorimetric method and method of radiochemical neutron activation analysis (RNAA). The results obtained with the RNAA method were lower than those obtained with the fluorimetric method, although the differences were statistically not significant. The mean selenium content in lyophilised samples determined with RNAA method ranged from 0.048±0.003 mg/kg to 0.061±0.002 mg/kg. The results obtained with fluorimetric method ranged from 0.057±0.004 mg/kg to 0.072±0.003 mg/kg.

Considering diet mass after reconstruction and dry mass content in diets before and after lyophilisation, the content of selenium was calculated in the daily diets. The mean selenium daily uptake calculated from RNAA results was from 19.02 μg to 27.33 μg. The mean selenium content in diets calculated on the basis of fluorimetric determinations was from 22.10 μg to 32.78 μg. The results indicated that the selenium content in Polish diets was relatively low. The coverage of the recommended intake of selenium varied from 40% to 70%, depending on the type of the household.

INTRODUCTION

Selenium is a micronutrient necessary for human and animal health. There is a positive correlation between selenium intake and its blood level [Gissel-Nielsen, 1989; Rea *et al.*, 1979; Watkinson, 1981]. A relationship between selenium intake and occurrence of some diseases has also been indicated. Typical examples are endemic diseases: Keshan (cardiomypathy) and Kashin-Back (articular cartilage dystrophy) in China – it has been proved that one of the causes of both diseases is selenium deficiency in diets of a population living in the area with the occurrence of the above-mentioned diseases [Litov & Combs, 1991; Yang *et al.*, 1984]. In other regions of the world (for example Finland, Germany or Sweden), a low blood serum level of selenium has been found in patients with heart strokes [Oster & Prellwitz, 1990]. A positive influence of selenium supplementation of a diet has also been found on protein-calorie malnutrition treatment and treatment of some neurological diseases [Robinson & Thomson, 1983]. A correlation between selenium intake and incidence of lung cancer has also been reported. A higher selenium intake is likely to lower the risk of its incidence [Food, Nutrition..., 1997]. There are also studies showing a preventive role of selenium with respect to gastric, hepatic, thyroid or prostatic carcinomas [Food, Nutrition..., 1997; Yoshizawa *et al.*, 1998]. A crucial role of selenium in lowering the toxicity of cadmium, mercury and methylomercury has also been indicated [Goyer, 1995; Johansson, 1991].

Considering the significance of this microelement in proper functioning of the organism, it is also important to know selenium content in food products and its intake with diets.

To determine selenium in food, various analytical methods are used nowadays, including spectrophotometry, fluorimetry, atomic absorption spectrometry, and neutron activation analysis.

Spectrophotometric methods are the oldest ones. They are based on selenium (IV) ability to form a yellow complex poorly soluble in water, called piazoselenol, with 3,3-diaminobenzidine (DAB) in acid medium [Cheng, 1956; Gawłoska & Masłowska, 2000; Marczenko, 1968; Służewska, 1964].

In fluorimetric studies into selenium determination in biological materials, most authors determine the fluorescence of a complex formed by selenium (IV) with 3,3-diaminobenzidine [Watkinson, 1966], 2,3-diaminonaphtalene [Rodriguez *et al.*, 1999; Watkinson, 1966] or 2,3-diamino-1,4-dibromonaphtalene [Rodriguez *et al.*, 1999]. The first step indispensable for spectrophotometric and fluorimetric selenium determination is mineralization of the sample and reduction of the selenium (VI) form to tetravalent selenium, as Se (IV) is the only form capable of forming colour complexes. The reduction is usually carried out by heating the mineralized sample with hydrochloric acid [Marzec, 1998; Rodriguez *et al.*, 1999; Służewska, 1964]. Various heating times and temperatures are used in that process.

One of the critical steps in selenium determination in organic substances is sample mineralization during which a

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considerable loss of the element can occur due to high volatility of selenium and its compounds or their precipitation [Gorsuch, 1959; Rodriguez *et al.*, 1999; Służewska, 1964; Stibilj *et al.*, 1994]. In spectrophotometric and fluorimetric methods for selenium determination, a considerable difficulty is caused by the control of hydrogen ion concentration – pH of the solution when selenium colour complexes are forming and are being extracted.

Among the atomic absorption methods for selenium determination, two techniques are used: a technique based on generation of hydrides (recommended by the Association of Official Analytical Chemists – AOAC) [AOAC, 1996], and an electrothermic technique with activation in a graphite oven (ETAAS) [Januzzi *et al.*, 1997; Kumpulainen & Saarela, 1992].

For the determination of low concentration levels of Se, neutron activation analysis (NAA), both in the instrumental (INAA) and radiochemical (RNAA) modes, is very expedient and is also well known as a reference method [Stibilj *et al.*, 1994]. In the case of instrumental or on-destructive NAA, determination is based on the measurement of the γ – energies either of short-lived isotope ^{77}mSe($t_{1/2}$=17.4 s) or more often, on long-lived ^{75}Se($t_{1/2}$=120 d) radionuclide. When using INAA, the matrix usually has a marked negative influence on the signal to background ratio. This results in poorer accuracy and higher limit of detection. The RNAA method, based on carefully selected experimental conditions and chemical yield determination, offers selective separation of the desired radionuclide. The method is highly sensitive and the possibility of sample contamination is minimal, blank free advantage, but it is, however, time-consuming and too costly to be used for routine selenium determination in food products. It is used as a reference method for comparative interlaboratory tests and for certification of reference materials [Stibilj *et al.*, 1994].

In few Polish studies on the quantification of selenium in diets, various analytical methods were used and results varied widely. For example studies in the 1980-ties have shown a quite high intake of selenium, exceeding recommended dietary allowances. Average Polish diets recalculated on the basis of data on food consumption obtained from studies of household budgets carried out by The Central Statistical Office in 1981, in which selenium content was determined by colorimetric method, contained from 110 to 182 μg of this microelement [Rutkowska *et al.*, 1987].

An analysis of selenium intake with daily diets of adults in 1998 (spectrofluorometric method) has shown a lower content of that element in diets. It accounted for 60.4±24.1 μg on average [Marzec, 2002].

As for selenium content in domestic food products, studies are also incomplete. There have been obtained different results pertaining to the same type of food products. For example the results obtained with spectrofluorometric method by Marzec [2002] for cereals, flour, bread and pastry were approximately twice as lower as the results obtained by Gawłowska and Masłowska [2001] who used three different spectral methods.

In the light of the above, it is advisable to select and verify a relatively available method of selenium determination in food and estimation of selenium intake with an average Polish diet.

The purpose of the reported study was to compare the results of selenium determination in the lyophilised reconstructed Polish diets – carried out by two methods: fluorimetric analysis, which is suitable for wide use, and radiochemical neutron activation analysis (RNAA), which is used as a reference method.

MATERIAL AND METHODS

The material were diets reconstructed in a laboratory on the basis of data on food consumption obtained from the studies of household budgets carried out by the Central Statistical Office in 1996 [Household budgets ..., 1997].

They were designated as follows: NR1 – diets in households of white-collar workers with the lowest incomes; NR2 – diets in households of white-collar workers with medium incomes; R1 – diets in households of blue-collar workers with the lowest incomes; R2 – diets in households of blue--collar workers with medium incomes; NZ – diets in households of people living on unearned sources.

The masses of the reconstructed diets were as follows: NR1 – 1385 g, NR2 – 1543 g, R1 – 1278 g, R2 – 1543 g and NZ – 1400 g. The dry mass of the diets was determined using the radiator method [Kunachowicz *et al.*, 1997]. Then, diet samples were lyophilised in a Modulyo (Edwards High Vacuum International) lyophilising cabinet and their dry mass was again determined.

Selenium content was determined with the method of radiochemical neutron activation analysis and by fluorimetry. The determination was done in five samples of each diet and each sample was analysed five times within one year.

The statistical significance of differences between the results of both methods was analysed using T-Student's test for paired variables and the SAS statistical pack.

Selenium determination by RNAA. A sample aliquot (250–300 mg) was transferred to a plastic ampoule, cleaned with HNO_3 (1:1) solution. Neutron irradiation of samples and appropriate standards (80 μg/g) was performed in the TRIGA MARK II Reactor at the "J. Stefan" Institute (Ljubljana, Slovenia). For the long-lived induced radioisotope ^{75}Se, the time of irradiation was 40 h in the rotary specimen rack at a neutron fluence of 1.1×10^{12} n.cm^{-2}s^{-1}. The radiochemical NAA procedure for selenium determination is based on the destruction of the irradiated sample with 0.1 g carrier (1 mg Se g^{-1}) using 3 g of $Mg(NO_3)_2$ in 3 mL H_2O in quartz crucible, heated on a hot plate at 250°C, transferred the crucible with a light yellow residue to an oven at 520°C, reduction of Se(VI) with 10 mL of 6 mol/L HCl at 100°C, reaction between Se(IV) and 4-nitro--1,2-diaminobenzene (1% in 1 mol/L HCl), and extraction of the chelate 5-nitro-2,1,3-benzoselendiazole with 6 mL toluene. The γ activity of the separated radionuclides ^{75}Se, arising from the nuclear reactions ^{74}Se(n,γ)^{75}Se, ($t_{1/2}$=120 d, E_γ=0.401 MeV) in 5 mL organic phase was measured with a well-type HP Ge detector connected to a 4096 channel Canberra 90 analyser. The yield was determined spectrophotometrically for each aliquot of sample from the absorption of Se chelate at 443 nm after γ activity measurement [Stibilj *et al.*, 1994, 1996]. The RNAA method with the added carrier offers the elimination of the losses of analyte during the procedure (due *e.g.* to volatility or absorption) and controls the experimental conditions for the separation and quantification of the analyte, as it enables determina-

tion of recovery of the analyte for each aliquot of the sample by the measurement of the yield of the carrier. In our case, the recovery was high, ranging from 80 to 90%. The detection limit was less than 5 ng Se/g. The standard deviation (RSD) of five repetitions was from 3% to 8%. The accuracy and precision of the method was checked with standard reference material Total Diet NIST 1548. The obtained result (0.255±0.004 mg Se/kg) was in agreement with the certified value (0.245±0.005 mg Se/kg).

Selenium determination by the fluorimetric method. For the determination, averaged weighted amounts of 2–3 g mass were used. The samples were covered with nitric acid (V) and left overnight. Then they were cautiously heated until the appearance of the first yellow-brown fumes when heating was stopped. After cooling, 5 mL of 10% chloric acid (VII) and 2 mL of concentrated sulphuric acid (VI) were added. Heating was gradually increased until the samples became colourless and white fumes appeared. Darkening of the sample ruled it out from further determination. To the sample, 1 mL of 30% H_2O_2 was added twice, heating it each time until the appearance of white fumes. At this stage, the reduction of Se (VI) to Se (IV) proceeded.

The mineralized samples were transferred quantitatively into 100 mL measuring flasks using 25 mL of deionized water and selenium determination was carried out. With constant stirring, 0.03 mol/L sodium versenate (EDTA) was added to the flasks and using ammonium hydroxide (NH_3H_2O) the pH was adjusted to such a level that after the addition of 5 mL of 2,3-diaminonaphtalene (DAN) solution in 5 mol/L sulphuric acid (VI), the pH of the resulting solution reached 3.0±0.5. The reaction must proceed in acid medium, if it is not acid the reaction of condensation and cyclization of piazoselenol (naphto-(2,3-d)-2-seleno-1,3-diazole) is very weak or absent, the results are then not reproducible. The mixture was then rapidly heated in a water bath and left standing for about 1.5–2 h. After that time cyclohexane was added and shaken vigorously for extracting piazoselenol. This treatment, followed procedures described in the Official Methods of the AOAC [AOAC, 1995]. Fluorescence was measured in a Cecil 6600 CE unit with fluorimetric attachment at exciting wavelength 366 nm, and measurement was done at 525 nm wavelength.

The parameters of fluorimetric method were checked on reference materials and with the method of the addition of an averaged mixture of powdered milk and flour to the reference sample. Selenium recovery from the samples studied ranged from 86.2 to 95.4%. The standard deviation (RSD) of five repetitions was from 3.4% to 6.3%.

For the sample, containing above 20% of fat, decomposition of wet ashing (HNO_3, $HClO_4$, H_2SO_4, H_2O_2) and digestion in fusion with $MgNO_3$ were used. The Se determination for both methods was based on the extraction of Se-chelate (naphto-(2,3-d)-2-seleno-1,3-diazole and 5-nitro-2,1,3-benzoselendiazole) from acidic solution to organic solvent.

RESULTS AND DISCUSSION

The selenium content in the analysed diets was very low, fluctuating between 0.048 and 0.072 mg/kg.

The Se contents obtained by the fluorimetric method were higher than those obtained by the RNAA method (Tables 1 and 2). It is evident that the samples were well homogenised, because the amount of the sample used for the fluorimetric method was 10 to 15 times higher than the amount used for RNAA. The samples were analysed within one year and the reproducibility of results was perfect for both methods.

The mean selenium content determined by the RNAA method ranged from 0.048±0.003 mg/kg in the households

TABLE 1. The results of selenium determinations in lyophilised samples of reconstructed diets obtained with RNAA method (mg/kg) determined within one year.

No.	NR1	NR2	R1	R2	NZ
1	0.061	0.065	0.054	0.062	0.051
2	0.058	0.061	0.050	0.057	0.050
3	0.055	0.061	0.048	0.060	0.045
4	0.056	0.059	0.044	0.058	0.044
5	0.051	0.060	0.052	0.061	0.048
Mean	**0.056**	**0.061**	**0.049**	**0.060**	**0.048**
SD	**0.004**	**0.002**	**0.004**	**0.002**	**0.003**

TABLE 2. The results of selenium determinations in lyophilised samples of reconstructed diets obtained with fluorimetric method (mg/kg).

No.	NR1	NR2	R1	R2	NZ
1	0.061	0.060	0.057	0.073	0.059
2	0.064	0.063	0.053	0.071	0.059
3	0.065	0.066	0.063	0.068	0.055
4	0.067	0.068	0.058	0.075	0.062
5	0.064	0.064	0.056	0.072	0.060
Mean	**0.064**	**0.064**	**0.057**	**0.072**	**0.059**
SD	**0.002**	**0.003**	**0.004**	**0.003**	**0.003**

TABLE 3. Mean selenium value in the reconstructed diets (in μg per daily diet).

Method	NR1	NR2	R1	R2	NZ
RNAA	22.54	27.33	19.02	27.21	19.67
Fluorimetry	25.70	28.71	22.10	32.78	24.44

of people living on unearned sources (NZ) to 0.061±0.002 mg/kg in the households of white-collar workers with medium incomes (NR2).

According to the determination with the fluorimetric method, the lowest mean selenium content was reported in the material prepared from the diet in the households of blue-collar workers with the lowest incomes (R1) – 0.057±0.004 mg/kg. The highest selenium level was found in the material prepared from the diet of the blue-collar workers with medium incomes (R2) – 0.072±0.003 mg/kg.

The differences between the mean values ranged from 5% in NR2 to 24% in the NZ diet. In the lyophilised diets NR1 and R1, the differences were by about 15%, and in the R2 diet by about 20%.

Anyway, the comparison of the results for the two methods used with T-Student's test showed that the differences between them were statistically insignificant ($p<0.001$).

Considering diet mass after reconstruction and dry mass content in diets before and after lyophilisation, the content of this element was calculated in the daily diets. The results are presented in Table 3. Based on the Polish recommendation for daily selenium intake [Ziemlański *et al.*, 1998] and share of different age groups in each of the mentioned households [Household budget.., 1997], the coverage of norm of the recommended intake was calculated.

The results indicated that the selenium content in the diets was below the recommended values. The coverage of the recommended intake of selenium varied from 40% to 70%, depending on the type of the household.

Similarly as in the case of selenium content in diet, the selenium daily uptake, calculated on the basis of fluorimetric determinations, was higher than that calculated from RNAA results. The differences between the results of selenium assessment in diets based on the results obtained by these two methods ranged from 5% to 24%. The greatest divergence of the results was in the NZ diet, and the lowest one in the NR2 diet. In diets NR1 and R1, the difference of the results was 14% and 16%, respectively. For R2 diet this difference was 20%.

The results obtained in this study show an average selenium intake in the households. Taking into account differentiation of food consumption in different populations, it may be supposed that in some groups of population selenium intake varies from that obtained in our study.

It is advisable to undertake further studies on selenium content in food products (with the purpose of updating and extension of databases on nutritional value of food) and studies on selenium intake in different groups of population in our country.

CONCLUSIONS

1. The differences between the results of selenium content determination with the RNAA method and with the fluorimetric method were statistically insignificant. It proves the possibility of using the fluorimetric method for selenium determination in diets and food products.

2. The results obtained indicate too low selenium content in the diets studied in comparison to the recommended values.

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Received September 2003. Revision received December 2003 and accepted February 2004.

OCENA ZAWARTOŚCI SELENU W DIETACH Z ZASTOSOWANIEM DWÓCH METOD ANALITYCZNYCH – BADANIA PORÓWNAWCZE

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Zawartość selenu w liofilizatach diet pięciu wybranych gospodarstw domowych w Polsce oznaczono dwiema metodami analitycznymi: metodą fluorymetryczną oraz metodą radiochemicznej neutronowej analizy aktywacyjnej (RNAA). Wyniki uzyskane metodą RNAA były niższe od otrzymanych metodą fluorymetryczną, chociaż obserwowane pomiędzy nimi różnice nie były statystycznie istotne. Średnia zawartość selenu w liofilizatach oznaczona metodą RNAA wahała się od 0,048±0,003 mg/kg do 0,061±0,002 mg/kg (tab. 1). Wyniki uzyskane metodą fluorymetryczną mieściły się w przedziale od 0,057±0,004 mg/kg do 0,072±0,003 mg/kg (tab. 2).

Oznaczoną analitycznie zawartość selenu w liofilizatach przeliczono na zawartość tego pierwiastka w dziennych dietach. Bazując na wynikach oznaczeń metodą RNAA uzyskano średnią zawartość selenu w dietach od 19,02 μg do 27,33 μg (tab. 3). Według przeliczenia wyników uzyskanych metodą fluorymetryczną, średnia zawartość selenu w dietach wynosiła od 22,10 μg do 32,78 μg. Wyniki uzyskane obiema metodami wskazują na stosunkowo niską zawartość selenu w badanych dietach. Realizacja norm zalecanego dziennego spożycia tego pierwiastka wahała się od 40% do 70%, w zależności od typu gospodarstwa.