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Original scientific paper

DETERMINATION OF SELENIUM IN SELECTED FOODS FROM NORTH MACEDONIA BY ETAAS FOLLOWED BY MICROWAVE-ASSISTED DIGESTION

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ABSTRACT

For the determination of selenium in selected food products produced or purchased in North Macedonia, a suitable electrothermal atomic absorption method (ETAAS) was optimized and validated due to its biological activity and importance in human and animal nutrition. In this study, a sample preparation method with microwave digestion was used to digest various food samples for Se determination. The limit of detection (LOD) and limit of quantification (LOQ) were 0.99 µg/L and 3.30 µg/L, respectively. The validation results showed a recovery of 96.13-99.60%, and the relative standard deviation (RSD) was less than 5.3%. It was found that the determined selenium content was lower compared to the values reported from other Balkan countries. The highest Se content was found in protein-rich foods such as fish muscle of an endemic species of Lake Ohrid trout, then in pork, chicken breast and offal; the content in nuts, beans, milk and dairy products was average, while grain-based samples, fruits and vegetables had the lowest Se concentration.

Key words: determination, electrothermal atomic absorption spectrometry, food, microwave-assisted digestion, North Macedonia, selenium.

INTRODUCTION

The effects of a healthy diet on human health have recently been the subject of numerous scientific studies, as an unhealthy and unbalanced diet can cause various health disorders (Shridhar et al., 2015). However, many social and economic factors such as food prices, individual preferences and beliefs, cultural traditions, geographical and environmental aspects, increasing production of processed foods, rapid urbanization, and changing lifestyles have led to a change in dietary habits. People now consume more high-energy foods, fats, free sugars, and salt, but not enough micronutrients (Cena & Calder, 2020).

Selenium is a trace element that is important from both toxicological and nutritional points of view. This element is essential in cells as a selenate, activated as a selenophosphate, and integrated into a serine tRNA leading to a seleno-cysteine tRNA. Selenium is the only trace element that is incorporated into proteins during translation, making it a unique trace element essential for most organisms (Legrain et al., 2014). It is an integral component of important selenoproteins, including glutathione peroxidases (GPx), an antioxidant enzyme that catalyses the reduction of hydrogen peroxide and other hydroperoxides, and iodothyronine deiodinases, which are involved in thyroid hormone metabolism (Rayman, 2012). In addition, selenium is a component of thioredoxin reductase, which is involved in the regeneration of antioxidants, regulation of cell growth and viability (Brown & Arthur, 2001).

Adequate selenium intake in humans reduces the risk of cardiovascular disease, protects the thyroid gland from oxidative damage, stimulates the immune system, and reduces the risk of certain cancers and inflammation (Hariharan & Dharmaraj, 2020; Shi et al., 2018). Selenium is also essential in animal nutrition because of its multiple roles in animal growth, fertility, and immune function. Selenium deficiency in animals is manifested by liver apoptosis in pigs and rats, decreased immune response and increased embryonic mortality in birds, and muscular dystrophy in ruminants and other animal species (Tórtora-Pérez, 2010).

The distinction between essential and toxic doses is very narrow. In the United States, the recommended daily allowance (RDA) for selenium is 55 μg/day and the tolerable limit (UL) is 400 μg/day for men and women. In contrast, in the European Union, the recommended daily allowance for selenium is 70 μg/day and the UL is 300 μg/day. Selenium toxicity has been observed after ingestion of a high single dose or after multiple doses, usually above 400 μg (Silva et al., 2018). High selenium intake can cause nausea, diarrhea, vomiting, strong garlicky odor, skin lesions, weakness, decreased cognitive function, pain in the extremities, tremors, confusion, coma, mucosal damage in the oral cavity and esophagus, and eventually death (Morris & Crane, 2013).

Because of the dual nature of this element, attention is given to the study of the distribution and accumulation of selenium in various foods and feeds as the main sources of selenium for humans. The selenium content in foods is highly variable and depends on its concentration in the soil in a given geographical area, the ability of plants to accumulate it, and the available feed (Finley, 2006). However, in most agricultural areas of northern Macedonia, soils contain a low concentration of available Se, which is taken up in trace amounts by cultivated plants (Krustev et al., 2019). Other factors such as climatic conditions, cultivation, and food preparation methods may also affect the Se content in food (Ivory & Nicoletti, 2017). In selenium-deficient areas, thyroid disorders have been noted, and selenium supplementation is becoming a part of medical therapy to treat them and prevent certain cancers and signs of aging (Ventura et al., 2017).

Routine and reliable methods are needed to determine low selenium concentrations in foods and food products. There are numerous methods for the determination of selenium, such as spectrophotometry (Wen et al., 2014), atomic fluorescence spectrometry (Ventura et al., 2009), electrothermal atomic absorption spectrometry (ETAAS) (Sherovski et al., 2022; Sun et al., 2013), hydride generation atomic absorption spectrometry (HGAAS) Chirita et al., 2021; Klapec et al., 2004), inductively coupled plasma mass spectrometry (Voica et al., 2012), high-performance liquid chromatography (Montes-Bayon et al., 2006), polarography, and voltammetric techniques (I˙nam et al., 2006). Electrothermal atomic absorption spectrometry is widely used for the determination of selenium in various matrices due to its low detection limit, high sensitivity and selectivity, and small sample volume. Selenium determination in complex matrices usually requires extensive sample preparation prior to instrumental quantification. Residues of acids and organics that remain after sample digestion can interfere with most selenium determination procedures. On the other hand, these residues do not interfere with ETAAS determinations due to the high atomization temperature during ashing. Many modifiers have been used to improve the thermal stabilization of selenium, and palladium is one of the most suitable and commonly used modifiers in biological samples. It is more sensitive compared to other modifiers, does not cause contamination of the graphite tube, and palladium is not determined unlike copper and nickel (Butcher, 2021).

Currently, there are very few data on selenium intake throughout Macedonia (Vrhovnik et al., 2013; Gjorgovska et al., 2012). Therefore, the aim of this study was to optimize and validate a method using microwave-assisted acid digestion and ETAAS to determine the total selenium content in selected foods produced or purchased in North Macedonia in order to facilitate the formal decision on dietary selenium intake.

MATERIALS AND METHODS

Instrumentation

In this study, a Varian SpectrAA 640Z Zeeman electrothermal atomic absorption spectrometer was used with a PSD-100 autosampler (Varian, USA) and GTA-100 graphite furnace (Varian, USA). Pyrolytically coated tubes were used as atomizers. A selenium hollow cathode lamp (Varian, USA) was used, and measurements were performed at 196.0 nm. Argon was used as protective gas, and 10 µl of the samples were injected into the graphite furnace (GF). The optimal operating parameters of the graphite furnace are given in Table 1. Integrated absorbance values (peak height) were used for quantification.

Table 1. Optimal parameters for Se determination by Zeeman ETAAS

Parameter	Se
Wavelength	196.0 nm
Lamp current	10.0 mA
Calibration mode	Absorbance, peak height
Background correction	Zeeman
Drying	
Temperature	85; 95; 120 °C
Time	5; 40; 10 s
Pyrolysis	
Temperature	1100 °C
Ramp time	5 s
Hold time	20 s
Atomization	
Temperature	2500 °C
Ramp time	1 s
Hold time	3 s
Cleaning	
Temperature	2500 °C
Time	2 s
Gas	Argon

Reagents

Working standard solutions were prepared by dilution of selenium standard solutions (1000 µg/mL) from Solution Plus Inc (USA). The palladium matrix modifier solution with a concentration of 500 µg/mL was prepared in 20% HCl (*V*/*V*) (Merck, Darmstadt, Germany) by dilution of 10 g/L $Pd(NO_3)_2$ (Merck, Darmstadt, Germany). Trace amounts of trace pure concentrated nitric acid (65% *V*/*V*) (Merck, Darmstadt, Germany) and hydrogen peroxide (Merck, Darmstadt, Germany) were used for sample digestion. Double distilled water with a conductivity of 0.3 µS/cm was used in all procedures.

Procedures

For microwave-assisted digestion, 0.25-0.5 g of food samples (depending on Se concentration) were placed in Teflon digestion vessels, 5 ml of concentrated nitric acid and 2 ml of H_2O_2 (30%, mass/volume) were added. The mixture was left overnight at room temperature, then the vessels were sealed and placed in a microwave oven (Mars CEM XP 1500) and mineralized according to the two-step procedure described in Table 2. The vessels were then cooled, carefully opened and the contents transferred to a calibrated 10 ml flask (Yang et al., 2013; Noël et al., 2003).

Table 2. Microwave oven digestion parameters

Power	400 W
First step: Ramp time	15 min
Second step: Hold time	10 min
Pressure	800 psi
Temperature	200 °C

A blank sample was also prepared using the microwave-assisted digestion procedure described above. For selenium determination, 10 µl of the previously prepared samples were added to the graphite furnace with 5 µL of palladium modifier solution.

Selenium content was determined by injecting three times from each beaker into the graphite furnace, which was operated under the conditions given in Table 1.

Accuracy was evaluated at two concentration levels by adding known amounts of a selenium standard solution (10 µg/mL and 20 µg/mL) to the sample in a digestion flask prior to microwave-assisted digestion.

Sample selection

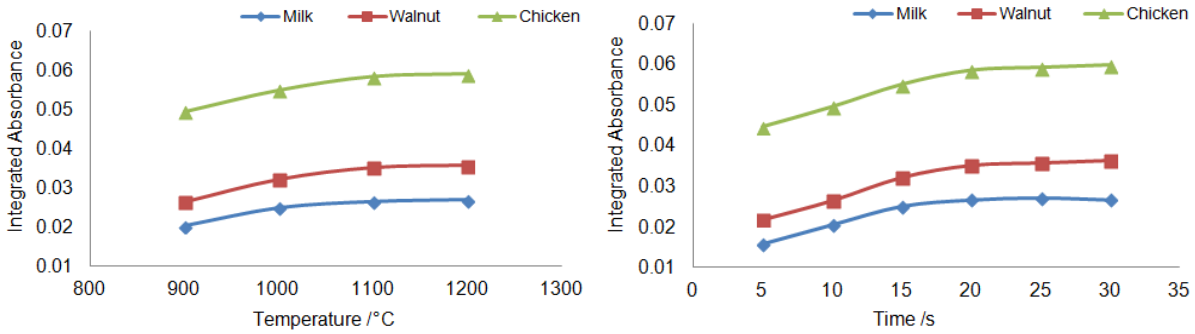
All food samples were of Macedonian origin: pasteurized cow's milk with 3.2% fat and dairy products such as yogurt with 2.8% fat and semi-hard yellow cow's milk cheese with 45% fat in dry matter from Bitola, pork from a farm in the central part of North Macedonia, hot dog and ham produced in Skopje, chicken breast and offal from Skopje, white wheat flour and bread produced in Skopje were randomly purchased at representative markets in Skopje. Rice was obtained from the Kochani area, beans from Tetovo, onions, garlic and nuts from local sources. Meat samples (pork and chicken) from pasture-raised animals and fish muscle from an endemic species of Ohrid trout (Salmo letnica) were also examined. Fruits and vegetables were randomly purchased from representative markets in Skopje. The criteria for the selection of the foods to be studied were either their frequent consumption, which constitutes a large proportion of the Macedonian diet, or the expected indispensable Se content.

Connective tissue and bones of meat and poultry were sorted out. Vegetables were rinsed several times with tap water to ensure that all contaminants were removed and then rinsed again with distilled water. All food samples tested that could not be consumed directly were precooked.

RESULTS AND DISCUSSION

ETAAS program optimization

Optimization of graphite furnace operating parameters for Se determination by graphite furnace atomic absorption spectrometry (GFAAS) in food samples was performed. According to our previous results, the parameters of the drying step were optimized to dry the sample drop slowly without sputtering. Ashing temperatures were optimized by generating pyrolysis-atomization curves of milk, walnut, and pork samples spiked with 10 µg/L Se in the presence of palladium (500 ppm) as a modifier for thermal stabilization in ETAAS. The modifier was added directly to the graphite furnace via the autosampler at a volume of 5 µl for 10 µl food sample. The ashing temperatures (900-1200 °C) and ashing time (5-30 s) were optimized to ensure complete decomposition and removal of the matrix during this step. The effects of pyrolysis temperatures and times on the integrated absorbance of milk, walnut, and pork samples with palladium modifier are shown in Figure 1.



(A) (B)

Figure 1. Effects of pyrolysis temperatures (A) and times (B) on integrated absorbance for milk, walnuts and pork samples with Pd modifier.

The optimal pyrolysis temperature was found to be 1100 °C, while the optimal pyrolysis ramp time was established to be 5 s and hold time was 20 s for all samples. The atomization was performed at 2500 °C by using Pd modifier. The atomization ramp time was 1 s and hold time 3 s.

Accuracy and precision

Under the optimized conditions, peak height absorbance measurements were used to determine the linear range, limits of detection (LOD) and limits of quantification (LOQ), precision and trueness for the determination of selenium in food by ETAAS. The results obtained are given in Table 4.

Table 4. Recovery results of different food samples

Sample	Addition (µg/L)	Found (µg/L)	Recovery (%)
Milk [a]	-	4.65	-
	10	14.42	97.74
	20	24.01	96.80
Walnuts [b]	-	6.95	-
	10	16.77	98.27
	20	26.71	98.80
Pork [c]	-	13.14	-
	10	22.75	96.13
	20	32.98	99.60

[a] Milk sample purchased from grazing cattle in North Macedonia.
[b] Walnuts obtained from local sources in North Macedonia
[c] Pork sample from grazing cattle from North Macedonia.

Calibration curves were obtained using seven selenium standard solutions (1, 2, 5, 10, 20, 30, and 40 µg/L). The correlation coefficient obtained was 0.9981. The values LOD and LOQ were calculated as 3.3σ / S and 10σ / S, respectively, and expressed in µg/L. Where σ is the standard deviation of the response and S is the slope of the calibration curve. The values LOD and LOQ for selenium were 0.99 µg/L and 3.30 µg/L, respectively. The linear range of the calibration function was in the range of 0.5-60 µg/L.

Intraday and interday precisions were evaluated by analyzing a sample six times under the same experimental conditions on the same day and on three different days. The intraday precision was 2.9%, and the RSD between days did not exceed 5.3%. The value obtained for the percentage RSD did not exceed 15% for all concentrations tested.

Method validation

The accuracy of the proposed method for direct ETAAS determination of selenium in food samples was tested using recovery experiments. Samples of milk, walnuts, and pork were spiked with Se at two different concentrations (10 µg and 20 µg) prior to microwave-assisted digestion. Se was added to the food samples at concentration ranges of 1-40 µg/L. Accuracy evaluation results are shown in Table 4 as averages of triplicate measurements. The recoveries ranged from 96.1 to 99.6%, which is within the acceptable limits for the accuracy of the analytical methods (95-105%).

Selenium content in food samples

The total contents of Se in the analysed food samples are shown in Table 5. The results are expressed in micrograms of Se per kg (wet mass) for all foods.

Table 5. Selenium concentration in selected foods grown or purchased in Macedonia and selenium concentration in various foods from the Balkan region

Food sample	Se concentration (present work) (µg/kg), average ± SD*	Se concentration in the Balkan region (reported by other authors) (µg/kg)
Fresh fruits and vegetables		
Apple	1.6 ± 0.5	7.8[a], 1.4 ± 0.2[b]
Pear	3.6 ± 1.3	6.3 ± 1.6[b]
Carrot	5.5 ± 2.6	8.1[a], 6.1 ± 2.4[b], 0.6–11.6[c]
Pepper	4.1 ± 1.2	9.1[a], 4.2 ± 0.3[b], 1 ± 1.4[c]
Tomato	1.3 ± 0.3	7.9[a], 2.3± 0.1[b], 1.1–29.1[c]
Onion	8.5 ± 0.9	15.3 ± 1.8[a], 7.3 ± 0.05[b], 1.1–10.5[c]
Garlic	32.7 ± 5.3	34.2 ± 5.1[a], 13.7 ± 0.9[b]
Beans	35.4 ± 6.6	24.4 ± 3.7[b], 52.6 ± 21.3[c]
Walnut	26.4 ± 5.4	46.1 ± 15.5[a], 19.12 ± 2.2[b]
Hazelnut	30.3 ± 7.1	
Cereal products		
Rice	7.1 ± 0.8	40.5 ± 9.6[a], 19.11 ± 1.4[b]
Flour	11.6 ± 0.9	58.8 ± 5.1[a], 11.9[c]
Bread	13.2 ± 1.1	47.7 ± 3.7[a], 73.9 ± 23.9[b]
Milk and dairy products		
Cow milk**	37.4 ± 8.2	28.7 ± 1.0[a], 14.2 ± 0.9[b], 12.5 ± 0.9[c], 12,1 ± 3,61[d]
Cow milk***	15.3 ± 1.2	
Cheese**	243.7 ± 38.9	72.6 ± 7.8[a], 50.31 ± 16.3 - 104.7[b], 23.2 ± 3.1[c]
Cheese***	83.5 ± 16.3	
Yogurt	68.6 ± 11.7	29.9 ± 10.4[a], 20.61 ± 6.3- 26.91 ± 6.1[b], 12.4 ± 0.5[c]
Meat, fish and products		
Pork**	112.8 ± 24.3	94.12 ± 4.1[b], 112,7 ± 27,64[d]
Pork***	95.6 ± 12.8	
Chicken**	143.6 ± 28.4	79.4 ± 3.1[b], 119 ± 31-128 ± 22[c]
Chicken***	121.1 ± 20.2	
Hot dog	84.7 ± 17.6	68.7 ± 17.0[a]
Ham	92.8 ± 16.8	106.5 ± 0.1[a]
Fish	140.1 ± 22.4	571.0 ± 22.0-859.2 ± 101.6[a], 62.7 ± 34.2-506.71 ± 13.2[b], 153–686[c]

*obtained by analysis of three samples per each food sample

** food sample purchased from local farm or market in Macedonia

*** food sample purchased from pasture-fed livestock or homemade product

[a] Croatia (Klapec et al., 2004)

[b] Greece (Pappa et al., 2006)

[c] Slovenia (Smrkolj et al., 2005)

[d] Serbia (Pavlovic et al., 2018)

Data on Se content in food consumed in Macedonia are limited, so it is not possible to compare the results obtained in this study. The selenium concentrations obtained in the present work were compared with those reported in the literature for foods grown or purchased in the Balkan region (Table 5).

The results for the wheat flour, rice and white bread studied indicate that rice and flour are poor sources of selenium, which can be explained by the low protein and high carbohydrate content of these products. These values were significantly lower than those obtained for the other countries in the region. In these countries, the Se concentration in rice and white wheat flour varied from 19 μg/kg to 58 μg/kg. These differences may be due to the variation in Se concentration in the soil. However, in most agricultural areas of Macedonia, the Se concentration in soils is very low, ranging from 0.3 to 0.6 mg/kg, which obviously affects the Se concentration in crops grown in Macedonia (Krustev et al., 2019). An exception is the city of Veles, where the soils around a lead and zinc smelter are contaminated with heavy metals including selenium (Stafilov et al., 2010).

The results of the present study show that the genus Allium (onion, garlic) tends to accumulate higher Se concentrations compared to rice and wheat, although the selenium concentration in the soil is the same. This is probably due to the higher concentration of sulfur-containing amino acids, analogs of which can be produced by replacing sulfur with selenium. These findings are in agreement with published results for Se concentration in onions and garlic in Croatia, Greece and Slovenia.

Protein-rich products such as beans, walnuts, and hazelnuts have higher Se contents than fruits and most vegetables, which are known to be poor sources of Se. These results confirm the relationship between protein content and Se content in foods of plant origin (Rayman, 2012). According to the results, animal foods for human consumption are a good source of selenium in Macedonia (Table 5). The Se levels in chicken meat, pork, chicken heart and liver, ham and hot dogs were similar to those reported for Serbia and Slovenia. However, these Se levels were lower than those previously reported for Croatia and higher than those reported for Greece. The Se content in meat and meat products varies depending on composition, Se concentration in animal feed, raw materials, processing methods, and many other factors.

On the other hand, Se concentrations in pasture-raised chicken and pork are significantly lower than in meat obtained from local farms in Macedonia. These differences in Se concentrations in meat products are probably related to selenium concentrations in animal feed. It is known that Se is present in animal feed in inorganic and organic forms. The addition of Se to commercial feeds can rapidly increase the selenium concentration in the tissues of cattle and pigs (Beale et al., 1990). This effect complicates the interpretation of results and is particularly evident in viscera such as liver and kidney. Studies of Se content in soil from pastures and in forages could be an important tool to identify the triggers for this difference.

The mean Se content in fish from Lake Ohrid is 140.1 μg/kg. Although fish contain high concentrations of selenium, its availability may be limited due to the high content of mercury (Hg) and other heavy metals that bind to Se and form insoluble inorganic complexes.

In general, selenium levels in milk may be lower than in meat because milk contains less protein than meat. The selenium concentration in whole cow milk purchased in Macedonian markets was 37.4 μg/kg, which is much higher than the concentration in whole cow milk from pasture-raised cows, which is 15.3 μg/kg. The Se concentration determined for whole cow milk from cows kept on pasture is comparable to that in other Balkan countries. The selenium concentration in milk could also be explained by the content of available Se in the soil, since the diet of dairy cows consists mainly of grazing and silage. According to the literature, feeding cows selenium-enriched feed would not significantly increase the Se content in milk because the microorganisms in the rumen convert the inorganic Se into its elemental form, which is biologically unavailable (Galbraith et al., 2016).

The reason for this difference is probably due to the use of selenium supplements or additives in the processing methods used in the dairy industry. This phenomenon can also be observed when we compare the Se concentrations of cheese purchased in the local market and homemade cheese. Indeed, the Se concentration in homemade cheese is three times lower than that in commercial cheese (Table 5). Also, the Se concentration in whole milk yogurt purchased at the local market is 68.6 µg/kg, which is much higher compared to the values found in Croatia, Greece and Slovenia (12-29 µg/kg).

It should also be remembered that good sources of selenium cannot be identified only on the basis of high selenium concentration, but the bioavailability of Se in food must also be taken into account. In general, Se concentrations in selected foods of Macedonian origin are relatively low compared to literature data from other countries. From the obtained results, it can be concluded that the daily Se intake is relatively low, suggesting a low Se intake in the Macedonian population. Selenium supplementation of consumers is recommended to compensate for nutritional deficiencies and to prevent various diseases associated with Se deficiency. A further, more comprehensive survey to determine selenium status in this country is strongly recommended.

CONCLUSIONS

The microwave-assisted digestion method followed by ETAAS analysis proved to be a suitable method for the determination of total selenium in selected foods of Macedonian origin (meat, milk and dairy products, vegetables, nuts and cereal-based samples). The optimised method provides reliable results for the different matrices studied using an aqueous standard calibration curve for quantification. In addition, the method exhibits low detection limits, good precision and accuracy, and is suitable for routine analysis of selenium in food samples. In this study, protein-rich foods were found to have the highest Se concentration; nuts, beans, milk and dairy products, cheese, and yoghurt were average, while grain-based samples and vegetables had the lowest concentration. The determined values for selenium in white wheat flour and rice were significantly lower than in some European countries, probably due to the very low Se concentration in the soil in the Balkans. This also affected the lower selenium content in cow's milk and pork derived from animals fed on pasture, while Se supplementation of commercial feeds can significantly increase concentrations in poultry and pork tissues.

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