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Features of plant material pre-treatment for selenium determination by atomic absorption and fluorimetric methods

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The features of plant samples mineralization by the method of autoclave decomposition under pressure were investigated. The optimal parameters of the procedure of plant material pre-treatment for selenium determination – temperature, time, oxidizing mixture, sample mass, deoxidizing agent – were established. The accuracy of selenium estimation related to the sample preparation varies from 9 to 4%. The possibility to apply the method of autoclave decomposition for the subsequent atomic absorption or fluorimetric determination of selenium in plant material was established.

Key words: selenium analysis, plant sample pre-treatment, FI–HG–AAS, fluorimetry

INTRODUCTION

The biological significance of selenium was first recognized in the 1930s when it was discovered that some well-studied and economically significant diseases of agricultural animals in fact were the result of chronic selenium poisoning [1]. Until the 50s scientists regarded selenium exclusively as a toxic element. The importance of selenium in human nutrition was first ascertained in 1957 [2]. Nevertheless, for a considerable period of time exact biochemical mechanisms of selenium compounds' activity were not specified. The process of their study was significantly accelerated after the discovery of selenium-bearing glutathione peroxidase in 1973 [3]. Nowadays selenium is attributed to antioxidants. As a component of glutathione peroxidase enzyme, together with vitamin E, selenium prevents cell destruction by peroxides, which are generated in the process of metabolism, and, thus, protects biological membranes and non-membranous proteins [4–7].

At present the volume of scientific information on selenium compounds' exchange, selenoproteins and their functions is rapidly increasing. To a great extent this is stimulated by the awareness of the fact that selenium deficiency in the nutrition is a reason for various health disorders. The following diseases are said to be related to the deficiency of selenium in an organism: Keshan disease (endemic cardiomyopathy), Kashin–Beck syndrome [8–10]; selenium proved to have a protective effect in the case of cardiovascular diseases [11, 12] and cancer [13, 14].

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Under normal conditions, selenium is mainly received by people and animals in the form of selenium-bearing amino acids – selenomethionine – and selenocysteine of the vegetative origin [15]. According to the available literary information, in order to prevent selenium deficiency in human and animal organisms, a minimum content of selenium in grain and fodder crops should be 100 μg/kg, but it should not exceed 2 mg/kg [16]. It is necessary to emphasize that selenium content in vegetative food considerably differs depending on the region of its growth. At the same time the number of regions in the world with insufficient selenium content is larger than the number of regions where there is an excess of this microelement [17]. Thus, in Non-Chernozyom zone extending from the northeastern borders of the USA through the whole Europe – northern Germany, Holland, Denmark, Poland, the Baltic countries, Central Russia – to the Ural, then through the whole territory of Siberia up to the eastern border of Russia (regions with Podzolic, sod-Podzolic and some kinds of swampy soils) biogeochemical provinces with selenium deficiency are especially common [18]. Due to this, at present particular attention is paid to the study of the of level of selenium supply for people and animals as well as possible means to increase the selenium content in their diets in the case when there is a deficiency of this microelement.

Various methods are applied to determine selenium content in vegetative objects [1]. However, the main difficulty here is sample mineralization. It is conditioned by the fact that selenium is mostly present in plants in the organic form as dimethylselenide and dimethyldiselenide, which volatilize from a

sample at a temperature exceeding 70 °C. Moreover, at different mineralization stages, other volatile selenium compounds may generate.

The present paper studies the peculiarities of vegetative sample mineralization by the method of autoclave decomposition under pressure and the possibility to apply this method for further atomic absorptive and fluorimetric measurements of selenium in vegetative material.

EXPERIMENTAL

Reagents. The following reagents were used in this research: hydrochloric acid (37%, Merck), nitric acid (65%, Merck), hydrochloric acid (3%, Merck), hydrogen peroxide (30%, Merck), 1.4 mol/L amidosulfuric acid (Merck), 0.2% alkaline solution of sodium tetrahydroborate prepared by dissolving $NaBH_4$ (>98%, Merck) in a 0.5% NaOH solution (pellets, Merck), 0.5% solution of 2,3-diaminonaphtalene (97% Acros Organics), n-hexane (Merck), selenium standard solution from Merck (1000 mg/l). All the reagents were of p. a. grade, unless stated otherwise. The accuracy of the measurements was controlled by analyzing standard vegetative material BCR-189 (wholemeal flour) with known content of selenium (132 ± 10 ng/g).

Apparatus. An atomic absorption spectrometer (Unicam M Series with Software Solaar, vapour system Unicam VP 90 and an electrical furnace EC 90, USA) and a fluorimeter (FluoroMax Spex, Jobin Yvon, USA) were used to carry out selenium determinations.

Procedure. The method of autoclave decomposition under pressure was applied for the mineralization of the vegetative samples. For this purpose, a charge of dry vegetative object was put into a fluoroplastic beaker ("closed vessel"), then aqua fortis was added and 10 minutes later a solution of hydrogen peroxide was added into the reaction solution. The fluoroplastic beaker was then hermetically sealed and put into an oven. The mineralization of the sample was carried out at a temperature of 180 °C for 2 hours. After the beaker got cold, its content was poured into a colorimetric test tube and a concentrated solution of hydrochloric acid and an amidosulfonic acid solution were added. Thereafter, the content of the test tube was kept at a water bath at a temperature of 70 °C for 1 hour.

Fluorimetric determination. An aliquot of a selenium solution was added into a test tube with a plug stopper, then a certain quantity of the reagent was added, the aqueous phase volume was brought to 5.0 ml and the test tube was left in the dark for an hour. Thereafter, 5.0 ml of n-hexane was added to the test tube and piazoselenol was extracted in 2 minutes. The organic phase was poured into a fluorimeter tray and the intensity of fluorescence relative to n-hexane was measured.

AAS determination. Atomic absorptive measurement of selenium with the generation of hydrides is based on the reduction of selenite ion by sodium tetrahydroborate with further atomization in a quartz tray. An alkaline solution of sodium tetrahydroborate (0.2%) was used as a deoxidizing agent. The rate of the thin gas (argon) flow was 200 ml per minute, the temperature of the quartz tray was 900 °C. The intensity of light absorption was measured at a wavelength of 196.0 nm relative to 3% solution of hydrochloric acid.

All the analyses were performed according to [19]. The atomic absorption method exhibited a determinable concentration, μg/ml, in range 0.005–0.04 and fluorimetric – 0.002–0.2; correlation coefficient – 0.999 for both methods.

RESULTS AND DISCUSSION

The present research studies the peculiarities of vegetative sample mineralization by the method of autoclave decomposition under pressure with the purpose to estimate the selenium content in the samples. The preparation of a sample for the analysis of selenium determination consists of the following stages: 1) converting the organic forms of selenium into the inorganic ones; 2) removal of the nitrite ions impeding selenium determination; 3) reduction of the selenate ion to the selenite ion.

In order to avoid the loss of selenium at the first stage, it is necessary to create highly oxidative conditions. Most authors suggest that HNO_3 + $HClO_4$ mixture should be used for this purpose [20, 21]. At the same time a significant number of researchers implement wet process at a temperature of 150–170 °C, but at certain points of mineralization the temperature may reach 180 °C, while some selenium compounds volatilize at a temperature of 70 °C [20]. The mineralization of a sample in pressurized autoclaves may solve this problem. In this study, hermetically sealed fluoroplastic beakers ("closed vessel") steadily effecting concentrated acids, high temperature and pressure were used for the sample mineralization. Aqua fortis and hydrogen peroxide were used as oxidizing agents. In order to avoid an impetuous process, the sample charge did not exceed 0.5 g.

The removal of the nitrite ions significantly impeding both atomic absorptive and fluorimetric determinations of selenium is an important element for the preparation of vegetative samples for the analysis. Several methods of nitrite ion removal from a mineralisate are mentioned in scientific literature: stripping with water, addition of chloric acid, treatment of samples with hydrogen peroxide [21]. However, the most preferable method is the application of amidosulforic acid, which is less dangerous at work than chloric acid, and more effective in the removal of the nitrite ions as compared to hydrogen peroxide.

The application of strong oxidizing conditions in wet reduction of vegetative samples to ashes leads to a partial transformation of selenium into the selenate ion. Taking into account that only Se(IV) enters into the reaction with sodium tetrahydroborate (in atomic absorptive estimation) and with 2,3–diaminonaphthalene (in fluorimetric estimation), the stage of Se(IV) reduction proved to be necessary. A common method of transformation of selenate into selenite is heating of the sample with concentrated hydrochloric acid during 20–30 minutes at a temperature of 100–150 °C [20–22]. However, while heating the mineralisate with concentrated hydrochloric acid at a temperature exceeding 70 °C, considerable losses of selenium occur because it generates volatile compounds Se_2Cl_2 and $SeOCl_2$. Therefore, in order to avoid the loss of selenium at this stage of preparation, after adding hydrochloric acid the vegetative samples were kept for one hour in a water bath at a temperature of 70 °C.

The accuracy of the measurements was controlled by analyzing standard vegetative material BCR-189 with known content of selenium (132 ± 10 ng/g). For that, 0, 5.0, 10.0, 20.0, and

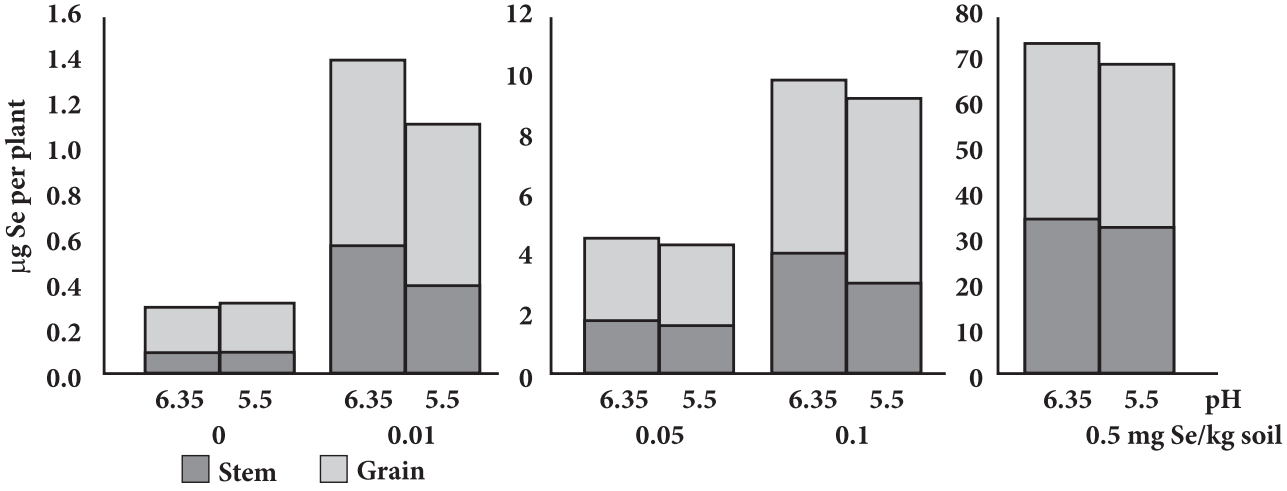

Figure. Selenium content in grain and stem of barley plants depending on selenium additive introduced and pH of the soil

Table 1. **Accuracy of selenium determination in standard vegetative material (M = 0.5 G, V = 25.0 CM³, N = 4, P = 0.95)**

Selenium content in standard material, µg/l	Selenium amount, µg/l		Recovery, %
	introduced	detected	
2.64	0	2.41 ± 0.05	91.29
2.64	5.0	7.2 ± 0.3	94.24
2.64	10.0	12.1 ± 0.3	95.73
2.64	15.0	16.8 ± 0.8	95.24
2.64	20.0	21.8 ± 0.6	96.26
2.64	30.0	31.4 ± 0.9	96.20

30.0 µg/l of selenium was added to the standard sample. The results of the analysis of standard vegetative material are shown in Table 1.

The data in Table 1 indicate that a relative error of selenium determination in the plant material is in limits of 4 up to 9%.

The elaborated method of the vegetative sample preparation was used both for atomic absorptive and fluorimetric determination of selenium in plants. Barley growing in selenium enriched soils was used as the object of the study. The analysis of the plants was carried out at phases of tillering, earing and milk-ripe. The results are presented in Table 2.

Thus, the difference between the results of fluorimetric and atomic absorption methods used in this sample pre-treatment method is statistically inadequate and both methods enable to determine the selenium content in vegetative material with equal accuracy. However, atomic absorption method is more expressive, it enables to carry out selenium determination without any preliminary extraction, which significantly reduces the analysis time and makes this method preferable in routine studies.

The application of the method of autoclave decomposition of vegetative material with the further selenium determination by atomic absorption enabled to study the peculiarities of the accumulation and distribution of this microelement in barley depending on the selenium additive introduced.

The findings show that the selenium content in the ear was larger than into stem of the plant (Figure). The difference in selenium content in grain and stem was especially significant at low selenium content in the soil. In the cases when higher volumes

Table 2. **Results of atomic absorption and fluorimetric measurement of selenium in barley at different vegetative phases (p = 0.95; n = 4)**

Vegetative phases	Selenium content in plants, µg/g		$t_{experimental}$
	Fluorimetric measurement (method 1)	Atomic absorption measurement (method 2)	
Phase of tillering	9.0 ± 0.1	9.12 ± 0.08	2.08
Phase of earing	4.75 ± 0.05	4.82 ± 0.08	1.89
Phase of milk-ripe	1.59 ± 0.03	1.61 ± 0.03	1.07

(At probability p = 0.95 and degree of freedom f = 6 the Student coefficient t = 2.57).

of selenium additives were put into the soil, the difference in selenium content in grain and stem was not significant. Thus, in the case when 0.05 mg of selenium was added per 1 kg of the soil, the grains contained by 1.6 times more selenium than the stems, whereas when 0.5 mg of selenium was added per 1 kg of the soil, this difference was considerably less (by 1.1 times).

These studies also show that the pH of the soil does not have any effects on the accumulation and distribution of this microelement in the grain or stem of barley (Figure).

Since at pH 6.35 and 5.5, selenium was mostly concentrated in the grain, and a major factor influencing the distribution of selenium between the grain and the stem was the concentration of selenium in the soil. This result is of great practical significance and should be taken into account for the selenium enrichment of cereals in order to increase selenium intake by animals and people.

CONCLUSIONS

The procedure of the preparation of a vegetative sample for selenium determination has been optimized: mineralization temperature – 80 °C, time – 2 hours, oxidising mixture – HNO_2 (concentrated) and H_2O_2, sample mass – not exceeding 0.5 g, deoxidizing agent – HCl (concentrated) in the presence of amidosulforic acid solution with further treatment in a water bath (t = 70 °C, time – 1 hour).

The accuracy of selenium determination related to the sample preparation varies from 9 to 4%.

This method of vegetative sample preparation could be recommended for the further fluorimetric and atomic absorption determination of selenium in this material.

It was determined that when the soil is enriched with selenium, the larger part of selenium is concentrated in barley grains, especially if the amount of selenium additives is not large.

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AUGALINĖS MEDŽIAGOS, PARUOŠTOS SELENUI NUSTATYTI ATOMINĖS ABSORBCIJOS IR FLUORIMETRINIU METODAIS, SAVYBĖS

S a n t r a u k a

Darbe buvo tiriama augalinės medžiagos, skirtos seleno kiekiui joje įvertinti, mineralizavimas ir skaidymas autoklavavimo būdu. Nustatytos optimalios mėginių paruošimo sąlygos: mineralizavimo temperatūra, laikas, oksidavimo mišinys, reduktorius, mėginio svoris. Seleno kiekio paklaida, susijusi su mėginių paruošimu, kito nuo 9 iki 4%. Parodyta galimybė taikyti autoklavavimo metodą augaliniams mėginiams, skirtiems seleno kiekiui nustatyti atominės absorbcinės spektrometrijos ir fluorimetriniu metodais.