1. Introduction

Mushrooms are still underestimated, and not much explored for nutritional elements. A general conviction is that they are worthless regarding their nutritional value, giving approximately 25–60 kcal per 100 g intake [1] and consisting of approximately 90% water. Their specific aroma and desirable taste accounts for their popularity in traditional menus in Europe. Mushrooms, assimilate many microelements and play an important role in human and animal life. The chemical composition of edible mushroom species from Basidiomycetes varies and is already known [2]. Dried mushrooms consist, on the average, of 19–35% proteins, approximately of 5% carbohydrates, of vitamins (B, A, H, D, carotene) and microelements [3,4]. The microelement content has been determined by many analytical techniques [5,6]. We know their bioavailability and environmental communes in geochemical or nutritional aspects. The elemental composition depends on many factors such as species, location, age of mycelium [7], soil composition and ecological living type (saprophytic, mycorrhizal or parasitic) [8]. Particular parts of the mushroom have a different ability to store mineral and organic compounds defined as a bioconcentration factor (BF). The layout of the mineral salts and organic compounds favors the caps in comparison to stalks [9]. One of the microelements appearing in most popular European mushrooms is molybdenum.

Molybdenum is a one of the trace element that plays an important role in living organisms as essential constituent of enzymes that catalyze redox reactions, e.g. oxidation of aldehydes, xanthine, and other purines [10,11], and reduction of nitrates and molecular nitrogen [12-14]. For plants and some groups of animals e.g. ruminants, molybdenum is the one of an essential element in nutrition. Some lands are barren for lack of this element in the soil. Typical deficiency symptoms of molybdenum in animals are poor hatchability, weak chicks, and poor feathering [15]. From the other hand too high concentration of molybdemun in organism leads to the delayed puberty, reduced growth rate and reduced egg production [16]. Because the concentration of molybdenum in plants, water and soil is generally at parts per billion levels, a sufficiently sensitive method is required for the determination of molybdenum.

Abstract: The highly sensitive method of adsorptive stripping voltammetry (AdSV), with differential pulse steps, was performed at a mercury film electrode and has been applied here for the determination of trace molybdenum content in selected wild mushrooms. Fruiting body caps of 12 selected macrofungi species from Basidiomycetes have been investigated. Molybdenum content of investigated samples was determined in the range of 0.006 to 0.38 mg kg⁻¹ of dry matter. The highest value of molybdenum content was found in *Leccinum rufum* and the lowest in *Lactarius deliciosus*.

Keywords: Molybdenum • Edible mushroom • Adsorptive stripping voltammetry
The most common methods for the trace determination of molybdenum are spectrophotometry [17], neutron activation analysis [18], atomic absorption spectrometry [19], X-ray fluorescence spectrometry [20], and first-derivative synchronous solid-phase spectrofluorimetry [21], which all require pre-concentration procedures such as coprecipitation, solvent extraction, ion exchange, or adsorption on activated carbon. Electroanalytical procedures allow the necessary pre-concentration of the analyzed element in-situ, which is not possible with the other analytical techniques. Many electroanalytical procedures have been proposed for the determination of trace amounts of molybdenum.

In particular, several adsorptive stripping voltammetric (AdSV) procedures have been developed for the determination of trace molybdenum with chloranilic acid and mercury drop electrode [22-26], mercury film electrode [27] and bismuth film electrode [28]. In this work, differential pulse adsorptive stripping voltammetry (DP AdSV) is applied for the molybdenum(VI) determination on a mercury film electrode in the presence of chloranilic acid as a ligand [27]. The presentation of the optimized methodology of the voltammetric determination of molybdenum in mushroom samples with short pre-concentration time is the main purpose of this paper.

2. Experimental Procedure

2.1. Measuring apparatus

A multipurpose Electrochemical Analyzer M161 with the electrode stand M164 (both MTM-ANKO, Poland) were used for all voltammetric measurements. The classical three-electrode quartz cell, volume 20 mL, consisting of a homemade cylindrical silver based mercury film electrode (Hg(Ag)FE), refreshed before each measurement and with a surface area of 1 – 12 mm², as the working electrode, a double junction reference electrode Ag/AgCl/KCl (3M) with replaceable outer junction (3 M KCl) and a platinum wire as an auxiliary electrode. pH measurements were performed with laboratory pH-meter. Mushroom material was digested with a microwave digestion system (Multiwave 3000, Anton Paar). Stirring was performed using a magnetic bar rotating at approximately 500 rpm. All experiments were carried out at room temperature.

2.2. Chemicals and glassware

All reagents used were of analytical grade. Acids: HNO₃, HClO₄ (Merck, Suprapur®), mercury GR for polarography (Merck). A 0.01 M standard stock solutions of molybdenum(VI) was prepared by dissolving the sodium salt Na₃MoO₄·2H₂O in four times distilled water (Aldrich). Solutions with lower molybdenum concentrations were made weekly by appropriate dilution of the stock solution. A 0.01 M solution of chloranilic acid (Aldrich) was prepared every two weeks by dissolving chloranilic acid in water. Prior to use, glassware was cleaned by immersion in a 1:1 aqueous solution of HNO₃, followed by copious rinsing with distilled water.

2.3. Standard procedure of measurements

Quantitative measurements were performed using differential pulse adsorptive cathodic stripping voltammetry (DP AdSV) and the standard addition procedure. The procedure of refreshing the mercury film silver base electrode (Hg(Ag)FE) was carried out before each measurement. A potential of −1.10 V was applied to condition the electrode (used surface area equals 6.6 mm²) prior to the refreshing step. An Hg(Ag)FE electrode conditioned in this way was used to determine molybdenum(VI) in the supporting electrolyte: HNO₃ + 100 µL chloranilic acid (0.01 M) and water (total volume 10 mL, pH 2.5) contained in a quartz voltammetric cell. The potential of the electrode was changed in the following sequence: conditioning potential −1.10 V for 7 s, pre-concentration potential E_{pre} = 0.00 V for t_{pre} = 20 s, and starting potential −0.250 V for 3 s. During the pre-concentration step, molybdenum(VI) was adsorbed while the solution was stirred (ca. 500 r.p.m.) using a magnetic stirring bar. Then, after a rest period of 5 s a differential pulse voltammogram was recorded in the cathodic direction from −0.250 V to −0.850 V. The other experimental parameters were as follows: step potential, 4 mV; pulse potential, 30 mV; time step potential, 30 ms (10 ms waiting + 20 ms sampling time). The measurements were carried out from degassed solutions.

2.4. Mushrooms

Twelve species of Macromycetes class mushrooms were picked from mixed and deciduous forests between July and November in Southern Poland; Agaricus was purchased at the market place. The examined species were from Basidiomycetes widely growing in Poland and European forests. Mushrooms were freeze-dried at low temperature, between 46°C – 52°C below zero at low pressure (0.01 hPa) for 72 hours. Afterwards, mushrooms were powdered by a mixer mill made of high hardness polymer materials and then dried at over 70°C for 8 h. Then, 500 mg of each sample material was weighed and transferred into a Teflon container and treated with 4 mL of nitric acid and 0.5 mL of perchloric acid. This mixture was left for 24 hours, after which time
4 mL of nitric acid, 0.5 mL of perchloric acid and 1 mL of perhydrol were added. Subsequently, the vessel was placed in a microwave oven (Multiwave 3000). Total digestion time was 95 minutes of max. temperature 240°C and max. pressure 60 bar. The digested sample was placed on a heating plate to let it evaporate the water and to remove the nitrate. The sampled solutions were cooled to room temperature and transferred quantitatively into volumetric flasks (10 mL) and filled up to the mark with double distilled water. All the procedures were repeated three times for each sample.

3. Results and Discussion

Adsorptive stripping voltammetric techniques are applicable for measuring ultra traces of molybdenum(VI). So far, one of the most sensitive methods are based on the adsorption of a complex of molybdenum with chloranilic acid on a hanging mercury electrode and mercury film electrode [23,27]. This method was used for the first time for determination of molybdenum in mushrooms. The sensitivity of the method with the mercury film electrode for mushroom samples is approximately 27% worse and the detection limit at about 30% worse than for the synthetic solutions described in [27]. But this is still enough to produce a molybdenum concentration determination in mushrooms from a complex matrix. Fig. 1 presents typical voltammogram of Mo(VI) obtained for sclerodermia citrinum. The shape and width of the peak was similar to that obtained for a synthetic solution. Determinations of molybdenum were performed using the standard addition method.

The examined species were divided into three working groups with regard to sporophore shape and living type. In the group with tubular hymenophore species forms are Xerocomus badius, Leccinum scabrum, Boletus edulis and Suillus luteus. This group manifested the highest molybdenum content in the examined probes. The highest molybdenum concentration determined for this group was in Leccinum scabrum (Fig. 2, Table 1) reaching the level of 0.39 mg kg⁻¹. Another group of saprofitic species living directly on the ground forming a platy-shaped sporophore generally had smaller molybdenum content than the first group represented by such species as Lactarius deliciosus, Agaricus bisporus and Tricholoma equestre. In the case of A. bisporus, the highest molybdenum level was observed 0.16 mg kg⁻¹ (quite high for this group). Molybdenum concentration in Agaricus bisporus varies depending on the origin of the culture, this species being the only one among the examined species that is cultivated. Two species growing on trees were assigned to the third group including Armillaria mellea and Laetiporus sulphureus. Armillaria sp. is the species which grows on trunks of dead wood but does not mycorise with live trees, which exhibits its parasitic origin. The second species Laetiporus is a typical mushroom which

Figure 1. The DP AdSV molybdenum(VI) calibration curve and obtained voltammograms for sclerodermia citrinum sample and (a) - 0; (b) - 7.5; (c) - 15; (d) - 22.5 nM of Mo(VI) in 100 µM chloranilic acid (pH of base electrolyte 2.5) obtained for pre-concentration time 20 s. Pre-concentration potential 0.0 mV, stirring rate during preconcentration step 500 r.p.m.
is found on plants forming different fruiting bodies and its pathogenic existence contributes to the death of the trees. Among all of the examined species, the lowest molybdenum content was in *Lactarius deliciosus* 0.006 g kg⁻¹. The species not mentioned above that forms neither platy nor tubular sporophores containing 0.26 mg kg⁻¹ of molybdenum is *Sarcodon imbricatus* but it micorises. The second species belonging to no group is *Scleroderma citrinicum*, which was selected as a control with 0.23 mg kg⁻¹ of molybdenum.

To validate the method, the certified reference material tobacco leaves (CTA-VTL-2 Institute of Nuclear Chemistry and Technology, Poland) was applied. The concentration of molybdenum determined was 1.93 ± 0.09 ppm. This result is in good agreement with the certified value (2.01 ± 0.15 ppm). Precision and recovery were determined using mushroom samples spiked with 0.01; 0.1 and 0.2 ppm of Mo(VI). The recovery of molybdenum ranged from 93–105%. The analytical usefulness of the presented method for the determination of molybdenum in mushroom samples was confirmed.

![Figure 2. Molybdenum content in investigated mushrooms.](image)

![Table 1. Results of molybdenum determination in the mushroom samples.](table)

| Group | Mushroom            | Mo determined [mg kg⁻¹] | Mo added [mg kg⁻¹] | Found [mg kg⁻¹], (Recovery %) |
|-------|---------------------|------------------------|-------------------|------------------------------|
| 1     | *Lactarius deliciosus* | 0.006 ± 0.0007      | 0.01              | 0.015 ± 0.0014 (94)         |
|       | *Agaricus bisporus*  | 0.178 ± 0.019        | 0.1               | 0.284 ± 0.022 (102)        |
|       | *Trichome eguestre*  | 0.088 ± 0.006        | 0.1               | 0.196 ± 0.011 (104)        |
| 2     | *Xerocomus badius*   | 0.114 ± 0.011        | 0.1               | 0.210 ± 0.017 (98)         |
|       | *Leccinum rufum*     | 0.384 ± 0.038        | 0.2               | 0.543 ± 0.041 (93)         |
|       | *Leccinum scabrum*   | 0.270 ± 0.012        | 0.2               | 0.484 ± 0.026 (103)        |
|       | *Boletus edulis*     | 0.294 ± 0.022        | 0.2               | 0.499 ± 0.033 (101)        |
|       | *Suillus luteus*     | 0.228 ± 0.023        | 0.1               | 0.318 ± 0.029 (97)         |
| 3     | *Scleroderma citrinum*| 0.230 ± 0.026        | 0.2               | 0.426 ± 0.035 (99)         |
|       | *Sarccodon imbricatus*| 0.258 ± 0.028        | 0.2               | 0.440 ± 0.031 (96)         |
| 4     | *Laetiporus sulphureus*| 0.024 ± 0.001        | 0.01              | 0.035 ± 0.002 (104)        |
|       | *Armillaria mellea*   | 0.008 ± 0.001        | 0.01              | 0.017 ± 0.002 (95)         |
4. Conclusions

The calibration graph for the presented method in synthetic solutions is linear from 2 nM (192 ng L\(^{-1}\)) to 200 nM (19 μg L\(^{-1}\)) for an accumulation time of 15 s. Satisfactory recovery (93–105%) shows that the method can be used for the determination of Mo in mushrooms. The results showed in the paper indicate differences between molybdenum assimilation in mushrooms species. Investigated molybdenum contents were greater in tubular hymenophore mushrooms in comparison with platy hymenophore mushrooms. Investigated species living directly on the ground, myccorhise facultatively, drawing water with mineral salts from soil have higher molybdenum content comparable to species growing on trees. This parasitical species growing on leafy trees selectively use mineral salts from the plant, and molybdenum uptake is limited. Environmental, geological, species-specific features produce different molybdenum concentration.

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