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Determination of heavy metals in mushroom samples by atomic absorption spectrometry

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The concentrations of heavy metals in the mushroom samples collected from the Batak mountain, Bulgaria have been determined by flame and graphite furnace atomic absorption spectrometry after dry ashing, wet ashing and microwave digestion. The study of sample preparation procedures showed that the microwave digestion method was the best. Good accuracy was assured by the analysis of standard reference materials. In all cases, quantitative analytical recoveries ranging from 92 to 104% were obtained. Results obtained are in agreement with data reported in the literature.

Keywords: *Atomic absorption spectrometry, Digestion, Heavy metals, Mushroom*

INTRODUCTION

Edible mushrooms are homely food for people in eastern and central Europe, while in western and northern European countries wild edible fungi are less popular [1-3]. Many studies have confirmed the high and balanced nutritional value of mushrooms [4-10], since they are rich sources of digestible proteins, vitamins B, D and K and in some cases vitamins A and C [11-15]. Carpophores are a good source of minerals, particularly K, P, Ca, Mg and Na [16-23]. Mushrooms are considered not only as spice and taste ingredients, but also as a nutritional supplement in the human diet and can also play a role as functional foods [24-29]. It is worth stressing here that many studies have focused on the medicinal properties of mushrooms [30-34]. They have also been reported to show anti-inflammatory, antibacterial, antiviral and antioxidant potential [35-36].

Mushroom has been used as a bioindicator by various researchers to determine the heavy metal pollutions [37-40]. Compared to green plants, mushroom can build up large concentrations of some heavy metals such as Pb, Cd, Hg, and a great effort has been made to evaluate the possible danger to human health from the ingestion of mushrooms [41-43].

Decomposition of solid samples is an important step in combined analytical methods. In most cases, when using highly sensitive measuring methods, such as flame atomic absorption spectrometry (FAAS), graphite furnace AAS, ICP-OES, ICP-MS, the sample is measured in an aqueous solution [44-46]. Combined analytical methods are favoured for multi element analysis of environmental and biological samples at very high speed. Sequential and simultaneous determinations of the elements can be made using the above analytical techniques [47-50].

In this study, the levels of heavy metals in wild edible mushrooms (*Lactarius deliciosus)* from the Batak mountain, Bulgaria were determined by flame and graphite furnace AAS after various digestion methods.

EXPERIMENTAL

Sampling

One hundred and fifty mushroom samples were collected in 2014 and 2015 from the Batak mountain by the authors themselves.

Mushroom samples were washed with distilled water and dried at 105°C for 24 h. The dried samples were ground, then homogenized using an agate pestle and stored in polyethylene bottles until analysis.

Reagents

All reagents were of analytical reagent grade unless otherwise stated. Double deionized water (Milli-Q Millipore 18.2 MΩ cm resistivity) was used for all dilutions. HNO_3, H_2SO_4, H_2O_2, HF, $HClO_4$ and HCl were of suprapur quality (E. Merck). All the plastic and glassware was cleaned by soaking in dilute HNO_3 (1+9) and rinsed with distilled water prior to use. The element standard solutions used for calibration were prepared by diluting a stock solution of 1000 mg l^{-1} (Pb, Cd, Co, Cr,

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Mn, Ni) supplied by Sigma and (Cu, Zn, Fe) by Aldrich.

Apparatus

A Perkin Elmer AAnalyst 800 atomic absorption spectrometer with deuterium background corrector was used in this study. Pb, Cd, Co, Cr, Mn and Ni in plant samples were determined by HGA graphite furnace using argon as inert gas. Other measurements were carried out in an airyacetylene flame. The instrumental parameters and operating conditions are given in Table 1.

Digestion procedures

Three types of digestion procedures were applied. Optimum digestion conditions are given below.

Dry ashing

One gram of mushroom sample was placed into a high form porcelain crucible. The furnace temperature was slowly increased from room temperature to 450 °C in 1 h. The samples were ashed for approximately 4 h until a white or grey ash residue was obtained. The residue was dissolved in 5 ml of HNO_3 (25% v/v) and the mixture, when necessary, was heated slowly to dissolve the residue. The solution was transferred a 25-ml volumetric flask and made up to volume. A blank digest was carried out in the same way.

Wet ashing

Digestion of mushroom samples was performed using an oxi-acidic mixture of HNO_3:H_2SO_4:H_2O_2 (4:1:1) (12 ml for a 1-g sample). This mixture was heated up to 150 °C for 4h and brought to a volume of 25 ml with deionized water. A blank digest was carried out in the same way.

Microwave digestion

Multiwave 3000 closed vessel microwave system (maximum power was 1400 W, and the maximum pressure in Teflon vessels - 40 bar) was used in this study. Mushroom samples (0.25 g) were digested with 6 ml of HNO_3 (65%) and 1 ml of H_2O_2 (30%) in microwave digestion system for 23 min and diluted to 25 ml with deionized water. A blank digest was carried out in the same way. All sample solutions were clear. Digestion conditions for the microwave system are given in Table 2.

In order to validate the method for accuracy and precision the certified reference material (CRM) - Virginia Tobacco Leaves (CTA-VTL-2) was analysed for the corresponding elements. The results are shown in Table 3.

Table 1. Instrumental analytical conditions of element analyses

FAAS						
Element	Wavelength (nm)	Lamp current (mA)	Slit width (nm)	Air (l min^{-1})	Acetylene (l min^{-1})	
Pb	283.3	30	0.7	17.0	2.0	
Cd	228.8	4	0.7	17.0	2.0	
Ni	232.0	25	0.2	17.0	2.0	
Cr	357.9	25	0.7	17.0	2.5	
Mn	279.5	20	0.2	17.0	2.0	
Co	240.7	30	0.2	17.0	2.0	
Cu	324.8	15	0.7	17.0	2.0	
Zn	213.9	15	0.7	17.0	2.0	
Fe	248.3	30	0.2	17.0	2.0	
GFAAS						
Instrumental conditions	Pb	Cd	Ni	Cr	Mn	Co
Argon flow (ml min^{-1})	250	250	250	250	250	250
Sample volume (µl)	20	20	20	20	20	20
Modifier (µl)	5	10	5	5	5	5
Heating program temperature, °C (ramp time (s), hold time (s))						
Drying 1	110 (1, 20)	110 (1, 20)	110 (1, 20)	110 (1, 20)	110 (1, 20)	110 (1, 20)
Drying 2	130 (5, 30)	130 (5, 30)	130 (5, 30)	130 (5, 30)	130 (5, 30)	130 (5, 30)
Pretreatment	850 (10, 20)	700 (10, 20)	1100 (10, 20)	1500 (10, 20)	1300 (10, 20)	1400 (10, 20)
Atomization	1500 (0, 5)	1400 (0, 5)	2300 (0, 5)	2500 (0, 5)	1900 (0, 5)	2400 (0, 5)
Cleaning	2400 (1, 2)	2400 (1, 2)	2400 (1, 2)	2400 (1, 2)	2400 (1, 2)	2400 (1, 2)

Table 2. Operating conditions for mushroom samples in microwave digestion system

Steps	Time (min)	Power (W)
1	2	250
2	2	0
3	6	250
4	5	400
5	8	550
Vent: 8 min		

Analytical procedure

Detection limit is defined as the concentration corresponding to three times the standard deviation of ten blanks. Detection limit values of elements as microgram per liter in flame AAS were found to be 0.025 for Cd, 0.127 for Co, 0.083 for Cr, 0.072 for Cu, 0.111 for Fe, 0.058 for Mn, 0.145 for Ni, 0.450 for Pb and 0.021 for Zn. The concentrations of Cu, Zn and Fe were determined in the plant samples using FAAS. The other elements (Cd, Pb, Co, Cr, Mn and Ni) were below the corresponding detection limits of FAAS. These elements in plant samples were determined using graphite furnace AAS by autosampler. During analyses, internal argon flow rate through the graphite tube was 250 ml min^{-1}; gas flow was interrupted during atomization. Sample volume, ramp and hold times for the drying, ashing, atomization and cleaning temperatures were optimized before analysis to obtain maximum absorbance and minimum background. Matrix modifiers were added 0.050 mg $NH_4H_2PO_4$ + 0.003 mg $Mg(NO_3)_2$ for Pb, 0.050 mg $NH_4H_2PO_4$ + 0.003 mg $Mg(NO_3)_2$ for Cd, 0.015 mg $Mg(NO_3)_2$ for Co, 0.005 mg Pd + 0.003 mg $Mg(NO_3)_2$ for Mn, 0.015 mg $Mg(NO_3)_2$ for Ni and 0.015 mg $Mg(NO_3)_2$ for Cr. Most of the matrix was removed before the atomization step and less interference occurred during atomization. Each graphite furnace AAS analysis calls for 20 µl of solution and 5–10 µl of the matrix modifier. As Table 1 shows, matrix modifier was used for all 6 elements determined by GFAAS. Characteristic mass for 0.0044 absorbance was found to be 1.3 pg for Cd, 17.0 pg for Co, 7.0 pg for Cr, 20.0 pg for Ni, 6.3 pg for Mn and 30 pg for Pb.

Statistical processing

SPSS (Statistical Package for Social Science) program for Windows was used for statistical data processing.

RESULTS AND DISCUSSION

It is desirable to use a higher ashing temperature in graphite furnace in order to remove the matrix efficiently for many analytes in food, biological and environmental samples. The ashing and atomization temperatures of heavy metals were increased using different chemical modifiers.

SPSS was used in this study. The comparison of dry, wet and microwave digestion methods showed no statistically significant differences in results. Therefore, the microwave digestion procedure was preferred because this procedure is more proper with respect to both time and recovery than dry and wet digestion. The disadvantage of the method consists in its expensiveness and need of some experience.

The standard deviations of the dry and wet digestion methods are higher than those of the microwave digestion method. The accuracy of the method was evaluated by means of heavy metals determination in CRM. The achieved results were in good agreement with certified values. The results from the analysis of CRM were all within the 95% confidence limit.

Table 3. Observed and certified values (µg g^{-1}) of element concentrations in the CRM (CTA-VTL-2) as average ± S.D.

Element	Certified value	Observed value					
		Dry ashing	Recovery (%)	Wet ashing	Recovery (%)	Microwave digestion	Recovery (%)
Pb	22.1 ± 1.2	22.5 ± 1.1	101.8	21.0 ± 1.3	95	23.0 ± 0.8	104
Cd	1.52 ± 0.17	1.44 ± 0.08	94.7	1.45 ± 0.09	95.4	1.50 ± 0.05	98.7
Ni	1.98 ± 0.21	1.89 ± 0.1	95	1.91 ± 0.06	96	1.94 ± 0.02	98
Cr	1.87 ± 0.16	1.87 ± 0.22	100	1.78 ± 0.13	95	1.91 ± 0.11	102
Mn	79.7 ± 2.6	76.4 ± 2.1	95.9	75.1 ± 2.0	94.2	77.5 ± 1.2	97.2
Co	0.429 ± 0.026	0.408 ± 0.009	95	0.408 ± 0.02	95	0.433 ± 0.006	101
Cu	18.2 ± 0.9	17.6 ± 0.8	96.7	18.9 ± 0.9	104	18.1 ± 0.7	99.4
Zn	43.3 ± 2.1	42.3 ± 3.0	97.7	43.9 ± 2.6	101.4	44.1 ± 1.6	101.8
Fe	1083 ± 33	1050 ± 48	96.9	996.36 ± 49	92	1160 ± 44	103

Table 4. Concentration of heavy metals in mushroom samples (*Lactarius deliciosus)* collected from Batak mountain, Bulgaria (n = 15)

	Pb	Cd	Ni	Cr	Mn	Co	Cu	Zn	Fe
$\overline{X}$ mg kg^{-1}	0.81	0.33	0.16	0.08	0.88	0.10	6.41	61.32	88.52
SD mg kg^{-1}	0.11	0.08	0.05	0.01	0.83	0.01	1.64	6.07	10.64
Min	0.63	0.21	0.08	0.06	0.18	0.08	4.11	51.72	74.56
Max	0.94	0.42	0.22	0.11	2.76	0.12	8.93	69.26	105.13
95% Confid. Level	0.06	0.04	0.03	0.01	0.46	0.01	0.91	3.36	5.89

According to this study, the edible wild mushroom Lactarius deliciosus could be used in human nutrition due to its good parameters. Heavy metal content of samples indicated that the Batak mountain was an ecologically pure region of Bulgaria, and therefore the mushrooms collected from this location could be consumed without any risk for human health.

CONCLUSIONS

The dry and wet digestion methods are more time-consuming and complicated than microwave digestion method without any advantage in terms of digestion efficiency. The use of microwave digestion system in mushroom samples provides a better, safer and cleaner method of sample preparation. The accuracy of the method was checked and confirmed by CRM.

From the obtained concentrations of heavy metals one can say that the locality Batak mountain is ecologically clean area and very suitable for collecting wild edible mushrooms that we can use in our daily menu.

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ОПРЕДЕЛЯНЕ КОЛИЧЕСТВОТО НА ТЕЖКИ МЕТАЛИ В ПРОБИ ОТ ГЪБИ ЧРЕЗ АТОМНА АБСОРБЦИОННА СПЕКТРОСКОПИЯ

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(Резюме)

Концентрациите на тежки метали в проби от гъби, събрани от Баташката планина в България са определени чрез атомноабсорбционна спектрометрия в пламък и графитна пещ след сухо опепеляване, киселинна минерализация и микровълнова минерализация. Проучването на процедурите за подготовка на проби показа, че методът на микровълновата минерализация е най-добрият. Добрата точност е доказана чрез анализ на сертифициран референтен материал. Във всички случаи на пробоподготовка се получават количествени извличания на елементите варирващи от 92 до 104 процента от сертифицираната стойност. Получените резултати са в съгласие с данните, докладвани в литературата.

Ключови думи: *Атомна абсорбционна спектроскопия, разтваряне, тежки метали,гъби*