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DETERMINING HEAVY METALS IN MUSHROOM SAMPLES BY STRIPPING VOLTAMMETRY

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Abstract. Industrial cultivation of mushrooms in Ukraine in recent years has been developing at a rather high pace. A promising consumer of Ukrainian mushrooms may be the European Union market. But mushrooms are not delivered there in significant volumes due to quite high requirements for the product quality. From the biosphere, heavy metals can enter the mushrooms and make them potentially dangerous for people. The content of heavy metals: zinc, cadmium, lead, and copper in edible mushrooms (ceps, chanterelles, butter mushrooms, saffron milk-caps, and champignons) has been studied by using the stripping voltammetry method after the destruction of the matrix of mushroom samples. Sample preparation was done by the method of "wet" mineralisation with oxidising mixtures of various compositions using inorganic acids (nitrate, chloride, sulfate), and an oxidiser – hydrogen peroxide. Besides, dry ashing was used. As a result of the experiment, it has been found that the most effective method of extracting the ions of heavy metals and ensuring the accuracy of the analysis is sample preparation using nitric acid and hydrogen peroxide. The concentrations of the metals under analysis are calculated on the dry basis (mg/kg). The concentrations found for bioelements that are contained in enzymes in living organisms (zinc, copper, and toxic elements – lead and cadmium), are within the range of 51.3–72.9; 3.0–10.3; 0.2–1.32, and 0.06–0.33 mg/kg, respectively. Thus, by arranging the samples of the mushrooms under study in ascending order by the specified total content of heavy metals, we obtain the following series: ceps – butter mushrooms – chanterelles – saffron milk caps – champignons. Besides, the relative error of analysis has been calculated, and the replicability of the selected research method has been estimated. Thus, the method of stripping voltammetry can be applied in quantitative determination of heavy metals in mushroom samples.

Keywords: mushrooms, heavy metals, stripping voltammetry, nitric acid, hydrogen peroxide.

ВИЗНАЧЕННЯ ВАЖКИХ МЕТАЛІВ В ЗРАЗКАХ ГРИБІВ МЕТОДОМ ІНВЕРСІЙНОЇ ВОЛЬТАМПЕРОМЕТРІЇ

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Анотація. Дослідження вмісту важких металів: цинку, кадмію, свинцю і міді у їстівних грибах (білих, лисичках, маслятах, рижиках та шампінйонах) проведено методом інверсійної вольтамперометрії після руйнування матриці грибних зразків. Пробопідготовка зразків виконано способом «мокрої» мінералізації і сухим озоненням окиснювальною сумішшю різного складу. У результаті експерименту було встановлено, що більш ефективним для вилучення іонів важких металів та забезпечення точності аналізу є варіант підготовки проб із використанням нітратної кислоти та перекису водню. Знайдено концентрації для біоелементів, які входять до складу ферментів в живих організмах, цинку та міді і токсичних елементів, свинцю та кадмію, знаходяться в межах 51,3–72,9; 3,0–10,3; 0,2–1,32 та 0,06–0,33 мг/кг, відповідно. Отже, метод інверсійної вольтамперометрії може бути застосований в практиці кількісного визначення важких металів у грибних зразках.

Ключові слова: гриби, важкі метали, інверсійна вольтамперометрія, нітратна кислота, перекис водню.

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Introduction. Formulation of the problem

The current economic situation in Ukraine has made people interested in broadening their diet with wild foods. An important source of them is picking wild mushrooms. Consuming wild edible mushrooms increases in developed countries, too, due to the protein and microelements content. Wild-growing and cultivated mushrooms are considered a popular food product in a number of countries, primarily in Europe and Asia. Exports of fresh champignons from Ukraine for the first nine months of 2017 amounted to 279 tons, which is 50 times more than in the same period a year before (5 tons). This is evidenced by the data from the

National Mushroom Agency UMDIS. Besides, mushrooms were registered as therapeutic food products, useful for diseases [1-4].

Metal concentration in mushrooms is considerably higher than that in agricultural crops, vegetables, and fruit. That proves that mushrooms have efficient mechanisms that allow them to absorb easily some metals out of the ecosystem, as compared to green plants which grow in similar places. It is known that some types of mushrooms accumulate big concentrations of heavy metals, such as lead, cadmium, iron, copper, manganese, zinc, chrome, nickel, aluminium, and mercury [1]. Since heavy metals can get into the food chain having been absorbed by edible mushrooms, then

it is necessary to estimate the levels of heavy metals content and inform about possible contamination, dangerous for people's health.

Analysis of recent research and publications

To define microquantities of heavy metals, it is necessary to convert them into a solution while destroying the organic matrix of the samples. To destroy the matrices completely, wet ashing is used. It is carried out with acids or acid mixtures (with or without an oxidiser – hydrogen peroxide). The mixtures used to this end are: mixture of nitric and perchlorate acids [2], of nitric, perchlorate acids, and hydrogen peroxide [3,4], of nitric, sulfate acids, and hydrogen peroxide [1], of nitric, chloride acids, and hydrogen peroxide [5], of nitric and chloride acids [6], of nitric acid and hydrogen peroxide [7,8,9-11], of chloric [12] or nitric [13-15]. But it is still a question which composition of an acid-oxidising mixture is the most rational one concerning the devices used and a certain analytical method of research.

Despite considerable progress in the development of spectroscopic methods of mushrooms analysis (atomic adsorption spectroscopy [2,4,7-9,12,13], flame atomic adsorption spectroscopy [1,5,10-11,14], atomic adsorption spectroscopy with inductively coupled plasma [3,6,15], dispersive X-ray spectroscopy [9,11]), a more modern method consists in using microwave heating devices for wet ashing under pressure described in the works [1,5,7-11]. But these analyses need complicated and expensive equipment, so cost too much.

Voltammetry is one of the methods which are currently being actively developed. The main advantages of the voltammetry method are a wide range of concentrations (10^{-9} to 10^{-2} M), high selectivity, comparatively short time for analysis, simple, available, and portable equipment. A peculiarity of the method is a possibility of determining several substances in complex matrices simultaneously [16]. Besides, as it was previously established, more effective for the stripping voltammetry method (SVA) is samples preparation combining wet and dry ashing [17].

That is why, **the purpose** of this study is to find out the possibility of using the stripping voltammetry method for determining heavy metals such as zinc, lead, cadmium, and copper in mushrooms, as well as determining the composition of the acid-oxidising mixture that ensures the maximum correctness of this method.

Objectives of the study:

1. To select an oxidising mixture for the stage of sample preparation by the SVA method using ceps as an example;
2. To determine the relative error of the analysis of the SVA method as in the case of ceps;
3. To find out the content of heavy metals in samples of mushrooms by the suggested method.

Research Materials and Methods

The research material was samples of mushrooms collected in the summer of 2016 in Chernihiv region

(ceps, chanterelles, butter mushrooms, saffron milk-caps) and bought in a shop (champignons). The mushroom bodies were thoroughly cleaned from foreign impurities and inclusions, dried in the dryer at the temperature 60°C for 4–6 hours till the air-dried state, then chopped. The received dried samples were then homogenised, stored in previously cleaned glass vessels for further analysis in a dry place protected from dust and humidity, in well-ventilated rooms.

To convert the elements to be determined into a solution in electromechanically active forms, a two-chamber programmable oven PDP-Lab was used.

Samples were prepared by the method of “wet” mineralisation and dry ashing. A weighed quantity of 0.5 g of mushrooms was mixed with 2.5 ml of concentrated acid (in the case of oxidising mixtures No. 1, 2, 4, 5 – HNO_3 , and for No.3 – H_2SO_4) in a quartz glass. It was heated up to the temperature 150 °C until gases stopped releasing, and evaporated up to 1/3 of the initial volume. Then, 2.0 ml of concentrated acid (oxidising mixtures No. 1–3) was added. In the case of oxidising mixtures No.4 and No.5, two acids were added, in an amount of 1.0 ml o–70 minutes at the temperature 150–350°C until dry.

The sample was ashed at the temperature 450°C for 30 minutes. Adding the acids and oxidiser, the evaporation and ashing were repeated two or three times until obtaining homogeneous ash of white, yellow, or grey color. The ash was dissolved in 1 ml of concentrated formic acid and was diluted with a bidistillate up to the volume 10 ml. In the quartz electro chemical chamber, 10 ml of distilled water, 0.2 ml of concentrated formic acid and 0.5 ml of sample aliquot were added.

The heavy metals content was determined with a voltammetry analyser TA-Lab (Research and Development Enterprise “Tomanalit”, RF) in a three-electrode electrochemical chamber. Of the three, the amalgam electrode was used as the indicator electrode, the silver chloride electrodes filled with a solution of 1M of potassium chloride, were used for comparison and as an auxiliary electrode.

The analysis was carried out on the background electrolyte containing 200 mkl of concentrated formic acid (chemically pure), under the following conditions: electrochemical purification of the indicator electrode at the potential +0.050 V for 10 seconds; accumulation of metals on the surface on the indicator electrode at the potential 1.500 V for 30–35 seconds; solution calming at the potential 1.300 V for 5 seconds; anodic oxidation with linear potential sweep at a rate of 80 mV/second.

Each mushroom's sample was analysed in three parallel experiments. Metals were determined by the one-additive method using standard solutions. The confidence interval (S_r) was calculated with the specialised computer program TA-Lab (version 3.6.10) and the metals concentration (c_n) by the formula:

$$c_n = \frac{I_n \cdot c_{ad} \cdot V_{ad}}{(I_{ad} - I_n)} \times \frac{V_{min}}{m \cdot V_{al}} \quad (1)$$

where c_n – content of the element to be determined in the sample, mg/kg;

I_n – peak current (height) of the element on the voltamperogram of the sample, uA;

I_{ad} – peak current (height) of the element on the voltamperogram of the sample with an additive, uA;

c_{ad} – concentration of the additive of the element's assayed mixture, mg/l;

V_{ad} – of the additive of the element's assayed mixture, ml;

V_{min} – volume of the mineralised substance, ml;

V_{al} – aliquot volume – volume taken from the solution of the mineralised sample and added to the analyser chamber for being measured, ml;

m – weight of the sample taken for the analysis, g.

The accuracy of the method (R) was determined by the samples of ceps with an affinity to cadmium [5]. They were divided into two parts, and one was left unchanged, and into the other, the elements to be determined were added and calculated by the formula:

$$R = \frac{(c_{n+ad} - c_n)}{c_{ad}} \times 100\% \quad (2)$$

where: c_n – content of the element to be determined, in the part of the sample remaining unchanged, mg/kg;

c_{ad} – concentration of the additive of the element's assayed mixture, mg/l;

c_{n+ad} – concentration of the element to be determined, in the sample with the addition of elements to be determined, mg/kg.

Results of the research and their discussion

In Fig. 1, examples of voltammetry curves of the background (1), mushroom samples without the additive (2) and with the additive (3) of the metal analysed.

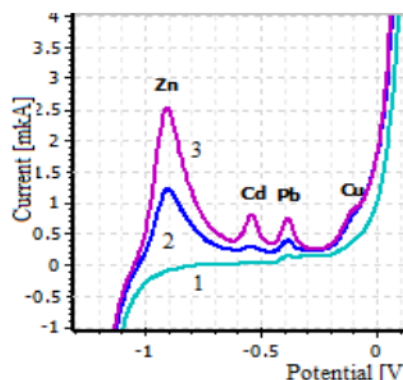


Fig. 1. Voltamperograms of the mushroom sample of ceps

You can see in Fig. 1 that on the voltammetry curves of the background solutions, there are no peaks of the oxidation current (curve 1) in the range of the potentials from -1200 mV to +100 mV. This shows the purity of the background electrolyte, that is about the absence of those metals in it. At the potentials -900, -550, -320, and -50 mV, on the voltammetry curves of the mushroom samples there are four peak currents corresponding to the processes of anodic oxidation of zinc, cadmium, lead, and copper, respectively. When into the sample solution the additive of the standard solution is introduced, which contains all the metals, peak currents of the oxidation of zinc, cadmium, lead, and copper increase on the curves proportionally to the increase of the concentrations of these metals.

The same voltammetry curves are registered for other samples of mushrooms under study.

The results of determining the content of zinc, cadmium, lead, and copper in the cep samples prepared with different kinds of the acid-oxidising mixture, and the analytical characteristics are given in Table 1.

Table 1 – Concentration of heavy metals (c_n), mg/kg, relative standard deviation (S_r),%, and level of heavy metals extraction (R),%, in cep samples

Content of acid-oxidising mixture	Parameters to be determined	Ions of the metals							
		Zn ²⁺	S_r , %	Cd ²⁺	S_r , %	Pb ²⁺	S_r , %	Cu ²⁺	S_r , %
1 HNO ₃	c_n	54±10	2.5	0.16±0.08	4.5	0.19±0.07	2.5	3.0±1.2	0.9
	c_{n+ad}	149±17	0.5	0.24±0.10	0.9	0.46±0.10	0.7	8.8±0.4	0.5
	R	110		78		84		107	
2 HNO ₃ H ₂ O ₂	c_n	66±11	2.6	0.33±0.09	2.8	0.15±0.04	3.2	3.7±1.0	0.6
	c_{n+ad}	120±12	0.5	0.76±0.04	0.4	0.35±0.04	0.5	4.9±0.6	0.7
	R	101		98		103		96	
3 H ₂ SO ₄	c_n	49±13	2.8	0.13±0.09	3.7	0.20±0.06	1.8	2.1±1.0	1.1
	c_{n+ad}	90±15	0.7	0.49±0.05	0.6	0.34±0.07	1.2	12.0±0.3	0.4
	R	118		80		82		112	
4 HNO ₃ HClO ₄ H ₂ O ₂	c_n	73±8	1.4	0.28±0.07	3.4	0.13±0.09	4.8	4.8±1.8	0.8
	c_{n+ad}	136±18	0.3	0.51±0.11	0.8	0.21±0.06	1.6	7.5±0.5	0.5
	R	94		93		96		106	
5 HNO ₃ H ₂ SO ₄ H ₂ O ₂	c_n	72±8	1.4	0.37±0.08	3.0	0.14±0.08	4.3	5.2±0.7	0.5
	c_{n+ad}	141±19	0.6	0.77±0.05	0.5	0.25±0.11	1.7	9.3±0.6	0.9
	R	95		105		88		92	

Since the limit of the relative error of the measurement of the metal ions concentrations (i.e. the degree of ion ex-

traction), for the SVA method should be 75–125%, as can be seen from Table 1, all varieties of the acid-oxidising mix-

ture of preparing mushroom samples by the combined method give satisfactory results.

But the results closest to ideal ($100\pm 5\%$) for all the metals in question are obtained with mixtures No. 2 and No. 4. However, perchloric acid is volatile, that is why the method of “wet” mineralisation and dry ashing with adding nitric acid and hydrogen peroxide can be

recommended as a method for preparing mushroom samples for further quantification of heavy metals by stripping voltammetry.

The results of determining the content of heavy metals in the mushroom samples prepared with acid-oxidising mixture № 2 based on nitric acid and hydrogen peroxide are given in Fig. 2.

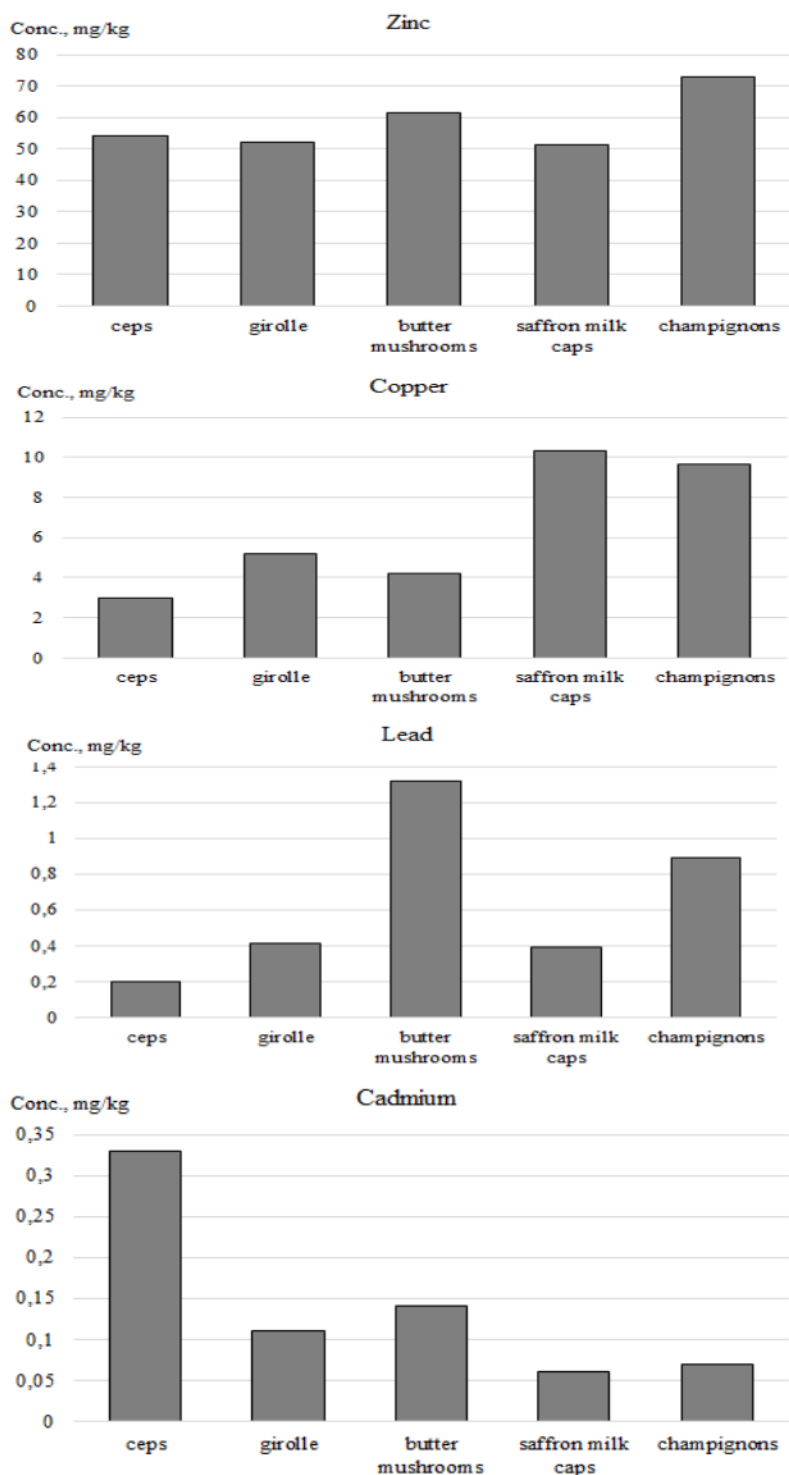


Fig. 2. Content of heavy metals in different mushroom varieties, mg/kg

As you can see in Fig. 2, all mushroom samples contain heavy metals. The biggest lead content has

been found in the butter mushrooms – 1.32 mg/kg. The biggest cadmium content is in the ceps – 0.33 mg/kg.

Zinc content in all samples is high, the maximum content of zinc has been found in the champignons – 72.9 mg/kg. Copper content is big in the saffron milk caps – 10.3 mg/kg.

Conclusions

Thus, the accumulation of heavy metals in mushroom is influenced by ecological factors, such as the amount of organic substance, soil acidity, concentrations of metals in the soils, and such factors as mushroom varieties, the morphology of a mushroom body, stages of development, the mycelium age and biochemical composition.

Thus, the study of the content of heavy metals in mushroom samples and the results received allow making the following conclusions:

1. To define the content of heavy metals (zinc, cadmium, lead, and copper) in mushroom samples, the

stripping voltammetry method can be used with a relative analysis error not more than 4.8%.

2. Preparing mushrooms samples prior to determining heavy metals by stripping voltammetry can be carried out by a method that combines wet mineralisation of nitric acid with adding hydrogen peroxide and dry ashing. The method is more effective in destroying organic matrix of mushroom samples and extracting ions of heavy metals into the solution and thus provides high accuracy of determining.

3. It has been established that all mushroom samples under study include heavy metals. For almost all the metals (but the copper), we can observe the exceedance of maximum permissible norms.

Therefore, the suggested method of determining the content of heavy metals can be used in laboratories that control the quality of products. Besides, the data obtained can be used in the system of monitoring research as a method of bioindication of the environment.

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