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The Monitoring of Heavy Metals in Fruits

M.N. Stefanut[*], I. David[**], Z. Stanoiev[***], C. Macarie[*]

[*] Institutul Naţional de Cercetare şi Dezvoltare pentru Electrochimie şi Materie Condensată, Timişoara,Romania, Electrochemistry Department Phone:+40256-222119, Fax:+40256-201328, E-mail: mariana_stefanut@yahoo.com, http//www.incemc.ro

[**] U.S.A.M.V.B., Facultatea de Tehnologia Produselor Alimentare, Timişoara, Romania,

[***] Universitatea „Politehnica" Timişoara, Facultatea de Chimie Industrială şi Ingineria Mediului, Timişoara, Romania

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Abstract: The paper proposes some possibilities for heavy metals monitoring in fruits. The heavy metals concentrations have been determinated by AA spectrometry and electrochemical methods: i = f(E) voltammetry and selective ion electrode determinations with a Thermo Orion 710A+ apparatus. The determination of heavy metals concentration in fruits is very important to be known because they both represent a natural and healthy source of vitamins, microelements, mineral salts, sugars, pectins, vegetable fibers necessary to human body equilibrium, and can be "small accumulators" of some toxic metals and their concentration in time can contribute to Alzheimer, hepatic, pancreatic, kidney diseases. The monitoring of heavy metals in fruits represents a possibility to evaluate the degree of medium pollution: soil, water, air.

Keywords: AA spectrometry, heavy metals, cyclic voltammetry, selective ion electrodes

1. Introduction

The modern life style has deeply changed the way of food preparation and consumption. The natural uptake of vitamins, minerals, sugars, pectins, fibres, etc. in humans is achieved by fruits and vegetables consumption.

The determination of heavy metals concentration in fruits is very important because the healthy and natural uptake of microelements, vitamins, minerals, sugars, pectins, fibres achieved by their consumption contributes to the equilibrium needed by the human body. In the mean time fruits are "small accumulators" of different heavy metals as a function of soil/air/water pollution degree which can, in time, when ingested, lead to severe liver, kidney, pancreas or Alzhmeier, etc. diseases.

Biologically speaking, heavy metals exhibit a high affinity towards carboxyl and sulfhydril groups as a function of their physico-chemical properties [1,2,3]. Thus oxidation and disulfidic bridge formation by the sulfhydril groups of membrane proteins from erythrocytes play an important role in the mechanism of cellular distruction and subsequent hemolysis caused by copper. Formation of active free radicals (hydrogen peroxide H_2O_2, superoxide anion O_2^-, hydroxil radical OH^-) in aerobic cells take place with rather small rates, but heavy metals determine the increase of these free radicals formation rate leading to a lipids peroxidation process initiation which determines the alteration of biomembrane functioning. Enzymic inhibition is also one of the effects of heavy metals based on the same principle of affinity towards the sulfhydril groups, necessary for catalytic activity, through their oxidation or substitution of some divalent cations from metaloenzyme structure.

Two main enzyme activity inhibition mechanisms under action of metals have been estsblished:
- bonding of metals to functional groups (-SH) important for the catalytic activity
- substitution of the ion from enzyme structure with a toxic one or the lack of appropriate ions of metaloenzymes.

2. Experimental

2.1. Samples preparation

Fresh or dried fruits have been weighed and treated by concentrated nitric acid (67 %, Merck, heavy metals free). Samples digestion has been achieved in a 1000W MWS-2 – Berghof type microwave oven using a three-step program: T_1 = 160 ºC, t_1 = 15 min., P_1 = 40 – 60 % from total power, T_2 = 210 ºC, t_2= 15 min., P_2 = 60 – 80 %, T_3 = 210 ºC →100ºC, t_3 = 15 min., P_3 = 0 %.

Thus resulted solutions have been completed with Millipore ultrapure water to equal volumes in 25 ml calibrated flasks.

2.2. Methods of analysis

2.2.1. AA Spectrometry

The heavy metals content has been determinated by AA spectrometry [4] and cyclic voltametry [5]. In addition lead content in fruits has been determinated with an ion selective electrode too.

AA spectrometry has been achieved with a novAA 400 G type spectrometer equipped with an Analytik Jena graphite furnace and provided with WinAAS 3.17.0 soft for evaluation, control and results display and Cookbook for all elements. Calibration curves have been plotted using standard solutions of metals in search.

2.2.2. Electrochemical Methods

Heavy metals such as Zn, Pb, Fe at the electrode surface are afflicted by characteristic redox phenomena with can be used to determine their concentration.

The i = f(E) voltammogrames have been plotted with

Radiometer Copenhagen Voltalab PG Z301 equipped with VoltaMaster 4 sofware.

Platinum (S_{work}=4 mm^2, S_{aux}=8 mm^2) and standard calomel electrod (ESC) have been used in a BEC/EDI X51V001 electochemical cell of 50cm^2 with 0.1M HNO_3 support electrolyte. Recording speed was 100 mV/min. at an apparatus sensitivity of 100 mA. Calibration curves for Fe and Zn have been plotted using metals standard solutions as I_{pic} = f(conc.).

Determination of Pb in fruits has been made also with a Thermo Orion Model 710A+ type pH / ISE mV / Temperature Meter using a Model 96-82-ionplus® Sure-Flow® type ion selective electrode, which does not require a separate reference electrode.

3. Results and disscutions

The heavy metal concentrations in fruits determined by AA spectroscopy are presented in Table 1.

TABLE 1. The heavy metal concentrations

No.	Fruits	Concentration, ppm			
		Cr	Cu	Ni	Pb
1.	Pears (fresh)	0.11	0.61	0.19	0.08
2.	Apples (fresh)	0.14	0.30	0.18	0.23
3.	Bananas (fresh)	0.13	0.62	0.09	0.19
4.	Raisins (dried)	0.28	2.0	0.22	*
5.	Rose hips (dried, Baia Mare)	0.35	1.91	2.80	0.10
6.	Underbrushs (dried,Cluj)	0.90	6,02	4.30	0.06

* under limit detection

TABLE 1. The heavy metal concentrations (continuation)

No.	Fruits	Concentration, ppm			
		Zn	Al	Fe	As
1.	Pears (fresh)	2.70	1.3	14,88	*
2.	Apples (fresh)	1.11	0.003	5,88	*
3.	Bananas (fresh)	4.40	-	5.76	*
4.	Raisins (dried)	2.77	5.2	59.37	*
5.	Rose hips (dried, Baia Mare)	8.89	1.8	142.44	*
6.	Underbrushs (dried,Cluj)	527.3	5.7	142.44	*

* under limit detection

Some fruits concentrates large amounts of Fe, Cu, Zn, metals which play an important role in humans. Their consumption in larger quantities and/or for longer periods can help in solving some lacks that may appear during or subsequent of some diseases.

Heavy metals concentrations in fruits also vary with the composition of the soil they are harvested from, with the pollution grade and depends on the *plant-fruit metals fixing capacity* (Table 2, Table 3).

Examples are presented for the fruits of underbrushs and Rose hips. Fruits of underbrushs have been subject of many researches due to their energizing, equilibrating, antitumoral, antioxidant, analgesic, anti-inflammatory, anti-asthmatic, antibacterial,etc. effects. They contain all the vitamins needed by the human body: A, E, F,D (unstable in acidic media), C, K, P, entire complex of B vitamins (unstable in alkaline media). The simultaneous presence in the fruit of these two types of vitamins at the pH=2-3 is possible due to some unidirectional membranes which can preserve the vitamins as long as they are not damaged.

TABLE 2. The repartition of heavy metal concentrations on Underbrushs vs. county harvest

No.	Underbrushs (dried)	Concentration, ppm			
		Cr	Cu	Ni	Pb
1.	Cluj county	0.90	6.02	4.32	0.06
2.	Mures county	0.99	6.26	3.25	0.41
3.	Orăştie county	0.97	5.57	3.73	1.00
4.	Vâlcea county	0.59	3.51	4.79	*
5.	Ilfov county	0.62	3.60	6.47	*
6.	Gioagiu county	1.72	13.07	35.07	0.11

* under limit detection

TABLE 2. The repartition of heavy metal concentrations on Underbrushs vs. county harvest (continuation)

No.	Underbrushs (dried)	Concentration, ppm			
		Zn	Al	Fe	As
1.	Cluj county	527.30	527.30	142.44	*
2.	Mures county	25.27	25.27	126.40	*
3.	Orăştie county	14.95	14.95	115.27	*
4.	Vâlcea county	15.54	15.54	106.13	*
5.	Ilfov county	57.68	57.68	126.44	*
6.	Gioagiu county	34.81	34.81	129.64	*

* under limit detection

The fruits of Rose hip also exhibit a rich content of vitamins (C, A, B1, B2, P, K) and can be used as a supplement in the treatment of liver and kidney diseases, as diuretics, antidiarrheals or simply as vitamins and minerals in food supplements.

TABLE 3. The repartition of heavy metal concentrations on Rose hips vs. county harvest

No.	Rose hips (dried)	Concentration, ppm			
		Cr	Cu	Ni	Pb
1.	Baia Mare county	0.35	1.91	2.80	0.10
2.	Valcea county	0.35	1.55	0.51	0.23

TABLE 3. The repartition of heavy metal concentrations on Rose hips vs. county harvest (continuation)

No.	Rose hips (dried)	Concentration, ppm			
		Zn	Al	Fe	As
1.	Baia Mare county	8.90	1.77	142.36	*
2.	Valcea county	9.05	3.85	36.89	*

* under limit detection

We expected higher heavy metals concentrations in Baia Mare county. A possible reason is that some of metals extracting mines were closed and for the others the best available technologies was applied, ths directly influencing the processing plants.

For the determination of heavy metals by electrochemical methods, the first step was plotting the calibration curves. The methods used for Fe and Zn by means of cyclic voltametry **i=f(E)** are presented in (Fig.1., Fig.2., Fig.3., Fig.4.).

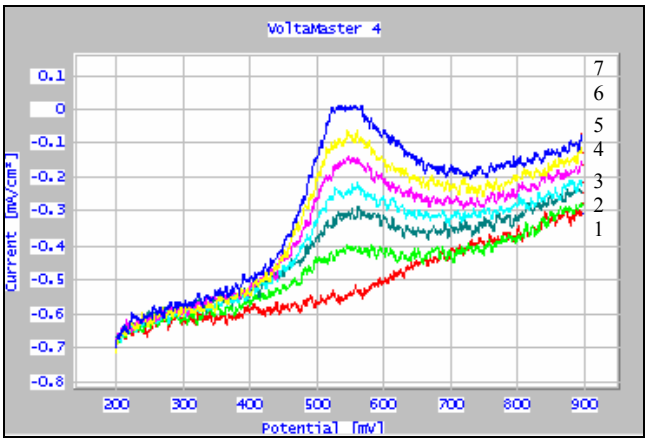

Figure 1. Cyclic voltammograme ciclice for equilibrium $Fe^{3+} + e^- \rightarrow Fe^{2+}$
1 – support electrolyte HNO3 0.1 M; 2 - c=24.39 mg/L; 3 - c=47.62 mg/L; 4 - c=69.77 mg/L; 5 - c=90.91 mg/L; 6 - c=113.64 mg/L; 7 - c=136.37 mg/L;

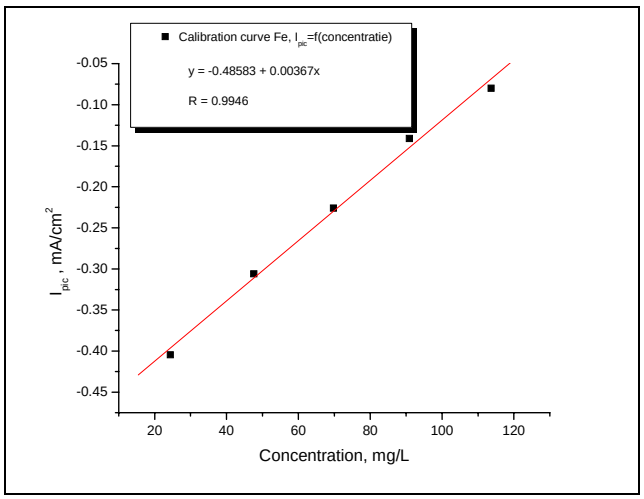

Figure 2. Calibration curve for Fe concentration determination in fruits, I_{pic} = f(conc.)

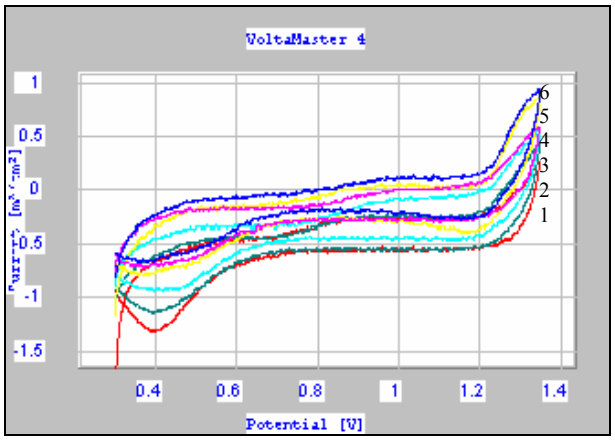

Figure 3. Cyclic voltammograme ciclice for Zn calibration
1 – support electrolyte HNO3 0.1 M; 2 - c=24.39 mg/L; 3 - c=69.76 mg/L; 4 - c=90.91 mg/L; 5 - c=111.11 mg/L; 6 –c=130.43

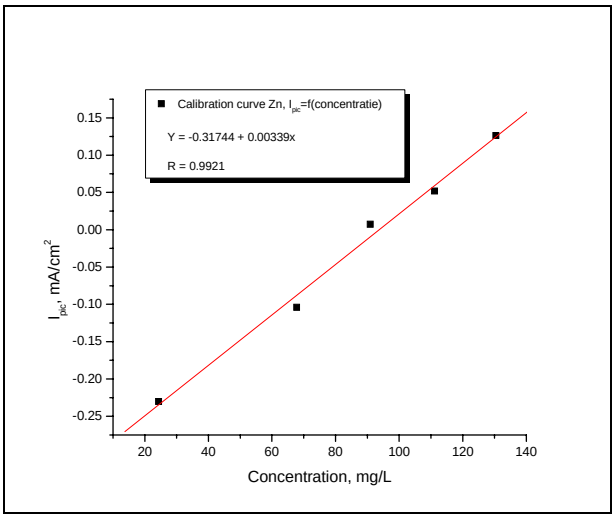

Figure 4. Calibration curve for Zn concentration determination in fruits, I_{pic} = f(conc.)

Results obtained are displayed in Fig.5 and Table 4. for Fe and Fig.6. and Table 5 for Zn, respectively

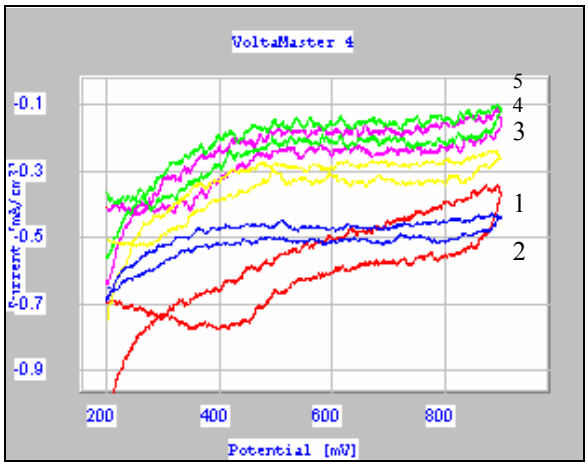

Figure 5. Determination of Fe concentration in fruits by cyclic voltammetry ($E_{pic, ESC}$= 530 mV); 1 – support electrolyte HNO3 0.1 M; 2 - pears; 3 – underbrushs from Ilfov county; 4 – underbrushs from Cluj county; 5 – Rose hips from Baia Mare county

The appearance of the voltammogrames for the real samples differs from that of the calibration curves probably due to interference with other metallic ions or some compounds undestroyed at the microwave digestion.
These differences appear for both Fe and Zn.

TABLE 4. Fe concentration in some fruits

Curve	Sample	I_{pic}, mA/cm^2	Concentration, mg/L
2.	Pears	-0.4706	4.1423
3.	Underbrushs, Ilfov county	-0.2974	51.3610
4.	Underbrushs, Cluj county	-0.1705	85.9237
5.	Rose hips, Baia Mare county	-0.1626	88.0806

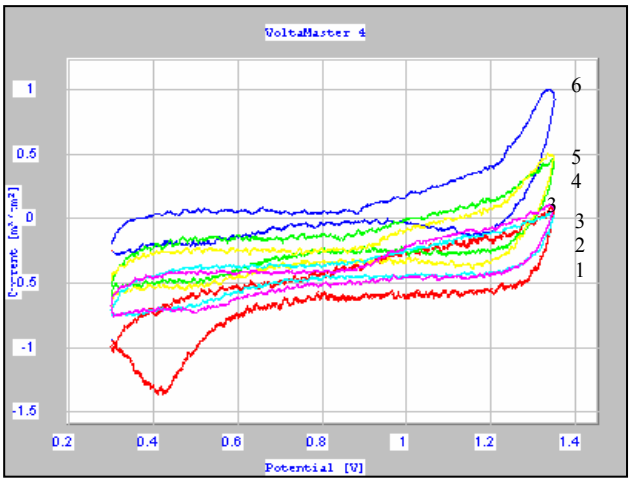

Figure 6. Determination of Zn concentration in fruits by cyclic voltammetry ($E_{pic, ESC}$= 1100 mV)
1–support electrolyte HNO3 0.1 M; 2 - raisins; 3 –pears; 4 - underbrushs from Gioagiu county;; 5 – underbrushs from Ilfov county; 6 – Rose hips from Baia Mare county.

TABLE 5. Zn concentration in some fruits

Curve	Sample	I_{pic}, mA/cm^2	Concentration, mg/L
2.	Raisins	-0.1414	51.9301
3.	Pears	-0.1045	62.8102
4.	Underbrushs, Gioagiu county	0.0556	110.0401
5.	Underbrushs, Ilfov county	0.1844	148.0506
6.	Rose hips, Baia Mare county	0.3197	187.9500

The results presented above show greater Zn concentration then legally admitted limits: 50 ppm [6].

Determination of Pb content in fruits has been made also with an ion selective electrode. The work electrode was a Model 96-82-ionplus® Sure-Flow® lead electrode. The electrode is filled with solution Thermo Orion, Optimum Results^{TM}B, no.900062.

The standard solution was 0.1M $Pb(ClO_4)_2$ (Merck Co.) treated as follows:

- a mixture methanol-formaldehyde is prepared by adding 3 drops of 37% formaldehyde to 1 liter reagent grade methanol; this reagent is to be added in 50:50 ratio to all samples and standards to decrease solubility and retard oxidation of pellet;
- an ionic strength adjustor (ISA) is obtained by adding 80.25 g reagent grade $NaClO_4 \cdot H_2O$ in 100 ml volumetric flask; add first 50 ml distilled water to dissolve solid and then dilute to mark;
- add 2 ml of ISA to 50 ml standard or sample and 50 ml mixture methanol-formaldehyde prepared as above; ISA was used for all lead measurements.

In the direct measurement procedure, a calibration curve is defined by the Nernst relation:

$$E=E^0 + 0.059 \cdot \lg[Pb^{2+}]/n \quad (1)$$

The electrode potential of standard solutions were measured and plotted on the linear axis against their concentrations on the log axis (Fig.7.) [7]. The samples have been preparated by burning to ash and take again with ultrapure Millipore water to 25 mL flascks.

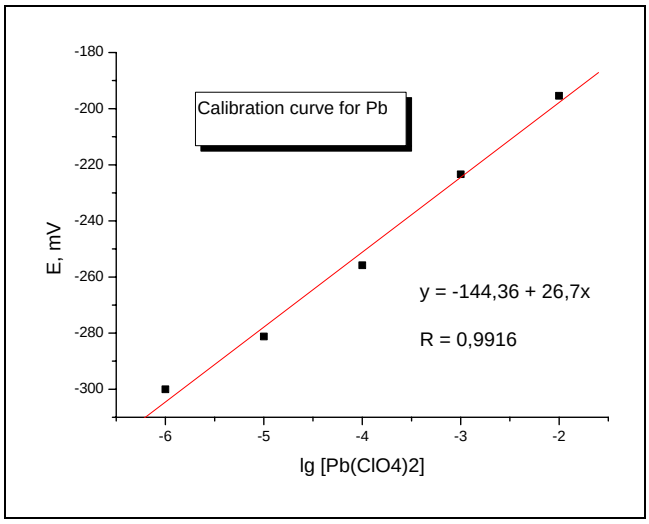

Fig. 7. Calibration curve for Pb determination with ion specific electrode

TABLE 6. Determination of Pb concentration with ion specific electrode

No.	Sample	Pb content, ppm
1.	Pears (fresh)	<1
2.	Apples (fresh)	<1
3.	Bananas (fresh)	<1
4.	Raisins (dried)	<1
5.	Rose hips, Baia Mare county (dried)	<1
6.	Underbrushs,Cluj county (dried)	<1

Lead registered concentrations by this method are under 1 ppm.

4. Conclusions

The environment pollution with heavy metals (Cr, Cu, Ni, Pb, Zn, Al, As, Hg, etc.) is due mainly to the activity of humans. High quantities of these metals can be toxic for all organisms. Still, some of them, called microelements, are necessary as components of enzymes or other proteins involved in major metabolic pathways.

The entry of heavy metals from the polluted environment in fruits and plants is influenced by different factors and stopped through several mechanisms. Their presence can have effects on different physiological processes: photosynthesis, respiration, transpiration, cell membrane permeability. Using heavy metal contaminated vegetal products in alimentation can have important effects on short or long terms, depending on the intensity and action period of the polluting factor.

The fresh fruits may contain max. 0,5 mg/kg As, 0,05 mg/kg Cd, 0,5 mg/kg Pb, 5 mg/k Zn, 5 mg/kg Cu, 0,05 mg/kg Hg. The determinated metals contents in examinated fruits are situated in legally admitted limits of Ordinul M.A.P.P.M. nr.756/1997 and Ordinul Ministrului Sănătății 276/1998, published in M.O. al României nr. 268/11.06.1999 [6].

The presented analyses methods offer the possibility to monitoring the content of heavy metals in fresh and dried fruits. The proposed electrochemical methods are safely and faster, but they are recommended for higher heavy metals concentrations (10^{-5} mol/L). From studied fruits some 25% errors of heavy metals contents have been registered because in fruits their concentrations are small.

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