THE ASSESSMENT OF Cd, Zn, Pb, Cu AND VITAMIN C IN PEACHES

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Scopul acestui studiu a fost determinarea cantitativă a ionilor metalici de Cd, Zn, Pb, Cu şi a vitaminei C (acid ascorbic) din speciile de piersici Prunus persica L. Cd, Zn, Cu şi Pb, recunoscute drept potenţiale elemente toxice, au fost determinate prin spectrometrie de absorbţie atomică în flacară (FAAS) după mineralizarea probei cu acid azotic şi apă oxigenată. Acidul ascorbic a fost determinat printr-o metodă nouă: HPLC/DAD (Cromatografie de lichide de înaltă performanţă cuplată cu detector cu reţea de diode).

The aim of this study was to quantitatively analyse the level of Cd, Zn, Pb, Cu and of vitamin C (ascorbic acid) in Prunus persica L. (peach) fruits. The peaches used in this work were collected from an unpolluted area outside Constanta city. The potential toxic elements Cd, Zn, Cu, and Pb were quantified by flame atomic absorption spectrometry (FAAS) after the chemical mineralization of the samples using nitric acid and hydrogen peroxide. Ascorbic acid was determined using a new method: High Performance Liquid Chromatography coupled with Diode Array Detector (HPLC/DAD).

Keywords: peach, vitamin C, Cd, Zn, Pb, Cu, HPLC/DAD, FAAS

1. Introduction

Peaches are classified as Prunus persica L. and belong to the Rosaceae family. A medium fruit should have 75 g, 30 Cal, 7 g of carbohydrate (6 g sugars and 1 g fiber), 1 g of protein, 50µg copper, 20µg zinc and 8% of the daily value for vitamin C (6.6mg% is the normal vitamin C content in peach) [1-5]. Peaches can be red, pink, yellow, white, or a combination of these. On one side of the fruit there is a distinctive vertical indentation [6].

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Monitoring of heavy metals in fruits is an important issue with regards to human health. The plants usually translocate larger quantities of metals to their leaves rather than to their fruits or seeds. Plants can contain metallic species taken from the soil, water and air. Some of them do not tolerate higher levels of metals but they hyper-accumulate Cd, Zn or Cu [7]. Lead is a naturally occurring element, but at levels higher than 0.5–0.8 mg/mL in the blood it causes different faults. Cadmium is a heavy metal, carcinogen to humans and toxic to plants. Cadmium ions are easily distributed in the liver and kidneys after ingestion. Copper is an essential trace element in plants and animals, but its toxicity is a much overlooked contributor to many health problems such as anorexia, fatigue, premenstrual syndrome, depression, anxiety, migraine, headaches, allergies, childhood hyperactivity and learning disorders. Although zinc is an essential requirement for good health, higher amounts could be harmful. Excessive absorption of zinc suppresses copper and iron absorption. The free zinc ion in solution is highly toxic to plants, invertebrates, and even vertebrate fish [8, 9].

A number of studies have shown heavy metals as important contaminants of the fruits [7-9].

The content of vitamin C in fruits is considered as an index of their health-related quality. Thus, the interest in the ascorbic acid (AA) has greatly increased in food analysis [10, 11]. Fruits are one of the richest sources of ascorbic acid, other antioxidants and produce-specific bioactive compounds. A general consensus from health experts has confirmed that an increased dietary intake of specific bioactive compounds found in some fresh product types may protect against oxidative damage and reduce the incidence of certain cancers and chronic diseases. [5]

The objective of present work was to investigate the presence of Cd, Cu, Zn, Pb and vitamin C in peaches collected from an unpolluted area outside Constanta city.

2. Experimental

2.1. Reagents and solutions

Analytical grade chemicals (HNO_3>69%, hydrogen peroxide 30%, Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} certified analytical standard solutions of 1000 mg/L purchased from Merck and Fluka) were used. The standard ascorbic acid was purchased from Fluka (Buchs, Switzerland). HPLC-grade methanol and acetic acid were purchased from Merck (Darmstadt, Germany). Solutions of 20 g/L methaphosphoric acid, 200g/L trisodium phosphate from Fluka (Buchs, Switzerland) were also used. All chemicals were of analytical reagent grade (purity > 98%) and used without further purification. All the solutions were prepared using ultrapure water from a Milli-Q Elix3 system. The solvents were filtered on 0.2μm membranes (Millipore, Bedford, MA, USA) and degassed before use.

2.2. Sampling and sample preparation

A homogeneous sample of fresh peaches from an unpolluted area outside Constanta city (Lazu village situated at 8 kilometers away from Constanta) was collected in 2011. In order to determine metal concentrations, the samples were washed with deionized water, dried and homogenized. 0.5-0.9 grams of each dry sample was subjected to digestion with 8 mL HNO_3 65% and 10 mL H_2O_2 25% at 150^oC in a Digesdhal device provided by Hach Company. After the complete digestion, the samples solution was filtered and transferred to a 50 mL volumetric flask with deionized water.

To analytically determine the ascorbic acid content, 5 g of homogenized material were homogenized with 10 mL metaphosphoric acid in a volumetric flask; then pH was adjusted to 2.5 -2.8 with trisodium phosphate. The homogenate was sonicated and centrifuged at 40 C and 5000 rpm for 10 min, using a Hettich Universal 320 centrifuge. The supernatant was filtered through 0.45-lm filters (Chromafil Xtra PFTE-45/25 particle size of 0.45 mm). Finally, samples were diluted with mobile phase, kept in dark colored vials and analyzed immediately after extraction.

2.3. Sample analysis and instruments

Cd, Cu, Zn, and Pb were determined by flame atomic absorption spectrometry (FAAS) in air/acetylene flame using an aqueous standard calibration curve (Fig. 1).

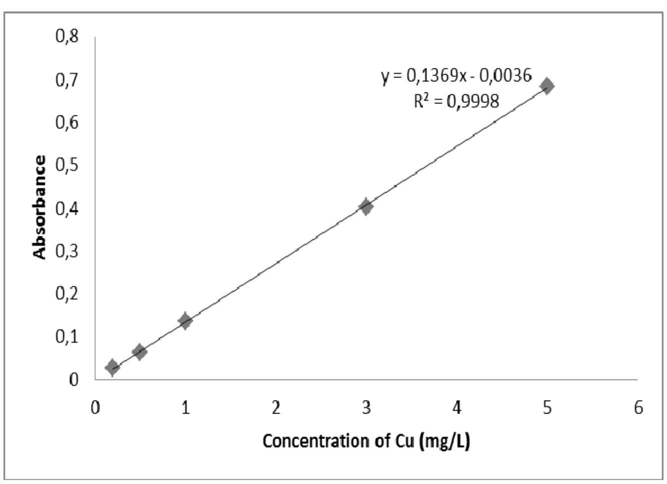

Fig. 1. A typical competitive curve for Cu

Analyses were made in triplicate and the mean values are reported. The FAAS instrument used was a GBC Avanta Flame Atomic Absorption Spectrometer. Concentrations of metals were measured using monoelement hollow cathode lamp.

The main characteristics of the equipment for metals determination are presented in table 1. Technical data of the vitamin C measurements are the following: HPLC Agilent 1200, with quaternary pomp, DAD, thermostat, degassing system, autosampler; chromatographic column C18, 250mm × 4.6mm; 5µm (Zorbax XDB or equivalent). Mobile phase consisted of (A) 0.1% acetic acid (v/v) in HPLC-grade/water and (B) methanol. The injected sample volume was 20µL at a flow rate of 0.25 mL/min. DAD detection was performed at 245 nm, the specific wavelength for ascorbic acid. Solutions of the standards of six different concentrations were analyzed and the calibration curves (y = ax + b) were constructed by plotting the peak area ratios (y) of analyte versus the respective concentrations. Analyses were made in triplicate and the mean values are reported.

Table 1

Technical characteristics of metal determination using FAAS

Ion	λ (nm)	Optimal concentration range (mg/L)	Gases flow (L/min)	
			Air	Acetylene
Cd^{2+}	228.8	0.01– 0.5	10.0	1.1
Cu^{2+}	324.7	0.2 – 5.0	10.0	1.2
Pb^{2+}	217.0	0.2 – 2.0	10.0	1.2
Zn^{2+}	213.9	0.2 – 2.0	10.0	1.2

3. Results and Discussions

The assessment of metals contents in peach represents one of the factors in the evaluation of their quality taking into consideration that some plants may accumulate particular metals, especially cadmium.

Cadmium and lead are the heavy metals of most concern because they may affect human health. Copper and zinc represents necessary microelements which could be harmful only if their concentration is too high.

Table 2 presents the average values of Cd, Cu, Zn and Pb concentrations in peaches. The highest metal concentration found is copper (2.47 mg/Kg) while lead was not detected in fruits assessed. The recommendable maximum limits for metals in fruits are 0.05 mg/Kg for Cd, 0.1 mg/Kg for Pb and 5 mg/Kg for Cu and Zn [12, 13].

Table 2

Heavy metals occurrence in peaches

Heavy metal	Cd (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)	Pb (mg/Kg)
Heavy metal in peach sample	0.0228	2.4700	1.5236	ND
Heavy metal accepted maximum limit	0.05	5	5	0.1

It can be noticed that the values of cadmium, cooper, lead and zinc concentrations in peaches are lower than the recommendable maximum limit of these metals in fruits. The content of Cd, Zn and Cu in peaches grown in an orchard in Constanta area was determined in a previous study [2]. In that study samples were collected in 2004. It can be observed that the Cu content is higher in 2010 than in 2004 (<LD Cu) and Cd and Zn levels are lower in the present study (0.43ppm Cd; 6.67ppm Zn in 2004 study). Zn is an essential element for plants and animals, just like Cu, but a slight increase in its levels may interfere with physiological processes. Sufficient Zn is essential to neutralize the toxic effects of Cd.

The value of cadmium concentration (0.0228 mg/kg) is below the recommendable maximum limit. Because the orchard is close to the highway and the town, the fuel combustion can be the major pathway through cadmium is released into the atmosphere. Plants can contain the metals from soil, water and air. High heavy metals uptake can also be found in some regions due to certain properties of soil (pH, organic matter) which favor the mobility of metals in soil and their availability of metals in soil and their availability to plants [7].

The heavy metals not only affect the nutritive values of fruits and vegetables but also have deleterious effect on human beings who eat them. In a previous study, the Egiptian Radwan and Salama [14] reported that the level of Pb and Zn in peaches was 0.38mg/kg and 6.22mg/kg respectively.

However, Cd (0.01mg/kg) and Cu (1.46mg/kg) levels were lower in Egypt [16] than those we found in our similar food samples. On the other hand, the concentrations of Pb and Zn we found in peaches were lower than those detected in Egypt.

The content of vitamin C in peaches was determined by HPLC/DAD method. The vitamin C is a strong antioxidant and is required for the synthesis of dopamine, noradrenaline and adrenaline in the nervous system or in the adrenal glands. The physiological functions of vitamin C are largely dependent on its oxido-reduction properties [15]. Under the previously described chromatography conditions, ascorbic acid eluted at 6.52 min, (Fig. 2 – from standard ascorbic acid and Fig. 3 – from peach sample).

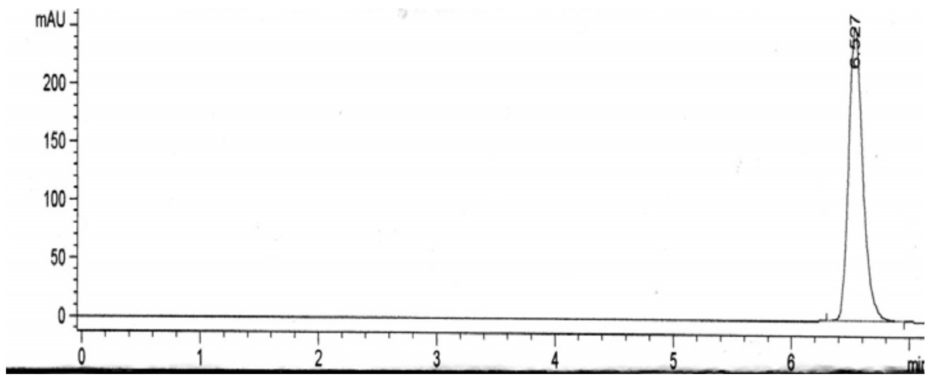

Fig. 2. The Chromatogram of Standard Ascorbic Acid solution of concentration 10 μg/mL

Good linearity was achieved in the concentration range 0.5-15 μg/mL for ascorbic acid with significant correlation factor ($R^2 \geq 0.997$).
The method was evaluated by determination of the correlation coefficient and intercept values (Table 3). LOD value obtained was 0.40 mg/L.

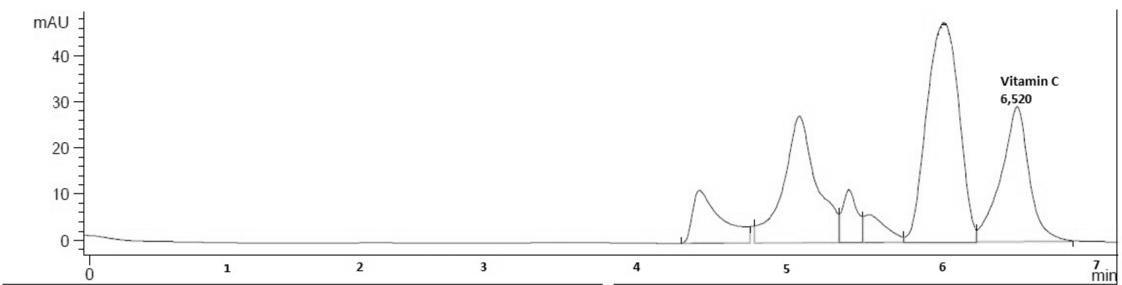

Fig. 3. The Chromatogram of ascorbic acid from peach sample.

15.12mgAA in100g peaches obtained in 2004 (only vitamin C was determined in 2004) is higher than the value obtained in the present research (5.46mgAA/100g product); both of them are in good agreement to the literature data (5-15mgAA/100g product) [1, 2, 5]. This large decrease in vitamin C content in peaches is probably due to air pollution that increases each year.

Findings of Chistyakov et al. [16] show that the ascorbic acid, a reducer, can potentiate the toxic effects of heavy metals if the results of their study were interpreted in terms of modern concepts concerning the mechanisms of reactive oxygen species (ROS) action.

Table 3

Calibration data and LOD of ascorbic acid using HPLC/DAD method

Compound	Retention time (min)*	Equation	LOD (μg/mL)	Range (μg/mL)	R^2
Ascorbic acid	6.54±0.01	A = 214.2c – 26.6	0.40	0.5-15	0.9979

At a zinc concentration of 0.1mg/L, ascorbic acid exerted a pronounced protective effect.

The strongest potentiation effect was observed in the case of copper ions, which caused almost 100% inhibition of bacterial luminescence at a concentration of only 0.05 mg/L (20 times lower than the minimal effective concentration in the absence of ascorbic acid) [16].

4. Conclusions

This paper presents original data concerning the Cd, Cu, Zn, Pb and vitamin C content in peach fruits. Cadmium and lead are the heavy metals of most concern because they may affect human health. Copper and zinc represent the necessary microelements which could be harmful only if their concentration is too high.

It can be noticed that the values of cadmium, cooper, lead and zinc concentrations in peaches are lower than the recommendable maximum limit of these metals in fruits.

The content of vitamin C in fruits is considered as an index of their health-related quality. Thus, the interest in the ascorbic acid (AA) has greatly increased in food analysis. The vitamin C content in peaches (5.46mgAA/100g product) is in good agreement to the literature data. The ascorbic acid, a reducer, can potentiate the toxic effects of heavy metals.

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