The influence of heavy metal content on superoxide dismutase and glutathione peroxidase activity in the fish meat originated from different areas of Danube river

CRISTIANA DIACONESCU, LAURA URDES, HANGAN MARIUS, DANIELA IANITCHI, DANA POPA
University of Agronomical Sciences and Veterinary Medicine, Faculty of Zootechnology, Bucharest, Romania -

Abstract

Heavy metals (Pb, Cu, Cd, Zn, Hg) and their compounds are considered cancerous for humans and animals. This is why the quantitative determination of such substances in food products and mainly in fish is a problem of great importance. For this reason, the present paper intends to biochemically investigate 5 sweet water fish species (bream, mackerel, carassius, tench, perch) , originated from the area of Sulina Arm and auxiliary canals, form a heavy metal content point of view, using atomic absorption spectrophotometry, and from the point of view of the enzymatic activity of superoxide dismutase (SOD) and of glutation peroxidase (GPx), using photocolorimetry. The results of the investigation proved that under the point of view of the copper, zinc and mercury content, all values are between normal values stated in the Sanitary Veterinary Norms for all the fish species investigated, with the note that the mercury level is close to the upper bound of this scale. Cadmium and mercury have not been put into evidence. The SOD and GPx activity is significantly low for the samples of fishes in which the mercury content was at the higher bounds (bream), being known the fact that heavy metal inactivate this enzyme.

Keywords: superoxide dismutase, glutathione peroxidase, heavy metals, atomic absorption spectrophotometry;

Introduction

Fish meat has a special nutritive value due to its content of high quality proteins, fats rich in poly-unsaturated fat acids with a high efficiency in the human body, vitamins (especially A and D) and a high level of minerals (iron, phosphor, potassium, magnesium)[1].
Aquatic life environment makes it very easy to contaminate the fish meat with harmful heavy metals that reach the river waters through industrial residual waste, metals like: plumb, cadmium, mercury, copper, zinc. Unfortunately, some heavy metals and their compounds are considered cancerous for humans and animals, and this is why, knowing their contented and the acceptable limit of presence in fish meat is a problem of national health for humans [2,3,6].
The present paper aims at determining the content of Pb, Cu, Cd, Zn and Hg from the meat of some sweet water fish species, originated from the area of Danube's Sulina Arm and nearby canals, known being the fact that the water of this river collects a series of affluent rivers that cross numerous industrial areas. Also, the enzymatic activity of SOD and of GPx was determined in these fish meat samples [7].

Materials and method

Biochemical determinations were made on around 50 individuals of each fish species (bream, mackerel, carassius, tench, perch), gathered during February-March 2007, and mackerel, gathered during March-May 2007. The biochemical investigations aimed at determining the heavy metal content by atomic absorption spectrophotometry, using a PERKINELMER-USA spectrophotometer.

In order to determine the Pb, Cd, Cu and Zn, tissue sample were taken from the homogenized fish with a weight between 12.80 and 15.65 g, which were burned (at 450°C±25°C, and the ash was brought to graded balloon with HCL diluted 1:4 with unionized water [5]. Unionized water was used as control sample.

For determining the Hg, the tissue sample was subjected to wet mineralization with concentrated nitrogen acid and oxygenated water, and brought to a graded balloon of 50 ml. Also, standard solutions were separated for all researched chemical elements.

The metal content was expressed in mg at 1 kg product (ppm) and was calculated with the following formula:

$$M(mg/kg) = \frac{C \bullet V \bullet 1000}{m \bullet 1000}$$

where M=the researched metal content; C= the concentration read on the balloon, in micrograms; V= the total volume of the solution that contains the control sample; m=the mass of the analyzed sample in grams;

The determination of SOD enzymatic activity was based on the enzyme's capacity to inhibit the generation of superoxide anions. The method was based on the inhibition of spontaneous adrenaline degradation in adenocrome by SOD. A unit of enzymatic activity was represented by an inhibition of 50%.

The GPx activity was determined in a spectophotometric manner, using the method described by Paglia and Valentine (1967)[4].

Results and discussions

The variation of Pb, Cu, Cd, Zn and Hg in Bream and Mackerel meat is presented in table 1.

Table 1. The variation of Pb, Cu, Cd, Zn and Hg content in Bream and Mackerel meat.

No.	Product type	U.M	Chemical element				
			Pb	Cu	Cd	Zn	Hg
1.	Sample1 Bream	mg/kg product	Not determined	0.78-0.82	Not determined	6.38-7.08	0.5789
2.	Sample1 Bream	mg/kg product	Not determined	0.54-0.59	Not determined	6.77-6.80	0.5189
3.	Sample1 Mackerel	mg/kg product	Not determined	0.80-0.85	Not determined	6.17-6.19	0.5612
4.	Sample1 Mackerel	mg/kg product	Not determined	0.83-0.84	Not determined	6.18-6.38	0.4673

By analyzing these parameters we can observe a lack of plumb and cadmium from both species that were investigated. The content of copper, zinc and mercury registers values comparable to the ones included in the Sanitary Veterinary Norms regarding the values acceptable for heavy metals in fish meat.

Still, we must observe that the mercury content is placed at the superior acceptable limit (between 0.4673-0.5789 compared to the value of 0.5 stated by the Sanitary Veterinary Norms) which suggests a slight pollution with this metal in the Sulina Arm waters.

Between the two species, the Mackerel meat has a lower zinc content, as it is known that it migrates to see waters.
Table two presents the measured content of the same heavy metals in Carassius, Tench and Perch meat.

Table 2. The variation of Pb, Cu, Cd, Zn and Hg content in Carassius, Tench and Perch meat.

No.	Product type	U.M	Chemical element				
			Pb	Cu	Cd	Zn	Hg
1.	Sample1 Carassius	mg/kg product	Not determined	0.57	mg/kg product	10.54	0.14
2.	Sample2 Carassius	mg/kg product	mg/kg product	0.86	mg/kg product	12.9	0.16
3.	Sample1 Tench	mg/kg product	mg/kg product	1.05	mg/kg product	4.91	0.14
4.	Sample2 Tench	mg/kg product	mg/kg product	0.59	mg/kg product	4.30	0.15
5.	Sample1 Perch	mg/kg product	mg/kg product	0.26	mg/kg product	6.36	0.35
6.	Sample2 Perch	mg/kg product	mg/kg product	0.37	mg/kg product	6.13	0.29

As for the first species, in the case of carassius, tench and peach, the plumb and the cadmium were not detected. For Carassius, a high level of zinc was detected (almost double than in Mackerel, Tench, Bream or Peach). The copper and the mercury content fits in the acceptable values stated by the Sanitary Veterinary Norms. It seems that in the waters of the Sulina Arm or in the nearby canals there is a high quantity of zinc, originated from different pollution sources and from all studied types of fish, the carassius is the most sensitive to this heavy metal.

Table 3. The variation of SOD and GPx enzymatic activity in the meat of Bream, Mackerel, Carassius, Tench, Perch.

No.	Product type	SOD (U/mg)	GPx(mU/mg)
1.	Sample 1 Bream	0,9	5,5
2.	Sample 1 Mackerel	1,9	7,8
3.	Sample 1 Carassius	3,1	9,8
4.	Sample 1 Tench	3,5	10,3
5.	Sample 1 Perch	3,2	10,2

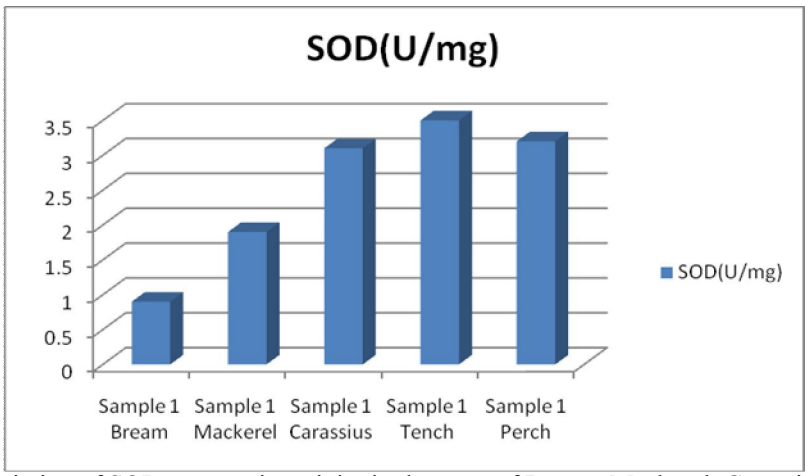

Figure 1. The variation of SOD enzymatic activity in the meat of Bream, Mackerel, Carassius, Tench, Perch.

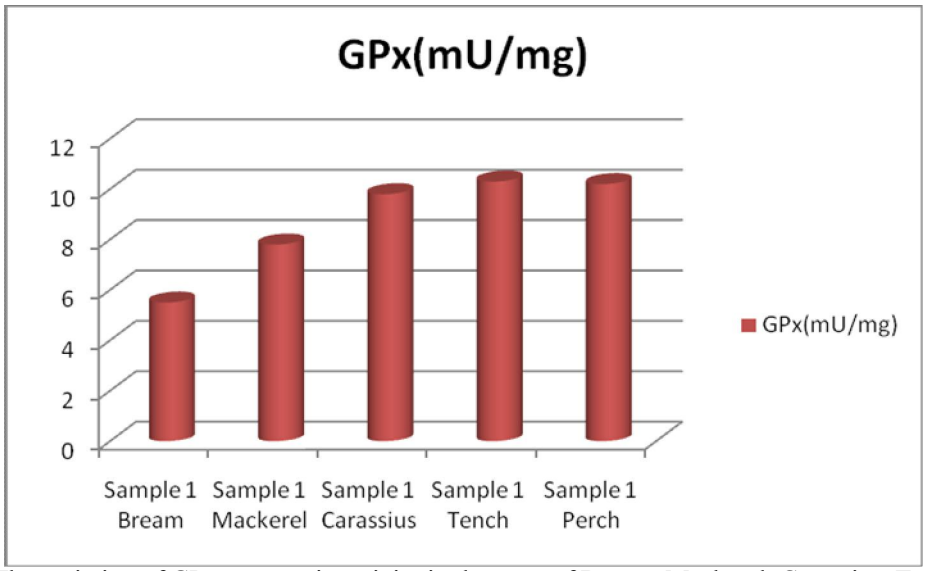

Figure 2. The variation of GPx enzymatic activity in the meat of Bream, Mackerel, Carassius, Tench, Perch.

By analyzing these values we can observe that the SOD activity was minimal in the case of the bream samples and even lower in the case of the mackerel sample, where mercury content was detected at the higher bound of acceptability. This is the result of the fact that the mercury acted as an inhibitor for the enzyme. The GPx activity varied between 5,5-10,3 mU/mg and the smallest values were registered in the bream and mackerel samples.

Conclusions

1. From all the heavy metals that were investigated, plumb and cadmium have not been present in measurable quantities.
2. The Carassius meat contains the highest zinc content and this indicates a sensibility of this species to this polluting metal.
3. In the case of Mackerel and Bream meat, the mercury content is situated at the upper acceptability limit.
4. The copper content is in the acceptability limits for all investigated species.
5. Bream and Mackerel meat register the highest level of SOD and GPx enzymatic activity.
6. Mercury had an inhibitive action on the antioxidant enzymes.

References

1. Banu, C. – Aliments, alimentation and health – Ed. Agir, Bucharest 2005.
2. Ghergariu S. and colab. – Manual for veterinary clinical laboratory – Ed. All, Bucharest 2000.
3. Huan, Wang. Eaves. H., - Food chemistry, 103, 1395-1402, 2007.
4. Paglia D. E., Valentine W. N. – J. Lab. Chim. Med. – Studies on the quantitative and qualitative characterization of erythrocyte gluthation peroxidase, 70, p. 158-169, 1967.
5. Popescu, N., Popa G., Stanescu V., - Physical-chemical laboratory determinations for animal origin food products – Ed. Ceres, Bucharest 1986.
6. Sanitary Veterinary and Food Safety Norms – Order 97/26 November 2005.
7. Stanescu V., - Food hygiene and control – Ed. Romania Tomorrow Foundation, Bucharest 1998.