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MICROBIOLOGICAL QUALITY OF FISH MEAT AND THE EFFECT ON THE HEAVY METALS CONTENTS

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ABSTRACT

In this study the microbiology quality and heavy metals content in fish meat of common carp (*Cyprinus carpio*) from fishpond of Budmerice (south-western Slovakia) was observed. In the experiments the total bacteria counts on the Glucose Tryptone Yeast agar after 72 hours cultivation at 30 °C, number of coliforms bacteria on the Violet Red Bile agar after 48 hours after 37 °C and counts of mesophilic anaerobic sporulating bacteria on the Meat Peptone agar after 72 hours after 25 °C were determined. The contents of selected heavy metals (Fe, Mn, Zn, Cu, Co, Ni, Cr, Pb, Cd) in fish meat using by AAS were determined. Evaluation of fish meat microbiological quality was determined in correspondence with microbiological standards in total bacteria counts and number of coliforms. The count of mesophilic anaerobic sporulating bacteria in monitoring samples exceeded standard values. Also the number of pathogen bacteria as *Staphylococcus aureus, Salmonella sp., Vibrio parahemolyticum* was monitored. Our results describe a good microbiological quality of fish meat samples and all values were in accordance with Codex Alimentarius of Slovak Republic. From the point of view of heavy metals contents, the fish meat quality corresponds with hygienic limits, except lead content with 40 % of exceeding samples. The positive and negative correlations between heavy metals contents and count of mesophilic anaerobic sporulating bacteria were detected, but without significant differences (P>0.05).

Key words: microbiological quality; heavy metals; coliform bacteria; mesophilic anaerobic sporulating bacteria; fish; *Cyprinus carpio*

INTRODUCTION

Meat, muscle and visceral organs of healthy, freshly caught fish are usually sterile but the skin and alimentary tract carry substantial numbers of bacteria. Reported numbers on the skin have ranged 10^2-10^7 cfu.cm^{-2} and 10^3-10^9cfu.g^{-1} in gills and the gut. These are mainly Gram-negatives of the genera *Pseudomonas, Shewanella, Psychrobacter, Vibrio, Flavobacterium* and *Cytophage* and some Gram-positives such as coryneforms and micrococci (Adams and Moss, 2002).

Iron is one of the most abundant metals on the earth and is essential to almost all organisms (Bury and Grosell, 2003). Manganese is a transition metal. Similar to the other transition metals, manganese occurs in a number of states in the environment. In surface waters is manganese a micronutrient, but elevated concentrations are toxic to fish (Krauskopf and Bird, 1995). For aquatic organisms, zinc is both an essential nutrient and environmental contaminant. The intestine is potentially the most important rout of zinc absorption, yet little is known regarding this uptake pathway for zinc in fish (Glover and Hogstrand, 2003). In contrast to copper and iron, zinc does not form free radical ion, and in fact has antioxidant properties (Powell, 2000). The diet is the major source of copper for fish under optimal growth conditions (Kamunde et al., 2002). High concentrations of copper in combination with low pH are believed to be fatal to fish (Cogun and Kargin, 2004). Nickel inhibits a number of enzymes and is bound to various proteins, including metallothioneins and albumins (Dallas and Day, 1993). Freshwater fish are exposed to nickel directly through the

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water and through the ingestion of contaminated food and sediments (Dallinger and Kautzky, 1985). In chromium, the valence state plays a significant role in its toxicity (Steihagen et al., 2004). The toxic effect of lead on the biota can be modified by different factors. Generally only the free metal ion is toxic to the ecosystem (Aardt and Venter, 2004). Cadmium is a heavy metal with unknown biological functions in animals (Xie and Klerks, 2004).

The target of this study was to assess level of some microbiological indicators (total bacteria count, mesophilic anaerobic sporulating bacteria and coliform bacteria) and concentration of selected heavy metals in the muscle of common carp (*Cyprinus carpio*).

MATERIAL AND METHODS

Totally 20 individuals of Carp (*Cyprinus carpio*), hailing from the fishpond of Budmerice (south-western Slovakia), were analysed for microbiological quality and heavy metals contents in muscle. Sampling site is showed in the Figure I.

Fig. 1. Sample Site of Analysed Fish

Analysed fish were evaluated by standard methods used in ichthyology (standard length and weight measurements, age determination by scales). After biometric data recording, the samples of fish muscles were obtained from the left side of fish body in dorsal part, without skins and bones. Before analysis the samples were kept at –18 °C. In the laboratory the samples were weighed (2 g) and washed with diluted nitric acid. The digested samples were analysed for the content of iron, manganese, zinc, copper, cobalt, nickel, chromium, lead and cadmium by using an atomic absorption spectrophotometer (AAS) Pye Unicam SP9. Values of monitored heavy metals are presented on a wet weight basis in $mg.kg^{-1}$ and compared with hygienic limits valid in the Slovak Republic. Maximal acceptable values in human food are as follows (in $mg.kg^{-1}$ wet weight): iron – not defined, manganese – not defined, zinc – not defined, cooper – 10.0, nickel – 0.5, cobalt – not defined, chromium – 4.0, lead – 0.2, and cadmium – 0.05.

Quantitative microbiological analysis

Plate diluting method was applied for quantitative CFU counts determination of respective groups of microorganisms in 1 g of substrate.

Gelatinous nutritive substrate in Petri dishes was inoculated with 1 ml of fish meat samples by flushing (total bacteria counts, mesophilic anaerobic sporulating bacteria) and on surface (coliforms bacteria) in three replications. Homogenized samples of meat were prepared in advance by sequential diluting based on decimal dilution system application.

Basic dilution (10^{-1}) was prepared as follows: 5 g of fish meat content was added to the bank containing 45 ml of distilled water. The cells were separated from substrate by shaking on a horizontal shaker (TE III. Chirana, Piešťany) (30 minutes). In this way prepared basic substance was diluted to reduce the content of microorganisms on the level below 300 CFU.

For statistical analysis Anova One Way and Regression Analysis in computer program Statgraphics Plus were used.

RESULTS AND DISCUSSION

In this study the microbiological quality of fish meat was evaluated. Fish meat was correspondent with microbiological standards in total bacteria counts and number of coliforms. The count of mesophilic anaerobic sporulating bacteria of monitoring samples was not exceeded with standards. Also was monitored number of pathogen bacteria as *Staphylococcus aureus, Salmonella sp., Vibrio parahemolyticum*. Our results showed a good microbiological quality of fish meat samples and all

Table 1: Media and cultivation conditions used for enumeration of bacteria (Holt et al. 1994)

Microbial groups	Medium	Length of incubations (h)	Cultivation method	Temperature (°C)
Total bacteria counts	Glucose Tryptone Yeast agar	72	aerobic	30
Coliforms	Violet red bile agar	24	aerobic	37
Mesophilic anaerobic sporulating bacteria	Meat Peptone agar	72	anaerobic	25

values were in accordance with Codex Alimentarius of Slovak Republic.

If fish used for consumption are not eviscerated immediately, intestinal bacteria early contaminate through the intestinal wall and pass into of the intestinal cavity. This process is believed to be aided by the action of proteolytic enzymes, which are from intestines and may be natural enzymes inherent in the fish intestines, or enzymes of bacterial origin from the inside of the intestinal canal, or both (Andreji et al., 2006).

In a study of 159 gramnegative isolates from spoiled freshwater fish with total aerobic flora of about 10^8 cfu.g^{-1}, about 46 % were pseudomonades and 38 % were *Shewanella* spp. (Jay, 2000)

Characteristics of analysed fish are listed in Table 3. Contents of monitored heavy metals in fish meat are listed in Table 4. Correlations between heavy metals contents and count of mesophilic anaerobic sporulating bacteria are listed in Table 5.

The hygienic limits of heavy metals in fish muscle for human food are defined only for copper, nickel, chromium, lead and cadmium. The detected values of copper, nickel, chromium and cadmium content in fish muscle had exceeded these limits. In the lead content, the overload of hygienic limit in 40 % of all analysed samples was recorded. The overload reached 1.01 – 1.27 times higher values. From this aspect the analysed fish muscle is unacceptable for human food. Comparable concentration of these metals in fish muscle from some

Table 2: Number of fish meat microorganisms

Number of sample	Coliforms bacteria	MASM	Total bacteria counts
1.	-	615	-
2.	-	365	-
3.	-	390	-
4.	-	330	-
5.	-	210	-
6.	-	425	-
7.	-	540	-
8.	-	425	-
9.	-	180	-
10.	-	510	-
11.	-	120	-
12.	-	760	-
13.	-	1854	-
14.	-	510	-
15.	-	920	-
16.	-	785	-
17.	-	735	-
18.	-	880	-
19.	-	900	-
20.	-	685	-

Table 3: Characteristics of analysed fishes

Species	N	Age	SL (mm)		BW (g)	
			Mean ± SD	Range	Mean ± SD	Range
Carp	10	3	387.3±16.06	360–412	1652.8±204.75	1354–2035
Carp	10	2	315.2±23.48	278–345	892.3±225.73	635–1283

Table 4: Contents of monitored heavy metals (in mg.kg^{-1} wet weight)

	Fe	Mn	Zn	Cu	Co	Ni	Cr	Pb	Cd
Mean	7.81	0.24	5.10	0.42	0.11	0.11	0.12	0.19	0.03
SD	2.98	0.05	1.34	0.19	0.02	0.01	0.02	0.03	0.01
Min	3.47	0.14	3.47	0.24	0.06	0.09	0.08	0.14	0.02
Max	13.80	0.33	9.52	0.93	0.15	0.14	0.17	0.25	0.05

Table 5: Correlation coefficients of relations between heavy metals contents and count of mesophilic anaerobic sporulating bacteria (MASM)

	Fe	Mn	Zn	Cu	Co	Ni	Cr	Pb	Cd
[1]MASM	0.218^{-}	0.097^{-}	-0.097^{-}	0.002^{-}	0.020^{-}	-0.377^{-}	0.246^{-}	0.246^{-}	0.056^{-}

$^{-}$P>0.05

ponds and water reservoirs are presented also by other authors (Svobodová et al. 2002, Stráňai and Andreji 2007, Andreji and Stráňai 2007, Andreji et al. 2006).

Also correlations between content of monitored heavy metals and count of mesophilic anaerobic sporulating bacteria were estimated. Generally, monitored heavy metals showed positive correlations to the count of mesophilic anaerobic sporulating bacteria, but without statistically significant differences (P>0.05). Zinc and nickel showed negative correlations also without statistically significant differences. Our results target, that the count of mesophilic anaerobic sporulating bacteria increase with increasing content of iron, as well as chromium and lead (P>0.05). On the unlike other, the counts of mesophilic anaerobic sporulating bacteria decrease with increasing content of nickel (P>0.05). The content of copper, cobalt and cadmium on the count of mesophilic anaerobic sporulating bacteria showed indifferent or like-indifferent effect.

CONCLUSION

MASB counts exceeded maximal acceptable value in all analyzed samples of fish meat. The value of 0 CFU.g^{-1} for pathogens (*Salmonella sp.* and *Vibrio parahaemolyticus*), and coliform bacteria, which are indicators of fecal pollution can be viewed positively. From the point of view of heavy metals contents, the fish meat quality corresponds with hygienic limits, except lead content with 40 % of exceeding samples. Because of the importance of fish in the human consumption, it is necessary to monitor fish raised for human consumption regularly to ensure continuous food safety.

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