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Effect of Boiling Water Treatment for Mitigation of Toxic Recalcitrant Heavy Metal Residue in Fish Commonly Consumed in West Bengal, India

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ABSTRACT

The major goal of this study is to identify and quantify the hazardous recalcitrant heavy metals present in three different fish. Experiments are conducted to study the identification and quantification of iron (Fe), lead (Pb), copper (Cu), cobalt (Co), nickel (Ni), chromium (Cr), and zinc (Zn) on three species of fish namely, Rohu (Labeo rohita), Tilapia (Oreochromis mossambicus) and Prawn (Penaeus monodon) using atomic absorption spectrophotometer. The results revealed that heavy metals, lead (Pb) and chromium (Cr) are present in toxic limits whereas all other heavy metals selected for the study are within the permissible limits. However, after giving boiling treatment, residual concentration of heavy metal lead (Pb) reduced from 3.35 to 2.4 ppm in Rohu fish, 11.16 to 2.17 ppm in Tilapia fish, and 2.74 to 1.5 ppm in Prawn (Chingri) fish. Similarly, residual concentration of heavy metal chromium (Cr) reduced from 25.4 to 5.7 ppm in Rohu fish, 29.7 to 17.8 ppm in Tilapia fish, and 18.6 to 6.4 ppm in Prawn (Chingri) fish, which is within the permissible limits. Boiling treatment is found to be an effective method for reducing the concentrations of recalcitrant heavy metals in fish fillets.

Key words: *Heavy metals, Atomic absorption spectrophotometer, Boiling treatment, Recalcitrant, Residual limits.*

INTRODUCTION

Fish is an aquatic animal found abundantly in all natural waters. It is a valuable source of food which has been used by man from antiquity. Fish is an excellent source of proteins, omega-3 fatty acid, vitamins and minerals which are essential for maintaining good health[2]. As far as key minerals are concerned, fish meat is a good source of copper, sulphur and phosphorous[15]. As an important, cheap and readily available source of protein, fish is expected to be a chief food item for national food security, meeting the nutritional needs of the increasing population. In India, Fish is the major source of protein for over one-third of the population especially for the rural poor in coastal areas. The per capita consumption of fish in India is 9.8 kg against the recommended intake of 13 kg.

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This is probably because the marine fish production has been stagnating over recent years[5]. In India also fish production by aquaculture has doubled to about 2.0 MT during the last decade[4]. India has a coastline of about 8,118 km and the continental shelf bordering the Indian coast has an area of about 2.6 million sq.km in which infinite varieties of fishes are found[17]. Total global fish production is about 5.9 mt which includes 36% contribution from India[13,14.] World aquaculture output has been growing at about 11% per annum and has reached 33.3 MT in the year 1999[9]. India's marine product exports boosted to 0.38 MT during 1996-97 earning a foreign exchange worth Rs 4,121 crores[6].

Fish is a much preferred food item in many places in India like West Bengal, Odisha, and Kerala etc. and in certain regions where livestock is relatively scarce. Though it is a good source of nutrition but it may contain high levels of environmental contaminants like heavy metals, such as Pb, Cr, Cu, Zn, Co etc. which may cause dangerous diseases and even death. Heavy metals come from the industrial effluents as well as from the agro-chemicals used in crop production which ultimately find their way into a variety of water bodies[11]. As a result, these water bodies become heavily polluted and fishes grown in such polluted waters absorb these heavy metals and often bioaccumulation reaches so high level that it may cause toxicity to the consumers. Accumulation of metals in different tissues of fish varies, depending upon the source of metal uptake, either waterborne or dietary exposures. Various fish species showed the ability to accumulate and concentrate cadmium and iron manifolds those found in their natural habitats. Consumption of such fishes may cause several lethal diseases such as "Ouch-Ouch" disease, characterized by extreme bone pain due to osteomalacia commonly found in Toyama city of Japan[12]. Further, intake of Arsenic has also been reported to increase the risk of cancer in the liver, bladder and lungs[10]. That apart, ingesting very high levels of cadmium severely irritates the stomach, leading to vomiting and diarrhea. Similarly, exposure to high lead levels can severely damage the brain and kidneys and ultimately cause death. According to the report on "Total Diet Study in West Bengal" submitted to World Health Organization in 2009, three species of fish namely, *Rohu* (*Labeo rohita*), *Tilapia* (*Oreochromis mossambicus*) and *Prawn* (*Penaeus monodon*) were abundantly available and mostly consumed in West Bengal[8], where agro-chemicals are extensively used for crop production and industrial and urban effluents are directly discharged into the water bodies frequently without any prior treatment. In spite of the above risk, fish continues to be the proteinaceous staple food in West Bengal. Therefore, it was considered necessary to ascertain the safety status of fish particularly with respect to and to find out ways and means of minimizing the toxic heavy metals residues in fish. In view of the above, the present study was undertaken with the following objectives: to find the safety status of fish by quantifying the toxic heavy metals residues, and to find out simple, easy, cheap and effective methods of mitigating toxic heavy metal residues in fish.

MATERIAL AND METHODS

Experiments are carried out in West medinipur districts of West Bengal, India. The samples were collected from three local markets viz., Technology market, Gole Bazaar and Talbagicha market. All the market places are situated in West Medinipur district of West Bengal province of India. Three mostly consumed fish species namely, *Rohu* (*Labeo rohita*), *Tilapia* (*Oreochromis mossambicus*) and *Prawn* (*Penaeus monodon*) were considered for the study. After collection, fish samples were washed with tap water until all unwanted materials were removed and thereafter fish scales were removed according to the common household practices. Then fish fillets were obtained by using a sharp stainless steel knife and the samples were divided into two parts: First part was prepared by washing with normal water and the second part was prepared after washing followed by 5 min and 10 min boiling in tap water (boiling treatment).

The muscle tissue was dissected as much as possible after removal of the skin from each fish. The tissue was homogenized with a mixer grinder. The mixing was repeated until the composite sample appeared to be homogeneous, then it was kept at 4ºC until used for drying. The samples were oven dried at 105ºC for 24 hours as suggested by Ajmal *et al*[1]. The dried samples were then grounded into fine powder and stored in borosil bottles. Digestion was done following standard procedure i.e., the fish samples (0.5 g each) were placed into 100 ml beakers separately, to which 10 ml of tri-acid mixture (70% high purity HNO_3,70% H_2SO_4 and 65% $HClO_4$ in 5:1:1 ratio) were added. The mixture was then digested at 80°C on hot plate till the solution became transparent. The digestion of discarded washed water samples were also done following standard procedure i.e., the washed water samples (5.0ml each) were placed into 100 ml beakers separately, to which 10 ml of tri-acid mixture (70% high purity HNO_3,70% H_2SO_4 and 65% $HClO_4$ in 3:1:2 ratio) were added. The mixture was then digested at 150°C on kjeldatherm apparatus till the solution became transparent. Then resultant solutions were filtered using whatman no.42 filter paper and the filtrate was diluted to 100 ml using distilled water. Determination of metal residues in the digested fish samples and discarded washed water samples were carried out by means of Perkin Elmer A Analyst 700 Atomic absorption spectrophotometer according to methods described by Alinnor and Obiji[3]. Hollow cathode lamps were used as excitation sources. The operating condition of the instrument is given in (Table 1).

After determination of the heavy metals in fish samples, the data were tabulated and standard deviation was calculated using the following formula:

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}$$

[x_i: Each value in the population, μ: mean of the values, N: Number of values(the population), σ: Standard deviation]

Where, $\mu = \frac{1}{N}\sum_{i=1}^{N} x_i$

[x_i: Each value in the population, μ: mean of the values, N: Number of values(the population)]

Table 1: Standard operating conditions for the analysis of heavy metals using Atomic Absorption Spectrometry

Metals	Wavelength (nm)	Lamp current (mA)	Flame	Slit width(nm)
Cr	357.9	30		0.7
Co	240.7	30		0.2
Ni	232.0	25	Air- acetylene	0.2
Fe	248.3	25		0.2
Zn	213.9	25		0.7
Pb	283.3	12		0.7
Cu	324.8	30		0.7

RESULTS AND DISCUSSION

The results of the present study clearly indicate that out of seven heavy metals, Pb and Cr were present in toxic limits whereas all other metals were within the permissible limits (Table 2). However, after giving boiling treatment for 5minutes, concentration of Pb came down from 11.16 ppm to 6.61 ppm in *Tilapia*, 2.74 ppm to 2.07 ppm in *Prawn* (*chingri*), 3.35 ppm to 2.9 ppm in *Rohu* and Concentration of Cr came down from 29.7 ppm to 23.8 ppm in *Tilapia*, 25.4 ppm to 15.5 ppm in *Rohu*, 18.6 ppm to 12.45 ppm in *Prawn* (*chingri*). Further, after giving boiling treatment for 10 minutes, concentration of Pb came down from 11.16 ppm to 2.17 ppm in *Tilapia*, 2.74 ppm to 1.5 ppm in *Prawn* (*chingri*), 3.35ppm to 2.4 ppm in *Rohu* and Concentration of Cr came down from 29.7 ppm to 17.8 ppm in *Tilapia*, 25.4 ppm to 5.7 ppm in *Rohu*, 18.6 ppm to 6.4 ppm in *Prawn* (*chingri*). However boiling beyond 10 minutes fish fillet samples were no longer useful for frying or cooking purpose because it became softer and formed paste. This shows that, 10 minute boiling treatment is useful in reducing toxic heavy metals in fish. The reduction in heavy metal content can be explained by the fact that, during boiling fish fat containing heavy metal is leached out, thereby reducing the heavy metal content in the fish fillets. This was confirmed by analyzing the rejected water samples used for giving boiling treatment. The results of analyzed washed water sample is given in (Table 3).This water contained 5.9 ppm of Cr and 4.55 ppm of Pb in case of *Tilapia* washed water, 9.9 ppm of Cr and 0.45 ppm of Pb in case of *Rohu* washed water and 6.15 ppm of Cr and 0.67 ppm of Pb in case of *Prawn* (*chingri*) washed water when boiling treatment was given for 5 minutes and 11.9 ppm of Cr and 8.99 ppm of Pb in case of *Tilapia* washed water, 19.7 ppm of Cr and 0.95 ppm of Pb in case of *Rohu* washed water and 12.2 ppm of Cr and 1.24 ppm of Pb in case of *Prawn* (*chingri*) washed water when boiling treatment was given for 10 minutes. This clearly confirmed that boiling water treatment helps in reducing heavy metal residue in fish. Therefore, it can be recommended that all dietary fish samples which are likely to have heavy metals in higher concentration or at toxic level due to various reasons such as indiscriminate use of pesticides, heavy metals present in fish feed, cultivation of fish in polluted water etc., should be given boiling treatment for 10 minute followed by draining of water before making it ready for household consumption.

Table 2: Heavy metal concentrations (ppm) in the fish samples in normal washing condition, washing followed by boiling treatment

Treatment	Heavy metals	Acceptable limits(ppm)	Rohu fish	Tilapia fish	Prawn(chingri)
Normal	Fe[1]	100	52.8±0.18	68.0±0.23	22.4±0.68
	Pb[2]	2.5	3.35±0.6	11.16±0.9	2.74±0.64
	Cu[2]	30	3.16±0.92	4.88±1.02	4.48±1.05
	Co[2]	7.5	ND	ND	ND
	Ni[2]	5.0	ND	ND	ND
	Cr[2]	20	25.4±2.4	29.7±2.5	18.6±3.48
	Zn[2]	50	19.48±0.4	21.88±0.32	25.04±0.26
Boiling treatment (5 min)	Fe[1]	100	47.4±1.2	50.4±2.0	19.63±2.34
	Pb[2]	2.5	2.9±0.4	6.61±0.21	2.07±0.6
	Cu[2]	30	2.6±0.4	3.64±0.6	3.38±0.52
	Co[2]	7.5	ND	ND	ND
	Ni[2]	5.0	ND	ND	ND
	Cr[2]	20	15.5±1.6	23.8±2.2	12.45±2.3
	Zn[2]	50	18.08±0.6	20.16±0.71	22.9±1.1
Boiling treatment (10 min)	Fe[1]	100	42.53±2.6	32.4±1.2	16.46±1.94
	Pb[2]	2.5	2.4±0.62	2.17±0.45	1.5±0.38
	Cu[2]	30	2.24±0.3	2.6±0.8	2.48±0.4
	Co[2]	7.5	ND	ND	ND
	Ni[2]	5.0	ND	ND	ND
	Cr[2]	20	5.7±0.61	17.8±0.8	6.4±0.93
	Zn[2]	50	16.84±0.9	18.64±1.1	20.92±2.12
	ND-Not Detected; Values in arithmetic mean±standard deviation				

[1]Acceptable limit (WHO, 1989).
[2]Indian Standards for Food (Awasthi 2000).

Table 3: Heavy metal concentrations (ppm) in discarded washed water samples

Sample	Heavy metals	Discarded washed water sample		
		Rohu fish	**Tilapia fish**	**Prawn(chingri)**
Boiling treatment (5 min)	Fe	5.4±1.32	17.6±3.3	2.77±1.1
	Pb	0.45±0.18	4.55±0.43	0.67±0.11
	Cu	0.56±0.4	1.24±0.2	1.1±0.35
	Co	ND	ND	ND
	Ni	ND	ND	ND
	Cr	9.9±2.4	5.9±2.1	6.15±2.3
	Zn	1.4±0.6	1.72±0.32	2.14±0.26
Boiling treatment (10 min)	Fe	10.27±1.1	35.6±2.0	5.94±2.4
	Pb	0.95±0.4	8.99±1.9	1.24±0.6
	Cu	0.92±0.2	2.28±0.6	2.0±0.28
	Co	ND	ND	ND
	Ni	ND	ND	ND
	Cr	19.7±2.5	11.9±3.2	12.2±0.18
	Zn	2.64±0.4	3.24±0.11	4.12±0.1
	ND-Not Detected; Values in arithmetic mean±standard deviation			

CONCLUSION

The following conclusions can be drawn from the present study: Out of analyzed seven heavy metals in fish, Pb and Cr were within toxic limits whereas all other metals were within the permissible limits. Boiling water treatment for 5 minutes reduced the concentration of Cr by 19.8% in *Tilapia*, 38.9% in *Rohu*, 33.0 % in *Prawn* (*chingri*) and that of Pb by 40.7% in *Tilapia*, 13.4% in *Rohu* and 24.4% in *Prawn* (*chingri*).Boiling water treatment for 10 minutes reduced the concentration of Cr by 40.0% in *Tilapia*, 77.5% in *Rohu*, 65.5% in *Prawn* (*chingri*) and that of Pb by 80.5% in *Tilapia*, 28.3% in *Rohu* and 45.2% in *Prawn* (*chingri*). Boiling treatment for 10 minutes was found effective for reducing concentration of heavy metals in all fish fillets.

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