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Determination of metal content in *Ethmalosa fimbiata, Pseudotolilthus epipercus, Clupea harengus and Merluccious merluccious* stored in commercial cold stores

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Abstract

The levels of some metals (Fe, Cr, Ni, Zn, Cu, Mn, Cd, Pb and Ar) in four different species of imported frozen fish; Atlantic Herrings (Culpea harengus), Merluza (Merluccius merluccius), Bonga (Ethmalosa fimbriata) and Croaker (Pseudotolilthus epipercus) three cartons each of the different species were bought. A carton of each species was kept in three commercial cold-stores in Benin City, Edo State and was investigated for a period of five months during this study. One piece of each species in triplicate were collected every three weeks from these cartons from the three cold stores and transported to the laboratory in coolers with ice to prevent deterioration. Microwave digestion system (Closed Vessels Acid Digestion – MARS System - CEM) procedure based on the organic extraction technique was used for the digestion of all the fish samples, the mineral content was then determined by the use of atomic absorption spectrometer (AAS). Data obtained were analyzed using computer software (SPSS version 22). One way analyses of variance test was used at 5% probability level and DMRT was used to analyze the significant differences between homogenous subsets. Significant differences were observed in the levels of the individual metal in the different fish samples. Levels were compared with safety standard levels set by FAO, WHO and EU legislation for fish and fishery products, and most of the values were within the recommended standards set by FAO, WHO and EU legislation for fish and fish products. However, Ni and Cr were detected at higher levels above the set permissible limits. Fe however was the most accumulated metal in all the fish species. This calls for concern by the relevant agencies and arm of government because of the attendant health implication if consistently consumed due to bio-accumulation of metal in man

Key Words: *Bio-accumulation, Cold-store, Edo State, Frozen fish, Imported, Metals.*

Introduction

Heavy and trace metal contaminations of fish species are potential ways of human exposure to these metals. Consumption of fish especially imported frozen fish as a source of animal protein has been reported to supersede many other sources of animal protein in Nigeria (Akintunde, 2012). Massive importation of frozen fish into the country has ranked Nigeria as the largest importer of frozen fish in Africa (FAO, 2012). Fish represents a valuable source of protein and nutrients of fundamental importance for diversified and healthy diets such as vitamins (D, A and B), minerals (calcium, iodine, zinc, iron and selenium) and polyunsaturated Omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid) (ICES, 2001). Consequently, different variety of frozen fish species are imported and sold, although fish species such as Scombers, mackerels and croaker fishes are the most common and commonly consumed among fish consumers in Nigeria (Daniel *et al.,* 2013). Fish and shellfish bio-accumulate metals in varied concentrations many times higher than the level present in the water and its sediment (Yilmaz, 2009).

The concern about the possibility of finding levels of metals in fish and other marine products prompted several statutory bodies such as the World health Organization and the Food and Agriculture Organization (FAO) to establish maximum allowable concentrations for some of these metals in food (FAO, 2006). Thus advocating the obligatory monitoring of metals in fish and other marine product are very useful, in assessing of metal burden not only in fishes but in water bodies so as to create greater environmental awareness (Staniskiene *et al.,* 2006; Adefemi *et al.,* 2008).

In Nigeria, there are limited reports on safety assessments of heavy metals in imported frozen fish, although it is a known fact that some of these fishes might have been harvested from heavily contaminated waters (Eddy *et al.*, 2006). Several studies had shown that marine fish bio-accumulates heavy metals to a greater extent thus making it an area of concern (Simon *et al.*, 2013). These marine species harvested mostly from the high sea are supplied in frozen form; as deep freezing them can only preserve it from decomposition by slowing down some biochemical activities, but do not have any impact on the presence of heavy metal contaminants (Mieiro *et al.*, 2012).

In Nigeria, there are limited reports on levels of heavy metal in imported frozen fish stored in commercial cold-stores. Several studies have shown that marine species have the ability to accumulate and bio-magnify metals to levels that could cause health related illnesses. With the ever increasing importation of frozen fish into Nigeria according to NFC (2014), there is the need to raise awareness of the probable presence of metals in these fish species imported and sold in the Nigerian market.

The objective of this study is to determine the level of some metals in four commonly consumed imported marine fish species sold in Nigeria and compare the level of these metals in these fish species. With a view to knowing how safe their consumption is with regards to health and wellbeing.

Materials and Method

Four different species of imported frozen fish: Atlantic Herrings (*Culpea harengus*), Merluza (*Merluccius merluccius*), Bonga (*Ethmalosa fimbriata)* and Croaker (*Pseudotolilthus epipercus)* were bought and kept in commercial cold-stores located in Edo State, Nigeria.

Microwave digestion system (Closed Vessels Acid Digestion – MARS System - CEM) procedure was chosen for the digestion of all the fish samples, because of shorter required time, smaller deviations, excellent recovery and precision than the other procedures. Homogenized fish samples were taken and digested in the laboratory based on the organic extraction technique described by Sreedevi *et al.* (1992). Using inductively coupled plasma atomic emission spectrometer (ICP-AES) with solar software, Acetylene flame was used as the oxidant and a hollow cathode lamp as a source of radiation. The ICP-AES was calibrated for each of the study metals. While a standard solution of each metal salt and blank sample were run with each set of experimental digests.

Statistical Analyses

Data obtained were analyzed using computer software (SPSS version 22). One-way analyses of variance ANOVA) and Duncan Multiple Range Test was used at 5% probability significant differences level for separation of means.

Results

The results of the investigation of the presence of metals in *Clupea harengus, Pseudotolilthus epipercus, Ethmalosa fimbriata* and *Merluccius merluccius* bought and stored in commercial cold stores in Edo State are as shown in Table 1.

The overall observation during the period of study (Table 1) for the different species of fish showed that Fe concentration was highest in *Merluccius merluccius* (105.78 mg/kg) while the least value of 57.70 mg/kg was observed in *Pseudotolilthus epipercus*. Croaker had the highest values in Zn (21.52 mg/kg), Cd (0.15 mg/kg) and Mn (5.19 mg/kg) Cd and As were absent in some of the study species and their presence in some of the species based on ANOVA and DMRT were also not significantly important. Though the highest value of 0.72 mg/kg of Pb was observed in *Clupea harengus*, and the least value of 3.32 mg/kg of Cu in *Pseudotolilthus epipercus*, Cd, Pb, Mn, Cu and As were not significantly different (p>0.05) during the period of study.

Table 1: Monthly and General Mean Metal Concentration (mg/kg) in the Imported Fish Species Stored in Commercial Cold-stores

		December	January	February	March	April	Gen. Mean	P
Herring	Fe	140.53±31.04	108.83±56.17	82.71±42.70	62.86±32.45	26.20±5.96	84.22±51.32	0.033
	Zn	21.56±4.76	16.70±8.61	12.69±6.55	9.64±4.98	7.33±3.78	13.58±7.29	0.092
	Cd	0.14±0.03	0.11±0.06	0.07±0.06	0.05±0.05	0.05±0.04	0.09±0.06	0.172
	Cr	13.68±3.02	10.60±5.47	8.05±4.16	6.12±3.16	4.65±2.40	8.62±4.63	0.093
	Ni	34.59±7.64	26.79±13.83	20.36±10.51	15.47±7.98	5.57±2.88	20.56±12.88	0.029
	Mn	5.33±1.18	4.13±2.13	3.13±1.62	2.38±1.23	1.81±0.93	3.36±1.80	0.093
	Pb	1.14±0.25	0.89±0.45	0.67±0.35	0.51±0.26	0.39±0.20	0.72±0.39	0.091
	Cu	9.69±2.14	7.50±3.87	5.70±2.94	4.33±2.24	3.29±1.70	6.10±3.28	0.093
	As	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
Melusa	Fe	176.49±38.99	136.68±70.55	103.87±53.6	78.94±40.75	32.90±7.48	105.78±64.5	0.033
	Zn	18.86±4.17	14.61±7.54	11.10±5.73	8.44±4.35	6.41±3.31	11.88±6.38	0.093
	Cd	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
	Cr	7.42±1.64	5.75±2.97	4.37±2.26	3.32±1.72	2.52±1.31	4.68±2.51	0.092
	Ni	42.34±9.35	32.79±16.92	18.36±9.48	13.96±7.20	5.02±2.59	22.49±16.32	0.008
	Mn	3.72±0.82	2.88±1.49	2.19±1.13	1.66±0.86	1.26±0.65	2.34±1.26	0.092
	Pb	0.65±0.14	0.50±0.26	0.38±0.20	0.29±0.15	0.22±0.11	0.41±0.22	0.090
	Cu	12.61±2.78	9.77±5.04	7.42±3.83	5.64±2.91	4.29±2.22	7.95±4.26	0.092
	As	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	
Croaker	Fe	96.28±21.27	74.56±38.49	56.66±29.25	43.06±22.23	17.95±4.08	57.70±35.16	0.033
	Zn	34.16±7.55	26.45±13.65	20.11±10.38	15.28±7.89	11.61±6.00	21.52±11.55	0.093
	Cd	0.23±0.06	0.18±0.09	0.14±0.07	0.10±0.06	0.08±0.04	0.15±0.08	0.093
	Cr	15.19±3.35	11.76±6.07	8.94±4.62	6.79±3.51	5.16±2.66	9.57±5.14	0.092
	Ni	22.51±4.97	17.43±9.00	13.25±6.84	10.07±5.20	3.63±1.88	13.38±8.38	0.029
	Mn	8.23±1.82	6.37±3.29	4.84±2.50	3.68±1.90	2.80±1.45	5.19±2.78	0.093
	Pb	0.86±0.19	0.67±0.35	0.51±0.26	0.38±0.20	0.29±0.16	0.54±0.29	0.097
	Cu	5.27±1.17	4.08±2.10	3.10±1.60	2.36±1.22	1.79±0.93	3.32±1.78	0.093
	As	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	0.01±0.01	
Bonga	Fe	113.90±25.16	88.21±45.54	67.04±34.61	50.95±26.30	21.23±4.83	68.27±41.59	0.033
	Zn	26.38±5.83	20.43±10.55	15.53±8.02	11.80±6.09	8.97±4.63	16.62±8.92	0.092
	Cd	0.15±0.04	0.12±0.06	0.09±0.05	0.07±0.04	0.05±0.03	0.10±0.05	0.091
	Cr	12.12±2.68	9.38±4.84	7.13±3.68	5.42±2.79	4.12±2.13	7.63±4.10	0.093
	Ni	52.00±11.49	40.28±20.79	30.61±15.80	23.26±12.01	8.37±4.32	30.90±19.37	0.030
	Mn	4.72±1.04	3.66±1.88	2.78±1.44	2.11±1.09	1.61±0.83	2.98±1.60	0.092
	Pb	0.52±0.12	0.41±0.21	0.31±0.16	0.24±0.12	0.18±0.09	0.33±0.18	0.099
	Cu	13.94±3.08	10.80±5.57	8.21±4.24	6.24±3.22	4.74±2.45	8.79±4.72	0.093
	As	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	

The highest values recorded for all the metals during the period of study was in December while the lowest values were observed at the end of the study period (April) as shown in Table 1. Although analysis of variance (ANOVA) showed that there was significant difference ($p<0.05$) between some months, Duncan Multiple Range Test (DMRT) revealed the specific months where there were significant differences ($p>0.05$). During the period of study Arsenic (Ar) was not recorded in Herring (*Clupea harengus*) and Bonga (*Ethmalosa fimbriata*) and was only observed at detection levels in Merluza (*Merluccius merluccius*) and Croaker *(Pseudotolilthus epipercus)* as shown in Table 1. Analysis of Variance (ANOVA) and DMRT showed that there was no significant difference ($p>0.05$) between months in these species Ar was observed. Cadmium was totally absent in Merluza (*Merluccius merluccius*),

Discussion

Heavy metals are intrinsic, natural constituents of the environment and an alarming increase in their levels has been attributed to domestic, industrial, agricultural, among other natural activities (Prabhat, 2009). And their uncontrolled increases beyond certain threshold, could result in their becoming chronically toxic (Nike *et al.*, 2001). According to Dodd (2013), when fish are exposed to high levels of heavy metals in the aquatic environment, they will absorb the bio-available metals either through their gills, skin and/or through ingestion of contaminated water or food. The uptake and loss of metals in fish are regulated by the fish body to a certain level beyond which bioaccumulation of the metals can occur.

This study shows the presence of all the study metals (Zn, Cu, Pb, Cd, Mn, Cr, Ni, Fe and Ar) in the four imported study fish species sold in commercial cold-stores in Edo State.

The levels of Fe observed during this study are comparable to levels reported by other authors (Alinnor and Obiji, 2010). Fe content observed in these fish samples were higher compared to the mean concentration value for Merluza and Herring species of 13.0 mg/kg reported by Wyse *et al*. (2003). Iron is an important constituent of haemoglobin, myoglobin and enzymes such as catalases and peroxidases, the FAO/WHO and USA recommended 18mgFe or more per day for women during their reproductive years, to help cover the losses through menstruation. Severe iron deficiency in human cause's anaemia, Fe is found naturally in fresh waters and has no health-based guideline value, although high concentrations in water can give rise to consumer complaints (WHO, 2004). The high mean values observed in species used in present study could be due to contamination of the water by these metals, age, health status of the fish, differences in dietary habits with Merluza being exposed to more Fe from sedimentary sources.

Zinc was present in all the fish species examined, no value observed exceeded the WHO/FAO maximum permissible limit of 40mg/kg in the different months except in the month of April, when the value exceeded the European Union (E.U) acceptable limit of 0.05mg/kg. Thus, the nutritional quality of these fish going by this set standard can be said to be impaired. This result compares with previous work by Manthey-Karl *et al*. (2014) who observed a ranged of 17.6 mg/kg - 27.3 mg/kg zinc in the fish samples they worked with. Zinc is known to be involved in most metabolic pathways in humans; essential for normal growth and development, playing important role in enzymatic processes and synthesis of protein and RNA. Its deficiency can however lead to loss of appetite, growth retardation, skin changes and immunological abnormalities.

The mean concentration of Cd in fishes analyzed did not differ much from earlier

0.14mg/kg reported by Heath (1995) and were lower than Cd value (2.0mg/kg) recommended for fish and fishery products (WHO 1984). Cadmium was present in trace amount in all samples except in merluza where it was completely absent. Cadmium wastes in aquatic environment, can be accumulated in biomass, becomes concentrated to be passed down in the food chain. Cadmium even at low levels when ingested can become toxic; chronic exposure to high levels can result in osteoporosis and osteomalacia among other medical challenges (Ekpo *et al*., 2008).

The total mean concentrations of Cr for all the fish samples analyzed were higher than values of 3mg/kg observed and reported by Wyse *et al.* (2003) while working on some marine fish species and the recommended value of 0.15-1.0mg/kg in fish and fishery products (WHO, 1984). All the observed values in all the samples were however below the USEPA limit of 8.0mg/kg. Chromium though a notable hazardous metal is also an essential metal whose biological usable form plays an important role in glucose metabolism. An average human requires about 1µg /daily of Cr. Water sources contribute a major part of Cr in humans, while its deficiency can cause impaired growth, disturbances in glucose, lipid and protein metabolism (US. Food and Drug Administration, 1993).

The mean concentration of Ni for all the species in this study were very high and even higher than the Ni value of 6mg/kg found in Herring fishes as reported by Wood (2001). The reason postulated for this could be due to marine pollution by crude oil spill in waters of capture (Osuji and Onojake, 2004). Kamaruzzaman *et al.* (2011) also reported Ni in some commercially imported marine fish species, among other metals; concluding that this phenomenon could be due to respiratory mechanism of fishes. The maximum allowable residue level for nickel is 70 – 80mg/kg (USFDA, 1993) and the samples analyzed during studied showed concentration levels of between 3.63 - 52.00mg/kg which were higher than that observed by Ikem and Egiebor (2005) which ranged between 0.0 to 0.78mg/kg. The normal range of oral intake of nickel for humans is 300 – 600µg/day. Maximum acceptable level of Ni in water is < 1.0 µg/g (FEPA, 1999) and < 0.05 µg/g (WHO, 1984) respectively. The migration of nickel to foodstuffs should be as reasonably low, < 0.1µg/g as a general rule should be the allowable migratory levels into foodstuffs (Council of Europe, 2001).

The results obtained for manganese in this study were higher compared to that observed by Wood (2001) while working with samples of mackerels, sardines, merluza and herrings with a range of 0.01 - 2.55 mg/kg. Trace amount of manganese can interfere with iron metabolism, especially haemoglobin formation. The results obtained for manganese and copper in all fish species were below the World Health Organization recommended limit of 2500mg/kg Mn and 30mg/kg Cu respectively in fish and fish products. Only in the last month of study was 13.94 mg/kg recorded for bonga fish that exceeded the Australian Food Agency standard of 10mg/kg Cu.

Copper is essential for good health but a very high intake can result in liver and kidney damage. Copper can be potentially toxic to aquatic organisms at high levels in water (Muriel, *et al*., 2016). Copper is released into the environment primarily through mining, sewage treatment plants, solid waste disposal, and industrial waste-water (Dudeney, *et al*., 2013). The mean concentrations of copper reported in this study were higher than previously reported values by several investigators (Tarley *et al.*, 2001; Canli and Atli, 2003). Set standards for Cu is 1.2 mg/day (COMA, 1991), while the US safe designation for adults is 1.5mg/day Cu for adult males and twice that value (3.0 mg/day) for adult females. This suggests that supplements through other foods can be taken orally.

Agyekum *et al.* (2012) have earlier reported values of 0.40 mg/kg in some imported frozen marine fish; the overall observed values of lead during this study were above the E.U. 0.2mg/kg acceptable limits. Pb is a well known toxicant, having a deleterious effect even at low concentration. Hence, the result revealed that Pb in consumed fish could become deleterious over time, posing significant health risk to the consumers of these fish species because of the observed values.

The statutory limit for arsenic in food is 1 mg/kg, the mean concentration of Arsenic (Ar) in Meluza was lower than ≤0.31mg/kg observed by Ikem and Egiebor (2005) during their study. Arsenic has become a highly politicized issue globally particularly in some affected countries due to its carcinogenic characteristics and being a possible natural contaminant of groundwater sources (ARSLAND, 2006). In addition, uptake of arsenic via ingestion is associated with dermal carcinomas and hepatic angiosarcomas in humans (Khairul, *et al*., 2003). The fishes analyzed in this study may not be polluted with As since the concentration found were below the statutory limit stated by environmental agency in United Kingdom.

Conclusion and Recommendations

The result of this study shows that these heavy metals were present in almost all the fish samples analyzed. Although considerable differences were observed in the levels of the individual metal in each sample, the levels of these study metals were within the recommended standards however, Ni and Cr were detected at levels above the permissible limits set by FAO, WHO and EU legislation for fish and fish products. These calls for concerns because of the health implication that may result from their consumption, government should therefore monitor imported frozen fish more effectively and create public awareness on the possible implication of the presence of heavy metal accumulation from fish on human health. Routine analysis on imported frozen fish should therefore be carried out continually by governmental, institutional and research centres to ensure quality of imported frozen fish on arrival and after certain period in commercial cold storage.

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