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RESEARCH PAPER

Heavy Metal Concentrations in Imported Frozen European Hake *Merluccius merluccius* (Linnaeus 1758)

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Abstract

This research looked at the concentration levels of some heavy metals in tissues and organs of imported frozen European hake *Merluccius merluccius* (Linnaeus, 1758). This to ascertain the safety of the fish for consumption and whether long time freezing could bio concentrate heavy metals in the fish. Fish were procured from the market, muscles, gills and liver removed, digested for analysis of Cu, Zn, Mn, Fe, Cd, and Pb using AAS. Fe was the most accumulated metal with 4.393±0.19 mg/kg, Cadmium (Cd) was the least with 0.004±0.00 mg/kg (Fe>Zn>Cu>Pb>Cd>Mn). Muscle accumulated the most metals. Concentrations of the metals were below maximum limit recommended by Food and Agricultural Organization/World Health Organization (FAO/WHO), which shows the fish is safe for consumption. It also showed that long time freezing does not bio concentrate heavy metals in the fish about a month of freezing. It is recommended that regulatory authorities should always conduct an assessment of the heavy metals in imported frozen marine fishes before it is cleared for human consumption. This will guide against any adverse health risk associated with consumption of fish with high levels of heavy metals.

Introduction

European hake *Merluccius merluccius* (Linnaeus 1758) is one of the major imported frozen marine fish in Nigeria and other developing countries, where the vast population consume it because of its flavour, texture, affordability, availability and nutritional qualities. The fish is mainly imported from Europe and some African countries lying along the Atlantic coast and Mediterranean Sea.

In recent time, the quality of the fish in terms of its levels of heavy metals has been a source of concern by the consumers and fish toxicologists alike. This is so because of reports of increasing heavy metals concentrations in oceans and seas and from fishes caught from these habitats (Ozuni et al. 2014). According to Bosch et al. (2016), the numerous health benefits provided by fish consumption may be compromised by the presence of toxic metals and metalloids such as lead, cadmium, arsenic and mercury, which can have harmful effects on the human body if consumed in lethal quantities. These sea fishes are imported and consumed by the people without any assessment of the levels of heavy metals in the fish by the regulatory authorities in charge of importation. Thus, assessment of heavy metals in these imported frozen marine fishes and its effects on human health especially in developing countries where such fishes are majorly imported and consumed now becomes imperative.

Heavy metals are known to be toxic and bioaccumulates in the organs of fish (Rajeshkumar and Li 2018) and their high concentration above permissible limits in fish poses serious health risks to consumers (Castro-Gonzales and Méndez-Armenta 2008).

Although many studies have been done on the levels of heavy metals in European hake *Merluccius merluccius* (Linnaeus 1758) (Aksu et al. 2011; Ozuni et al. 2014; Brkic et al. 2017), most of these studies were done on fresh fish samples. There has not been any study of the levels of heavy metals in frozen European hake *Merluccius merluccius* (Linnaeus 1758) which is exported or imported for human consumption after long time of freezing and cold storage. The objective of this research is to determine the concentration levels of some heavy metals in the tissues and organs of frozen European hake *Merluccius merluccius* (Linnaeus 1758) that has been exported from Europe into sub–Saharan Africa for human consumption. This is to ascertain the safety of the fish for consumption based on the concentration levels of the heavy metals contained in the fish and to evaluate whether long term freezing could bio concentrate heavy metals in the fish.

Materials and methods

Sampling

Imported frozen European hake *Merluccius merluccius* (Linnaeus 1758) samples (n=20; mean weight 440±1.50g; mean total length 38.40±1.20cm) were procured from the open fish market and cold storage rooms in Ilorin, Nigeria. The fish samples were stored at -18°C in the cold rooms for a week after importation before been sold to consumers. The fish samples were thawed at room temperature in the laboratory and the muscles, gills and liver were dissected out of the fish and washed with distilled water. Quantification analysis of digestion was done using acid digestion method.

Sample Digestion

Each of the organs was dried at 105°C to a constant weight of 1.00 g. Each dried organ was homogenized to fine powder and then digested using 5 ml nitric acid, after which it was placed on a hot plate and heated at 100°C until the solution became clear and colourless. The digested solution was diluted with distilled water to 25 ml and filtered. The digested samples were analysed in triplicate after the method adopted by Food and Agricultural Organization/Swedish International Development Cooperation Agency (FAO/SIDA) 2003 for the following heavy metals: Cu; Zn; Mn; Fe; Cd; and Pb using Perkin Elmer Analyst 300 Atomic Absorption Spectrophotometer (AAS). The AAS validation for the parameters fit the criteria required by International Conference on Harmonization (ICH).

Statistical Analysis

Statistical analysis was done using one-way ANOVA and Duncan Multiple Range Test at P<0.05. The statistics was performed using SPSS Version 18.0.

Results

The fish samples were recovered from cold storage of -18°C before been thawed at room temperature in the laboratory where the muscles, gills and liver were dissected out of the fish. The result of the mean heavy metal concentrations in the muscles, gills and liver of European hake *Merluccius merluccius* (Linnaeus 1758) is presented in Table 1.

There were significant differences (P<0.05) in the concentrations of the heavy metals among the organs of the fish. Iron (Fe) was the most accumulated heavy metal in the fish with 4.393±0.19 mg.kg^{-1} accumulated in the muscles, followed by liver and the gills which accumulated 3.210±0.010 and 2.787±0.02 mg.kg^{-1} respectively. Zinc (Zn) was the second most accumulated heavy metal, while Cadmium (Cd) was the least heavy metal concentration in the fish with 0.004 ± 0.00 mg.kg^{-1} accumulated. Manganese (Mn) was undetected in all the organs of the fish. The trend in the level of heavy metal concentration is Fe>Zn>Cu>Pb>Cd>Mn. The muscle is the organ that showed the highest level of the heavy metals' concentrations followed by the liver and the gills respectively (Muscles>liver>gills).

Discussion

There were significant differences (P<0.05) in the concentrations of zinc and lead between the muscles, gill and liver with the muscles showing highest concentration, while gill and liver showed no significant difference (P>0.05) among the two heavy metals. Similarly, similar scenario was seen in the

Table 1. Mean level of heavy metal concentrations in different organs of European hake *Merluccius merluccius* (Linnaeus, 1758)

Organ	Zn (mg.kg^{-1})	Pb (mg.kg^{-1})	Cd (mg.kg^{-1})	Cu (mg.kg^{-1})	Fe (mg.kg^{-1})	Mn (mg.kg^{-1})
Muscle	3.113±0.023^{a}	0.068±0.00^{a}	0.004±0.00^{a}	2.080±0.021^{a}	4.393±0.19^{a}	0^{a}
Gill	1.437±0.015^{b}	0.013±0.00^{b}	0.013±0.00^{a}	1.0230±0.006^{b}	2.787±0.02^{b}	0^{a}
Liver	1.680±0.020^{b}	0.021±0.00^{b}	0.002±0.00^{a}	0.857±0.021^{b}	3.210±0.010^{b}	0^{a}

Values are Means ±SEM, Values in each column with the same superscript are not significantly different at P<0.05

concentrations of copper and iron among the three organs of investigation. No significant difference (P>0.05) was seen in cadmium and manganese concentrations in the three organs.

The heavy metals analysed in this work are known to be toxic to humans at certain concentrations. The numerous health benefits provided by fish consumption may be compromised by the presence of toxic metals and metalloids such as lead, cadmium, arsenic and mercury, which can have harmful effects on the human body if consumed in toxic (Bosch et al. 2016).

The concentrations of all the metals found in the fish were below maximum permissible limit recommended by Food and Agricultural Organization/World Health Organization (FAO/WHO) (2011) for human consumption (Table 2). This shows the fish is safe for consumption, in spite of it coming from seas and oceans where increasing heavy metals concentrations has been reported (Ozuni et al. 2014). The levels of heavy metals in fresh *Merluccius merluccius* (Linnaeus, 1758) caught from the Mediterranean seas have been reported to be within FAO/WHO (2011) permissible limit (Olgunoglu et al. 2015). Freezing of the fish after a long time does not seem increase the levels of its heavy metals' concentrations.

The trend in the heavy metal concentration in the fish was in conformity with the report of Ahmed et al. (2014), Olgunoglu et al. (2015), Gbogbo et al. (2018), Yang-Guang (2015; 2017). The heavy metals were more accumulated in the muscles because the food (such as crustaceans and fish which themselves accumulate heavy metals) eaten by the fish becomes converted to flesh. Liver was able to accumulate the metals because of its role in metabolism (Zhao et al. 2012). Low level of heavy metals in the gills (despite the gills being the first contact with the heavy metals in water) was due to the low level of the metals in the sea. This shows that heavy metals pollution from seas and oceans where the fish comes is still minimal.

The high concentration of iron (Fe), Zinc (Zn) and copper (Cu) in the fish could be due to their roles in physiological and metabolic functions. These metals are gotten from the habitat on which the fish lives and from the prey on which the fish feed. Lead concentration in the fish is low which portrayed the habitat of the fish to be pollution-free of lead. The source of lead in the fish could be from the sediments, since the fish is a bottom feeder. Low concentration of cadmium (Cd) has been established in many marine fishes (Olmedo et al. 2013). The low concentration of Cd obtained is therefore in agreement with this observation. The Scientific Committee of Food (SCF) recommended that greater efforts should be taken to reduce dietary exposure to Cd. The fish is therefore safe to eat based on its Cd level. The undetectable concentration of manganese in the fish could be linked to its non-availability in its dietary constituents and low concentrations in the habitat of the fish. Elnabris et al. (2013) have noted very low level of Manganese in *Merluccius hubbsi* (Marini 1933). Although, Mn is an essential metal in enzymes and cellular activities, its high intakes can cause adverse health problems (Demirezen and Uruc 2006). The low level of Mn in the fish does not make the nutritional qualities of the fish less important.

In conclusion, this research has allayed consumers' fear of heavy metal poisoning from the consumption of imported European hake *Merluccius merluccius* (Linnaeus 1758). Though, highest concentrations of the metals were found in the muscle which is the edible part of the fish, the concentration levels were well below the limits proposed for fish by FAO/WHO (2011) guidelines. Therefore, the fish is safe for consumption. Miri et al. 2017 have also reported safe level of Pb, Cd, Cr, and Ni contents in the fish consumed by the Sistan population in Iran. The research has also showed that long time freezing does not bio concentrate heavy metals in the fish going by the levels of the heavy metals recorded after about a month of freezing and cold storage. However, it is highly recommended that regulatory authorities in charge of importation of sea fishes should always conduct an assessment of heavy metals in these imported frozen marine fishes before it is cleared for human consumption. This will guide against any adverse health risk associated with consumption of fish with high levels of heavy metals.

Ethical Statement

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures performed in this study were in accordance with the ethical standards of the University of Ilorin, Ilorin, Nigeria, where the study was conducted and who granted the ethical approval for the conduct of the research.

Conflict of Interest

The author declares no conflict of interest

Table 2. FAO/WHO (2011) recommended limits of heavy metals in fish ($mg.kg^{-1}$)

Limits	Zn	Pb	Cd	Cu	Fe	Mn
Minimum	0.5	0.01	0.01	0.4	3.0	0.01
Maximum	5.0	0.5	0.05	3.0	20.0	0.5

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