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EFFECTS OF DIFFERENT COOKING PROCESSES ON BIOAVAILABILITY OF MERCURY AND ARSENIC IN EXPOSED AFRICAN CATFISH (*Clarias gariepinus*, Burchell, 1822)

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ABSTRACT

Fish consumption is one of the major routes of human exposure to environmental contaminants. This study was conducted to assess alternative ways of reducing the bioavailability of mercury and arsenic in *Clarias gariepinus* and to evaluate ameliorating effects of cooking processes (boiling, frying, and roasting) on the bioavailability of these metals in exposed Catfish. Thirty-six *Clarias gariepinus* (3 months; 100g-150g; 10-15cm length) were randomly divided into three groups (n=12). 0.2mg/L of mercury chloride and 20µg/l were used as test salts for Mercury (group A) and Arsenic (group B) respectively while Group C was the control. Exposed fish were subjected to various cooking processes on a 7 days interval basis for four consecutive weeks after which they were digested before analysis. The levels of mercury and arsenic in fish samples were determined using titrimetric methods and atomic absorption spectrophotometer (AAS) respectively and the data were analyzed using analysis of variance and the least significant difference (LSD) was accepted at p=.05. A statistically significant difference (p=.05) was observed as a reduction in the mercury level of the processed fish across the weeks as compared with the raw sample and in this order: raw>roasted>fried>boiled. For arsenic-treated fishes, there was no statistically significant difference (p=.05) across the weeks, but an increase in concentration was observed in processed samples as compared with raw samples for the four weeks and in this order: raw<roasted<fried<boiled. None of the cooking procedures has glaring reduction effects on both Mercury and Arsenic. The liver also recorded the highest concentration of both Mercury and Arsenic followed by gills and Muscle of Catfish samples. Since this study and others alike have established little effects of cooking procedures on heavy metals in food, further studies need to be conducted using combined cooking methods at different conditions designed at mitigating the effects of heavy metals in food.

Keywords: Cooking processes; *Clarias gariepinus*; bio-availability; mercury; arsenic; titrimetric methods; atomic absorption spectrophotometer.

INTRODUCTION

Heavy metals are natural components of the earth's crust [1-3], present in the environment (air, soils, and waters). They are one of the major contaminating elements in our food supply [4] and serious health hazards can be created as a result of an extreme dietary accumulation of heavy metals [5]. In areas with high anthropogenic activities, some metals can also be environmental pollutants [6,7]. According to

Chang, 1996, Cobalt (Co), Chromium (Cr), Iron (Fe), Manganese (Mn), Zinc (Zn) e.t.c are essential for humans while Arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb) have no useful effects and no known homeostasis mechanism in humans [8]. Although, previous researches have shown that the adverse health effects of a particular metal depend upon its concentration in the media, for instance, chronic exposure to metals such as Arsenic (As), Cadmium (Cd), Mercury (Hg), and Lead (Pb) can cause toxic effects at relatively low levels [8].

Mercury and Arsenic have been shown to exert serious health effects on humans and one of the major exposure routes is ingestion through food. Mercury is highly toxic at even low concentrations and in any of its forms while arsenic could still be tolerated to an extent in the body at certain concentrations [9]. Exposure to Mercury and the level of toxicity among humans depend on the chemical forms of the mercury (elemental or metallic mercury, inorganic or organic mercury compounds) [10]. Arsenic, a naturally occurring metal found in rocks, soil, water, air, plants, and animals can also be released into the environment from agricultural and industrial sources. According to Lièvremont et al. [11], inorganic arsenic is a known carcinogen, and epidemiologic studies have revealed that people with impaired arsenic metabolism are at higher risk for cancers some of which include skin and bladder carcinoma. Chronic health effects have been recorded since arsenic is highly poisonous in nature [12]. There is increasing concern regarding methyl mercury exposure in populations that consume large amounts of fish and this situation poses a dilemma for those who choose to consume fish for its beneficial effects on heart disease risk as well [13]. Mercury was a major water pollutant mostly from industrial waste discharge into water bodies. With very high toxic effects and accumulative properties, it tends to concentrate in aquatic organisms [14] due to reduced biodegradation of its derivatives.

Fish is a major delicacy and a rich source of protein for humans. Catfish are commonly consumed in Nigeria because it is cheap, abundantly available, easily cultivated and very adaptive in nature. Fishes are usually not consumed raw but processed before consumption [15] and methods of fish processing vary between countries and within the same country depending on the species of fish used and the type of derived product [16]. Despite records of various cooking methods with their effects on palatability, there is a scarcity of information on the effect of different cooking processes on heavy metals, especially Mercury and Arsenic concentration fish. Therefore, this study attempts to evaluate the effect of different cooking methods on the Mercury and Arsenic concentrations in African Catfish under different cooking processes.

METHODOLOGY

Thirty-six (36) African catfish (3 months weighing between 100 g-150 g and 10-15 cm length) were acclimatized for seven days after which the experimental groups were exposed to 0.2mg/L (0.025 to 0.70 mg/l) of mercury chloride (group A), 20µg/l (3 to 30 mg/l) as the test salt for arsenic (group B) while group C was the control group.

The fish were weighed and the mean weight was determined. The concentrations of the test dosage for the test salt were calculated using the formula below:

Dose = Concentration of test salt (g) X weight of fish (g) / Volume of water (L)

The fish were subjected to various cooking processes on a 7 days interval basis for four consecutive weeks after which they were digested before analysis. One fish sample each was taken from groups A and B every week. It was washed several times to remove the blood and mucus, the gut was eviscerated, removal of the head and then divided into four parts (for boiling, frying, roasting, and the last portion were left as raw). The above procedure was done in triplicate and biometric and weight tests were performed.

Frying was done using soy vegetable oil (200 ml) in a clean frying pan on an electric stove at 100 ^{0}C for 10 minutes, according to [16], smoking was done at 200 ^{0}C for 15minutes on a charcoal grill [16] while boiling was done in 200 ml of clean water at 100 ^{0}C for 10 minutes [16]. Data was collected for a period of four weeks for the two metals, the samples of each group were weighed equally and kept in polyethylene bags, which were closed tightly for digestion. Gills, liver, and fish muscles were also analyzed to determine the organ with the highest accumulation rate.

5 g of the homogenized samples were digested in 5 ml HNO_3 and heated in a water bath until the sample completely dissolved. The mercury samples were further subjected to 12 hours of extraction before analysis. Determination of the heavy metal concentration in the digested samples was done using a titrimetric method with potassium Iodate for mercury and Atomic Absorption Spectrophotometer (USING BUCK 200 AAS (AOAC, 975.23) for Arsenic. The metal concentration was expressed as mg /kg dry weight (ppm).

Data Management and Statistical Analysis

Descriptive statistics were used to determine the mean concentration within the groups and one-way Analysis of variance (ANOVA) across the various cooking process groups to determine if there is any significance on the concentration of the heavy metals in the processed fish samples [16]. Duncan's multivariate test was done using SPSS (p=.05).

RESULTS

The mean concentrations of Mercury (Hg) and Arsenic (As) in all the analyzed samples in the present study are shown in Tables 1 and 2 respectively. Generally, the Mercury mean concentrations obtained showed there is a consistent increase as the week progresses with week 1 having the lowest mean concentration and week 4 having the highest concentration in all the cooking processes (Table 1). The raw sample exposed to mercury had the highest concentrations across the week (0.027±0.016 - 0.078± 0.01mg/kg) except in week 2 where boiled catfish sample had the highest mean concentration (0.33±0.01mg/kg) followed by roasted (0.30±0.01mg/kg) catfish sample (Fig. 1) when compared with other cooking processes. The fried samples had the least mean concentration (Table 1) across the week (0.01±0.0001 - 0.058±0.04mg/kg). Aside from the progressive increase in mean mercury concentration, there were significant differences between raw Catfish and those that underwent cooking processes (Fried, Boiled, and Roasted). The mean mercury concentrations of the cooked samples were lower than the raw samples except for boiled and roasted samples in week 2 (Fig. 1).

Unlike the results obtained in mercury samples, the Arsenic mean concentrations of all the samples decrease as the week progresses except the fried catfish sample which only increased in week 2 (0.010±0.006mg/kg) and followed the same pattern with other cooking processes in weeks 3 and 4 (Fig. 2). The boiled catfish samples had the highest arsenic mean concentration (0.015±0.003 - 0.0005±0.0001 mg/kg) across the week while raw catfish samples had the least mean concentrations when compared with other cooking processes. The mean Arsenic concentrations of catfish samples exposed to cooking processes significantly higher than raw samples (Table 2).

Table 1. Effects of cooking processes on Mercury Concentration (mg/kg) in *Clarias gariepinus* across the weeks

Samples	Week 1	Week 2	Week 3	Week 4
Raw	0.027±0.016	0.037±0.03	0.07±0.01	0.078± 0.01
Fried	0.01±0.0001	0.033± 0.02	0.038± 0.01	0.058±0.04
Boiled	0.01±0.0001	0.33±0.01	0.045±0.087	0.055±0.01
Roasted	0.01±0.0001	0.30±0.01	0.062±0.013	0.06± 0.05

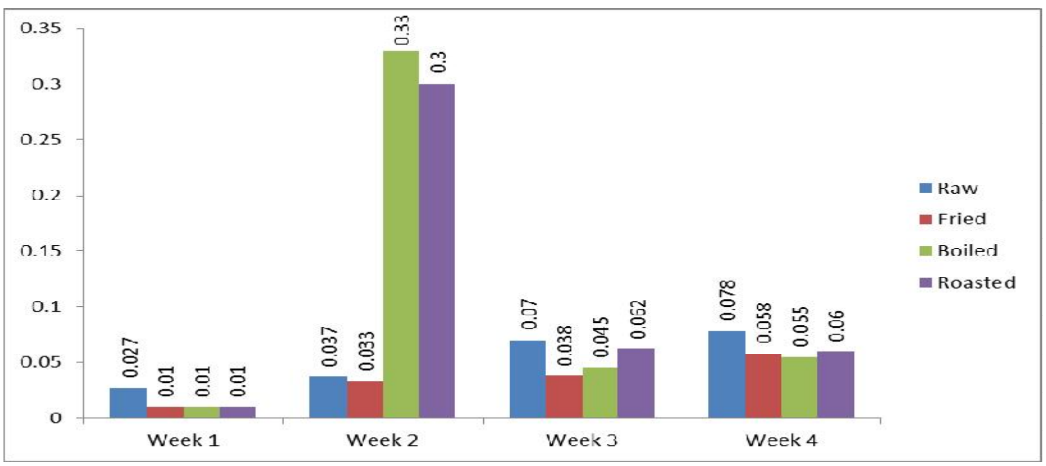

Fig. 1. Graphic Representation of Effects of Cooking Processes on Mercury Concentration (mg/kg) in *Clarias gariepinus* across the weeks

Table 2. Effect of cooking processes on Arsenic Concentration (mg/kg) in *Clarias gariepinus* across the weeks

Samples	Week 1	Week 2	Week 3	Week 4
Raw	0.008±0.005	0.007±0.004	0.001833±0.0006	0.0005±0.0001
Fried	0.009±0.056	0.010±0.006	0.002±0.0005	0.0008±0.0057
Boiled	0.015±0.003	0.009±0.003	0.003±0.0001	0.0005±0.0001
Roasted	0.014±0.001	0.008±.001	0.002±0.0005	0.0005±0.0001

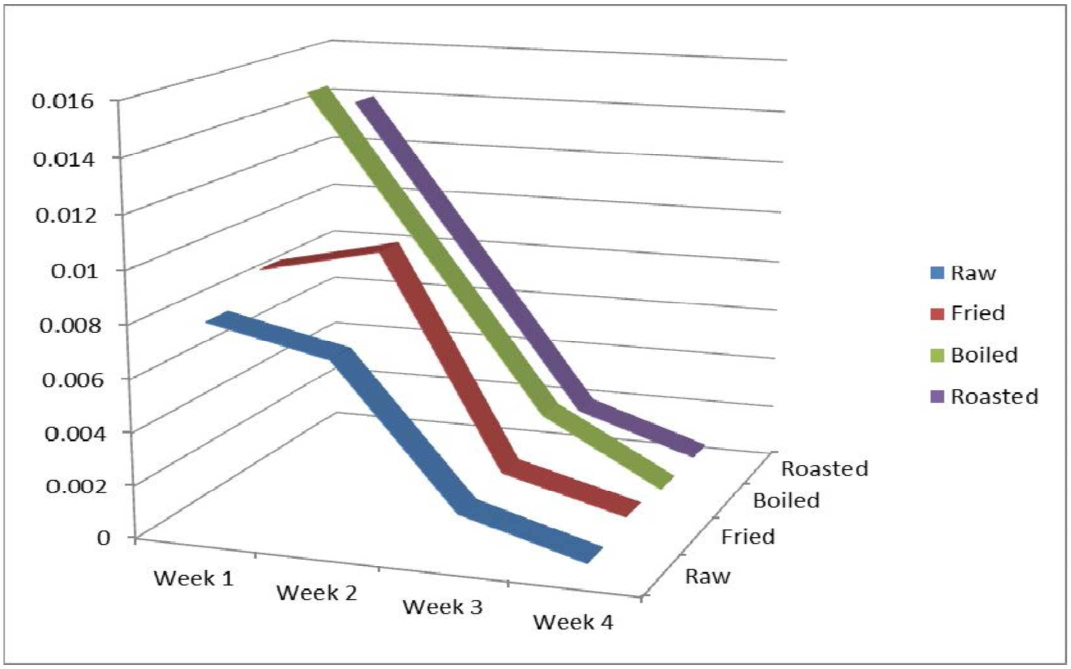

Fig. 2. Graphic Representation of Effects of Cooking Processes on Arsenic Concentration (mg/kg) in *Clarias gariepinus* across the weeks

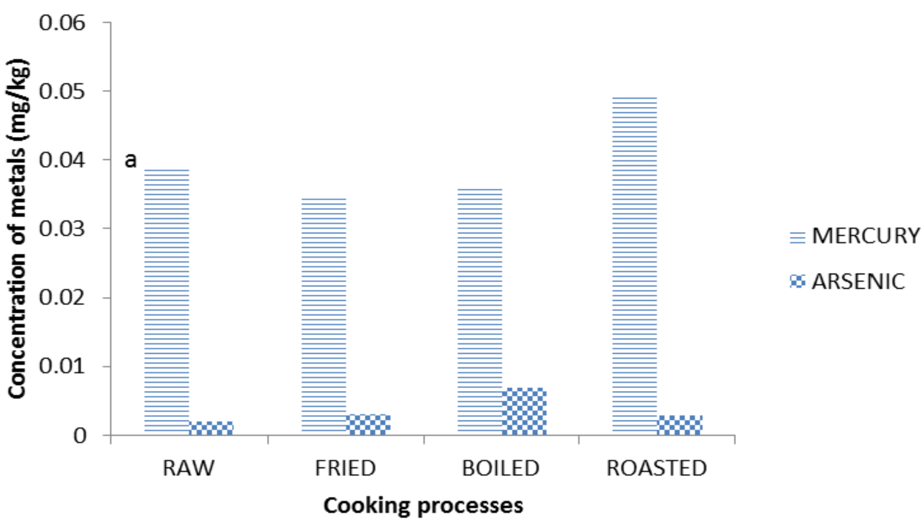

Fig. 3. Effects of cooking process on the mean concentration of mercury and arsenic in *Clarias gariepinus* across four weeks

The Concentration of Mercury and Arsenic in Fish Organs

Gills, Liver, and Muscle are parts of the major target organ for water-borne pollutants with much emphasis on gills because of their role as the site for metal uptake. In this study, the mean concentration of mercury was significantly highest (p=.05) in the liver (0.0081±0.0006mg/kg) followed by gills (0.004±0.0003mg/kg) while the muscle (0.0037±0.0029) was the least (Fig. 4). Likewise, Arsenic concentration was significantly high in the liver (0.0015±0.0003 mg/kg) 0.0015±0.0008 mg/kg and 0.0015±0.0008 mg/kg, respectively. The concentration of mercury was significantly greater in the liver than the gills followed by the concentration in the flesh (p=.05) as shown in Figs. 4 and 5 below:

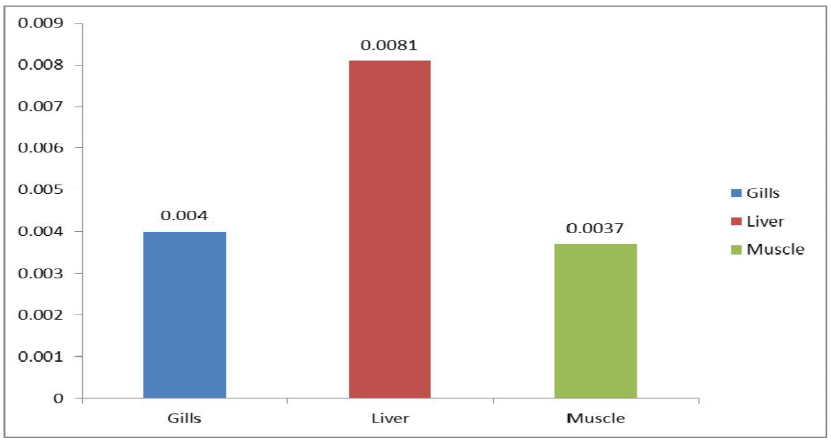

Fig. 4. Graphical Representation of Mean Mercury Concentration (mg/kg) in Organs of *Clarias gariepinus*

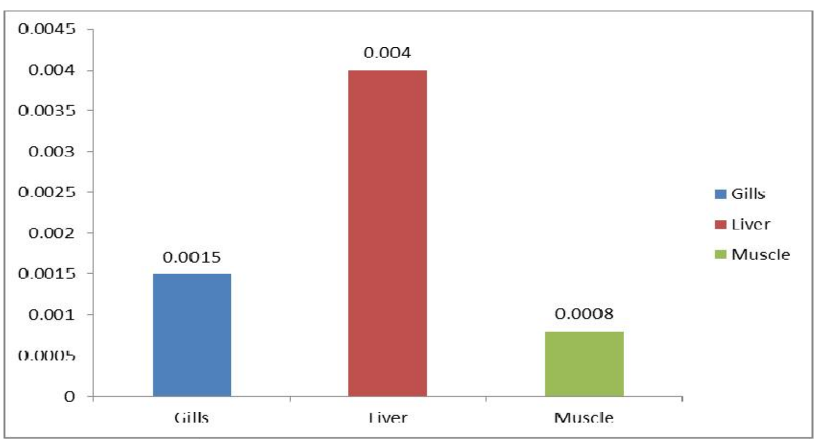

Fig. 5. Graphical Representation of Mean Arsenic Concentration (mg/kg) in Organs of *Clarias gariepinus*

DISCUSSION

In this study, heavy metals (Mercury and Arsenic) were not detectable in the cooked and uncooked samples of the control group. There was a significant ($p<0.05$) disparity in the bioavailability pattern of both Mercury and Arsenic in the tissues and organs of fish. This finding is in agreement with the earlier report by Olaifa et al. [17].

The increase in the mercury concentration as the week progresses indicated the high rate of bioaccumulation of mercury in fish with an increase in the number of periods of exposure. According to the result in this study, there were reductions in mercury concentration in the cooked samples when compared with raw samples. This was in agreement with the report of Inobeme et al. [18] which found out that grilling (a form of the cooking process) brought about a reduction in the content of some heavy metals (especially Zinc, Cadmium, Copper, and Manganese) and Devesa et al. [19] which reported a decrease in the concentration of chromium in catfish due to cooking irrespective of the cooking processes used. This same result was in contrast with the report of Ziarati et al. [20] who reported that heavy metal contents were higher in the processed sample (edible mushroom) when compared to the raw and Gremiachikh et al. [21] with the findings that absolute content of mercury in fish (smoked perch and manufactured and homemade canned salt and fresh-water fishes) remained unchanged during cooking irrespective of the procedure and duration of cooking.

Reduction in the mercury concentration as a result of cooking may be due to an increase in the rate of evaporation of mercury brought about by heating. It may be as a result of the duration at which the fish were subjected to the cooking process. This finding was in agreement with reports from Atta et al. [22] and Ersoy et al. [23] where a reduction in the concentration of heavy metal in the fish was observed after being processed. Though, Jortiem et al. [24] reduced metals (Nickel, Cobalt, and Cadmium) concentrations in crayfish on cooking, it was attributed to the effect of the applied heat in bringing about the degradation of proteins which thereafter affected the heavy metals present in the fish. Atta et al. [22] also reported that cooking processes (baking and steaming) bring about a reduction of heavy metal contents in different organs of fish.

Though, the difference in arsenic concentration across the weeks was not statistically significant ($p>0.05$), the mean concentrations of arsenic in cooked fish samples were higher than the raw samples. This finding was similar to the reports of Ersoy et al. [23] where arsenic concentration was significantly increased in fried fish samples. This increase in concentration could be due to the loss of moisture and the resultant concentration of arsenic in the fish. An increase in arsenic concentration as reported in this study is in contrast with previous reports where the impact of different cooking methods was observed on the content of heavy metals in vegetables and cereals [25] and some foodstuffs [26] revealed that that boiling method helps in reducing the content of Arsenic. The Higher Arsenic concentration recorded in this study is in agreement with the work of Kalogeropoulos et al. [27] where it was observed that the concentration of metals in cooked fish has a higher metal concentration than the raw samples. Part of the potential reasons for this finding may be due to the size of fish exposed to cooking because fish size is inversely proportional to the oil uptake and loss of water during the cooking process. Therefore, a higher loss of water leads to higher metal concentration when a smaller size of fish is cooked. Loss of water and weight occurs during thermal treatment bring about a change in the chemical substances and inorganic contaminants [28].

Concentrations of both Mercury and Arsenic in sampled fish organs were observed in this order: liver>gills> muscle (Figs. 3 and 4 respectively) which is in agreement with the findings of Benson et al. [29]. According to Akueshi, [30], the liver is a primary organ for the storage and detoxification of metal toxicants as well as an organ where the specific metabolic and enzyme-catalyzed processes related to each heavy metal take place, and thus it readily accumulates heavy metals.

Cooking can therefore be related to the reduction of metals in fish tissues with the removal of water and free salts, which are with the soluble amino acids and non-coagulated protein. As reported by Kris - Etherton et al. [31], another approach of reducing mercury concentration in fish and its ingestion through fish consumption could be by removing skin and fat from such exposed fish before cooking. The difference in the levels of accumulation of mercury and arsenic in different organs of fish sampled can primarily be attributed to the differences in the physiological role of each organ as reported by Eneji and Annune, [32].

Anatomically, gills are considered to be the dominant site for contaminant uptake and have physiological properties that maximize absorption efficiency from water accounting for the slightly high level of mercury observed in the gills as well [33]. Also, mercury taken in by the fishes is usually absorbed in the gastrointestinal tract and is available in the water-soluble form in which form it is transported through the circulatory system to the liver as reported by Clarkson [33]. Moreover, since the metabolism of mercury is very slow, it could account for its higher level in the liver.

CONCLUSION

There were effects of different cooking processes (Fried, Boiled, and Roasted) on Catfish samples exposed to Mercury across the week but the effects were not significant when compared with the concentration of mercury present in the raw sample of the same catfish under the experiment. The only cooking method with a better result was the frying procedure (when compared with the mercury concentration in the raw) at the third week of exposure. In the Arsenic exposed samples, none of the cooking procedures was able to reduce but rather increased the Arsenic concentration in the samples.

Comparing the results from this study to previous similar studies, there have been no consistencies concerning the effects of different cooking procedures on heavy metal concentrations in fish and other food items. Since this study and others alike have established minimum effects of cooking procedures on heavy metals in food, then further studies need to be conducted using combined cooking methods per food product or item at different conditions (such as time, temperature, cooking mediums) designed at mitigating effects of heavy metals in food.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author OOT designed the study. Authors AJK, AMM and ASA wrote the protocol, carried out the laboratory experiment and performed the statistical analysis. Authors AJK and AMM wrote the first draft of the manuscript. Authors OOT and ASA managed the analyses of the study. Authors AMM and ASA managed the literature searches. All authors read and approved the final manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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