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COMPARATIVE ANALYSIS OF NUTRITIONAL, ANTI-NUTRITIONAL AND HEAVY METAL COMPOSITION OF FRESH AND SMOKED CAT FISH *(Clarias gariepinus)* FROM HADEJIA RIVER, JIGAWA STATE

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Abstract

Fish are good source of quality nutrients such as protein, unsaturated fatty acids, important minerals and vitamins. Fresh fish requires preservation to increase their shelf live and requires a number of processing techniques for human consumption and safety. This research aimed at comparative analysis of nutritional, antinutritional and heavy metal composition of fresh and smoked catfish (*Clarias gariepinus*) caught from Hadejia River, Jigawa state. The experimental result was compared with recommended dietary allowance (RDA) and maximum permissible limit of intake (MPI) / tolerable upper intake level (UL) to ensure consumer safety and healthy living. The research adopted standard scientific methods for the analysis of the samples. The result on proximate composition showed a significant different in concentration based on moisture, ash, crude fibre and carbohydrate between the smoked and fresh fish at $P < 0.05$. The content of fat, protein, moisture and crude fiber is found to be higher in fresh fish than in smoked fish. The result on metals indicated that concentration of Zinc (Zn), Iron (Fe), magnesium (Mg) and calcium (Ca) significantly differs at $P < 0.05$. Zn, Fe, Mg and Hg were higher in fresh than in smoked fish, and in contrast, calcium was significantly higher in smoked than in fresh catfish. The result on vitamin contents revealed high constituent of vitamin A, vitamin B1, B2 and vitamin C in fresh than in smoked *C. gariepinus* and the smoked fish contained higher concentration of vitamin B3 and B9. Antinutrients analysis showed that there was a statistically significant difference in the concentration of tannin and cyanide (HCN) in the fish samples at $P < 0.05$. Tannin was significantly higher in smoked than in fresh fish. Flavonoids was slightly higher (Mean=4.864mg) in fresh than in smoked (Mean=4.854mg) catfish. In comparison with RDA, *C. gariepinus* contained daily value (DV) of 25% and 20% of energy for adolescents and adults respectively. It contained adequate protein (18.51%), fat (24.89%), carbohydrate (45.31%), thiamine (1.2 mg) and riboflavin (1.8mg). The Ca (32.69mg), Mg (8.4mg), Fe (0.64mg) and Zn (0.41mg) contents in the fish were low compare to their respective RDA. In contrary Hg (Mean=0.055mg), Pb (Mean=0.124mg) and Cd (Mean=0.025) conc. exceeded their respective MPI. The mean conc. of phytate (4.511mg), Cyanide (1.536mg), Oxalate (7.131mg) and Tannin (21.606mg) was low compare to their respective UL (mg/day) of 100-400, 50-60, 200-300 and 0-0.6mg/kg body weight. This research revealed that, both fresh and smoked catfish are of good nutritional sources, however the fresh catfish is higher

in nutritional quality than the smoked. Therefore, the consumers should be encouraged to consume more of the fresh fish than the smoked fish. Heavy metals are hazardous to health, therefore, the consumers of *C. gariepinus* caught from Hadejia river should always apply appropriate and adequate processing and preparation to reduce the concentration of heavy metals in the fish for safe consumption.

Index terms- Quality nutrients, unsaturated fatty acid, *Clarias gariepinus*, consumers and heavy metals

1. INTRODUCTION

Hadejia is an emirate town in Eastern Jigawa State, Nigeria. Hadejia River is a tributary of Yobe River (Komadugu, Yobe), Nguru and Hadejia are among the cities that lie on the banks of the river (Goes, 2002). The river is dammed for the purpose of irrigation for agriculture which is the major activities surrounding the river. The fishing activities in Hadejia contribute to increase availability of fish in the town fish market and in northern Nigeria as a whole leading to price drop in smokery preserved fish (Nigeria Fisheries Committee, 2018)

Fresh fish are the most perishable products if not adequately preserved since they can easily be attacked by microbes. Preserving foods make them to be safe for consumption and also increase their shelf lives. A number of processing techniques are utilized for the preservation of fish and these include chilling, freezing, salting, canning, drying and smoking (Stumbo, 2013; Akintola, 2014). Smoking is the most popular method used in developing countries like Nigeria for fish preservation. Smoking as a food processing technique is temporary, but an effective method of preserving food products, it helps to improve texture and also adds flavor to them (Nizio, et al., 2023). The nutritional compositions of food are important for assessing the economy viability of any nation, because a healthy man brings about a healthy economic development and invariably a healthy nation. Preservation of food by smoking often affect some of the essential minerals, vitamins and proximate compositions of meat and fish by either denaturing them depending on the smoking temperate and nature of woods type used (Adeyeye *et al.*, 2015). Most diets of man are deficient of the essential minerals derivable from animals and this has led to malnutrition and increase in various health challenges amongst the populace (Choge, 2020). Global changes in consumer's life style marked by the increasing demand for nutritional and healthy food products has resulted in the continuing rise in demand for fresh and ready-made food such as fish and meat, fish and meat account for the greater percentage of total protein intake in our diet (Akinsegun *et al.*, 2014; Onyango *et al.*, 2017). The knowledge of their proximate and essential mineral compositions is very important for the estimation of their quality and adequacy in the diet of the consumer. Also, heavy metals are elements of consideration naturally found in the environment and diet, some of them are required in small amount to support good health but when ingested in increase amount (in excess) above level required by the body, they become toxic and dangerous (Jarup, 2003). Therefore, this study assessed the nutritional composition (proximate, vitamins minerals), anti-nutrients contents and heavy metals concentrations of the food products; fresh and smoked catfish (*Clarias gariepinus*) and compared the experimental result obtained with the recommended limits so as to ensure consumer safety and healthy living.

2. NEED OF THE STUDY

The nutrient content in the consumer's food is used to estimate the adequacy of dietary intake of the population, diet disease relationship, health and nutritional status and for achieving the dietary intake of goals of population. One of the complex issues faced in developing countries is food security where animal derived proteins, such as fish and fish items, meat and meat items are lacking in most diet of populace leading to chronic malnutrition. Likewise, metals are required by the body at threshold limit value to support body physiology, however, bioaccumulation of heavy metals lead to a diversity of toxic effects on a variety of body tissues and organs.

3. RESEARCH METHODOLOGY

3.1. Sample Collection

The cat fish used for this research were caught from Hadejia River. The total of ten fish were collected and divided into two, one part was used as fresh sample and the other part used as smoked sample. Smoking of the fish was carried out in Kazaure town Jigawa State, using dried neem tree (*Azadirachta indica*). Analysis of the

fish samples were conducted at Bayero University Kano, Kano State and National Research Institute Zaria, Kaduna State. Nigeria.

Preparation of Fresh and Smoked Catfish Samples

Both fresh and smoke catfish samples were washed and rinsed with distilled water to remove adhering substances and then drained. The fresh fish was dismembered using clean knife and the guts removed. It was oven-dried at a temperature of 105°C, then pulverized into powder and stored in a capped plastic container. The smoked sample was deboned, pulverized and stored in another capped plastic container for further analysis.

3.2. Digestion of the Fish Samples for Essential Mineral Analysis

The digestion of samples (Fresh and smoke fish) was carried out using a mixture of concentrated HNO_3 and $HClO_4$ in ratio two to one (2:1). Ten-centimeter cube (10 $cm^{3)}$ of the mixture was added into each of the digestion flasks containing 2.0 g of the pulverized fresh fish sample and smoked fish sample. The samples were digested in a fume hood until a clear solution/digest was obtained. The digests were allowed to cool, then filtered using Whatman No1 filter paper, kept in digestate containers and refrigerated for two days before analysis.

3.3. Proximate Analysis of Fresh and Smoked CatFish *(Clarias gariepinus*)

The Moisture, Ash and Crude fiber were determined using grading drying methods and weight differences according to the standard method of Association of Analytical Chemists (AOAC) as described by Wu and Wu (2017).

3.4.1. Moisture: for each of the samples, a clean crucible was dried to a constant weight in an oven at 110°C, it was cooled in a desiccator, weighed (W1). Two grams of the prepared sample was added into the crucible and reweigh (W2). The crucible containing the sample was dried in the oven to a constant weight (W3). The percentage moisture content was calculated using the formula below:

$$\% \text{ Moisture content} = \frac{W2-W3}{W2-w1}\ x\ 100$$

3.4.2. Ash content: for each of the samples a porcelain crucible was dried in an oven at 100°C for 10 min, cool in a desiccator and weigh (W1). Two grams of the finely ground sample added into the porcelain crucible and reweigh (W2), it was ignited and then transferred into a furnace which was set at 550°C. The sample was left in the furnace for eight hours to ensure proper ashing. Then the crucibles were removed from furnace, cooled in a desiccator and weighed (W3). The percentage ash content was calculated using the following formula:

$$\% \text{ Ash Content} = \frac{W2-W3}{W2-w1}\ x\ 100$$

3.4.3. Crude fiber: Two grams of each sample was weighed into separate round bottom flask. To each of the flask, 100cm^3 of 0.25 M sulphuric acid solution was added and the mixture boiled under reflux for 30 min. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it became acid free. The insoluble matter was transferred into the flask and 100 cm^3 of 0.31 M Sodium Hydroxide solution was added, the mixture was boiled under reflux for 30 min and filtered. The residue was washed with boiling water until it became base free, the content was dried to constant weight in an oven at 100°C, cooled in a desiccator and weigh (C1). The weighed sample (C1) was incinerated in a muffle furnace at 550°C for 2 hours, cooled in a desiccator and reweigh (C2).

Calculation: The loss in weight on incineration correspond to the crude fiber present in the sample which is equal to C1-C2

$$\% \text{ Crude fibre} = \frac{C1-C2}{\text{Weight of original sample}}\ x\ 100$$

3.4.4. Crude lipid was determined using Soxhlet Extraction Method; a clean, dried 500cm^3 round bottom flask containing few anti-bumping granules was weighed and labelled (W1), 100g of the sample was placed in the flask and petroleum ether for extraction was poured into the flask and fixed with soxhlet extraction unit. The extractor thimble weighing twenty grams was fixed into the soxhlet unit. The round bottom flask and a condenser were connected to the soxhlet extractor with cold water circulation connected. The heating mantle was switched on and the heating rate was adjusted till the solvent refluxing at a steady rate. The extraction was carried out for 6 hours. The solvent was recovered and the oil was dried in an oven set at 70°C for 1 hour. The round bottom flask and oil was weighed (W2). The lipid content was calculated using formula:

% Crude Lipid content = $\frac{W2-W1}{Weight\ of\ Sample}\ x\ 100$

3.4.5. Protein content was obtained using conversion factor of Nitrogen concentration multiply by 6.25. Nitrogen concentration was determined using Kjeldahl method which involves three stages; digestion of the samples, distillation and titration. The ground defatted fish sample (100g) in an ashless filter was dropped into a 300cm^3 volume Kjeldahl flask, and 50ml of concentrated H_2SO_4 was added. The flask was transferred to the Kjeldahl digestion apparatus and was digested until a clear green color was obtained. The digest was cooled and diluted with 100cm^3 of distilled water. Distillation of the digest was carried out using 500cm^3 Kjeldahl flask containing anti bumping chips, 40cm^3 of 40% NaOH was slowly added to the flask containing mixture of 50cm^3 of 2% boric acid and 3 drops of mixed indicator was used to trap the ammonia being liberated. The conical flask and the Kjeldahl flask were placed on Kjeldahl distillation apparatus with the tubes inserted into the conical flask, heat was applied to distill out the NH_3 evolved with the distillate collecting into the boric acid solution. The distillate was titrated with 0.1 M HCl. Nitrogen (N_2) concentration was calculated using the formula below:

% N_2 = $\frac{14\ x\ M\ x\ Vt\ x\ V}{Weight\ of\ sample\ (mg) x\ Va}\ x\ 100$

% Crude Protein = % N_2 × 6.35

Where; M = Actual Molarity of Acid, V = Titer Value of HCl used, V t = Total volume of diluted digest and Va = Aliquot volume distilled

3.4.6 Total carbohydrate was determined by difference (Birch, 1985). The sum of the percentage moisture, ash, crude lipid, crude protein and crude fiber was subtracted from 100 as written below:

% carbohydrate = 100 - (% moisture + % Ash + % fat + % Protein + % Fibre)

3.4.7 Calorific value was calculated by summing the multiplication values for crude protein, fats, and carbohydrate (exclude crude fibre) by the factors 4, 9 and 4 respectively.

3.5 Analysis of Metal Concentration (Mercury, Lead, Cadium, Calcium, Iron Magnesium, Chromium and Zinc) in fresh and smoked fish

The metals were analysed using a black model 200A flame Atomic Absorption Spectrometer (AAS) as described by Bisergaeva and Sirieva (2020). The major underline principle of AAS is that the ground state atoms are capable of absorbing radiant energy of their own specific resonance wavelength when passed through a solution containing the atoms in question and part of the light will be absorbed. The extent of absorption is proportional to the number of ground state atoms present in the flame. The procedure involved setting the apparatus according to the instructions; AAS was calibrated using the respective standard solution for each metal and the reagent blank solution run with the sample. At each run a calibration curve was plotted indicating the absorption values against the metal concentration in µg/ml and reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption values of the samples.

3.6 Vitamins (A, B1, B2, B3, B9 and C) Contents of Fresh and Smoked Fish Analysis

The vitamins were analyzed using UV-Visible spectrophotometry. This method is based on interaction between light and matter. The principle of UV-Visible spectrophotometry is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectrum. Each compound absorbs light over a certain range of wavelength. The method measures how much a chemical substance absorbs light passes through the sample solution. The amount of light absorbed is proportional to the concentration of the absorbing substance. The sample solution for each of the vitamins was prepared accordingly with the control. The control blank was used to calibrate the spectrophotometer and sample solutions were run in their respective order. The standard table and standard curve from which, reading was taken displayed by the digital reader. The concentration of each vitamin in the fish samples was obtainable at its

correspondent wavelength of 325nm, 269nm, 242nm, 261nm, 282nm and 478nm for retinol, thiamin, riboflavin, niacin, folic acid and ascorbic acid respectively.

3.7 Analysis of Anti-nutritional Factors of Fresh and Smoked Fish *(Clarias gariepinus)*

3.7.1 Phytate content was determined using colorimentric mehod as described by McKie and McCleary (2016). The reagents; colour reagent, ammonium molybdate and phytase were prepared. Colour reagent was prepared by mixing 10g ascorbic acid, 90ml distilled water and 5.35mL conc. Sulfuric acid. Ammonium molybdate (1.25g) was dissolved in 20ml distilled water and the phytase assay buffer (200mM sodium acetate, at Ph 5.5) was prepared by dissolving 27.2g acetate tryhydrate acid in 0.9L of distilled water.
Phytic acid was extracted from the fish samples by adding 20ml HCL acid (0.66M), mixed vigorously for 3-24 hours under room temperature. Then 1ml of the extract was centrifuged at 11000 rpm. Then, 0.5ml supernatant was transferred to a fresh tube and neutralized with 0.5ml NaoH (0.75M). The solution was used in the enzymatic dephosphorylation of acid reaction. Supernatants obtained from the enzyme dephosphorylation of the phytic acid was used for colorimetric assay; the colour reagent (0.5ml) was added to 1ml of the supernatant in a microfuge tube and mixed using vortex mixer and incubated for 1 hour in water bath at 40^{o}c. After incubation, all reaction solutions prepared were added and mixed thoroughly with vortex mixer, then, 1ml of the mixture was transferred to a 1cm path-length microcuvette and measured using colorimeter. The absorbance of each solution was measured at 655 nm, the absorbance values of each sample and phosphorus standard solutions were used in the calculation of total phosphorus and phytic acid.

Total phosphorus (g/100g) = $\frac{meanM \times 20 \times 55.6}{10{,}000 \times 1.0 \times 1.0} \times \Delta A_{phosphorus}$

The phytic content of a sample was calculated as follows:

Phytic acid (g/100g) = $\frac{phosphorus(g/100g)}{0.282}$

3.7.2 Oxalate content in the fish samples was analyzed using the method described by Amaechi (2009) which involves three steps procedure of digestion, precipitation, and permanganate titration.
Digestion: Two grams of samples of each treatment in triplicates were suspended in 190ml de-ionized water in separate 250ml volumetric flasks; 10ml of 6 M HCl was added to each and the suspension digested at the boiling point of water (100^{o}C) for 1 hour, it was cooled and made up to 250ml and filtered.
Precipitation: Duplicate portion of filtrate (125ml) was measured into a beaker and four drops of methyl red indicator added followed by the addition of concentrated NH_4OH solution drops, until the test solution changes from salmon pink color to faint yellow color (pH 4-4.5). Each portion was heated to 90^{o}C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was heated again to 90^{o}C and 10 ml of 5% $CaCl_2$ solution was added while stirring constantly. It was cooled and left overnight in refrigerator. The solution was then centrifuged at a speed of 2500 rpm for 5 min, the supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% H_2SO_4 solution. At this point the total filtrate resulting from digestion of 2g of sample was made up to 300ml. Titration: Aliquots of filtrate (125 ml) was heated until near boiling, and then titrated against 0.05ml M standard ($KMnO_4$) solution to a faint pink color which persists for 30 seconds. The concentration of oxalate was calculated as:
1ml of 0.05ml M standard $KMnO_4$ = 2.2mg of oxalate/weight of sample used

3.7.3 Determination of condensed tannin content: Tannin was determined using spectrophotometric method as described by Nair, *et al.* (2015).

The reagent used were Folin-Denis reagent, saturated sodium carbonate solution and tannin standard.
Folin-Denis reagent was prepared by adding sodium tungstate (100g), phosphomolybdic acid (20g) and 85% phosphoric acid (50ml) into 750ml of water. Saturated sodium carbonate solution was obtained by adding anhydrous sodium carbonate (35g) into 100ml of water, the content was dissolved at 70-80^{o}c and kept overnight, then the clear liquid on it was decanted before use. Tannin standard was prepared by dissolving tannic acid (100mg) into 1 litre of water. Fresh solution of standard was prepared for each determination (1ml=0.1mg of tannic acid).
Aliquots of standard tannic acid (0-10ml) solution were pipetted into 100ml volumetric flasks containing 75ml of water, 5ml of Folin-Denis reagent and 10ml sodium carbonate ($NaCo_3$) solution was added into each of the flasks, the filtrate containing 0.1mg of tannic acid was added to each flask and made up to 100ml with distilled

water. The samples were added and mixed well and the colour was measured after 30 minutes at 760 nm against experimental blank. Tannin conc. was calculated using formular:

$$\text{Tannic acid} = \frac{\text{mg of tannic acid x dilution x100}}{\text{ml of sample x weigh of sample x 1000 colour development}}$$

3.7.4 Determination of cyanide content: Cyanide was determined using simple picrate method as described by Nwokoro, et al. (2009). Alkaline picrate reagent was prepared using 2ml of 2% KOH and 1ml of picric acid. Whatman no 1 paper were dipped into the reagent for 15 minute, after which the paper were removed and used for the analysis. 10g of each fish samples was diluted with 20% of HCL solution in glass bottles. Strips of the papers impregnated with the picrate reagent were used in suspension above the content to seal the glass bottles containing acidified fish samples and left at room temperature for 24hours. The paper strips were then removed and rinsed with 50% ethanol solution measured at 510nm using a spectrumlab 23A spectrophotometer. Graph was plotted; absorbance against concentration and the cyanide levels of the samples were extrapolated from the standard curve

3.7.5 Determination of flavonoids content: Aluminum chloride colorimetric method as described by Chandra, et al, (2014) was adopted to determine total flavonoid; using quercetin as standard for calibration, quercetin standard solution was prepared by serial dilution using methanol (5-200ug/mL). Stock solution was prepared by dissolving 5g quercetin in 1.0ml methanol. In triplicate, Aluminum chloride (0.6ml) was added to the extract and also added to the diluted standard separately and mixed. The mixtures were incubated for 60 min at room temperature. The absorbance of each mixture was measured against blank at 420nm wavelength. The concentration of total flavonoid content in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent (QE/g).

3.8 Descriptive Statistic

Data obtained were subjected to Analysis of Variance (ANOVA) to obtain the square mean, f – value and p – value. And $p < 0.05$ was considered significantly different. The descriptive statistic was also used to find the mean, standard deviation Least Significant Difference (LSD) was used to identify significant differences between the means. Results were presented in tables as mean ± standard deviation.

4. RESULT AND DISCUSSION

4.1 Comparison of the Proximate Composition between the Fresh and Smoke Catfish

Table 4.1: Descriptive statistics of the proximate composition in fresh (F) and smoked (S) *C. gariepinus*

Proximate composition	Mean	Standard deviation	Total mean ({F+S}÷2)	Mean square	F-value	P-value
Moisture (%)	F=9.019 S=5.797	0.527 0.689	7.408	15.569	41.368	0.003
Fat (%)	F=25.284 S=24.505	0.697 0.368	24.895	0.910	2.931	0.162
Ash (%)	F=2.495 S=3.792	0.306 0.229	3.144	2.521	34.569	0.004
Protein (%)	F=19.068 S=17.946	0.651 0.271	18.507	1.886	7.598	0.051
Fiber (%)	F=0.841 S=0.626	0.0675 0.0291	0.733	0.070	25.744	0.007
Carbohydrate	F=43.293	0.717	45.314	24.495	17.877	0.013

(%)	S=47.334	1.492				

The mean, standard deviation, mean square, f-value and p-value of the proximate composition of the fresh and smoked *C. gariepinus* are presented in Table 4.1. The results shown that Fat, protein, Moisture and Crude Fiber were significantly higher in fresh fish with (Mean=25.284, SD=0.697), (Mean=19.068, SD=0.651), (Mean=9.019, SD=0.527) and (Mean=0.841, SD=0.068) respectively than in the smoked fish with (Mean=24.505, SD=0.368), (Mean=17.946, SD= 0.271), (Mean=5.797, SD=0.689) and (Mean=0.626, SD=0.029) respectively. While Ash and Carbohydrate content were significantly higher in smoked fish with (Mean = 3.792, SD = 0.229) and (Mean = 47.334, 1.492) respectively than in fresh fish with (Mean=2.495, SD=0.306) and (M=43.293, SD=0.717) respectively. The two fish samples differ significantly based on, Moisture Content (%), F = 41.368, p = 0.003, Ash Content (%), F = 34.569, p = 0.004, Crude Fiber (%), F = 25.744, p = 0.007 and Carbohydrate (%), F = 17.877 at p = 0.013.

Macronutrients are the essential nutrients needed by the body in large quantities to remain healthy. The macronutrients; Protein, Fat, and Carbohydrate are high in both samples of the fish. The protein content in smoked fish was lower compared to protein content in fresh fish. This difference in value could be associated with the effect of smoking heat on the protein as affirmed by Abraha *et al.* (2018) that smoking is a processing technique which has an impact on fish protein denaturation leading to changes in physical and chemical structure of protein. The result correlate with that of researches conducted by Abraha *et al.* (2018) and Khalil *et al.* (2018) who established on general note that fish is made up of 15-24% and 18-20% protein respectively. The moisture and fat content are found higher in fresh fish than in smoked fish, these reduced values in smoked fish could also be associated with the drippings that occurred during smoking process. Both samples were high in fat content as established by Khalil *et al.* (2018) who said fat content of fish ranges from 0.2 to 25% especially polyunsaturated fatty acids (PUFA) which are essential for the proper growth of children and are not associated with occurrence of cardiovascular disease. The essentiality of macronutrients to the body cannot be over stressed as they remain the ingredient for survival and healthy living. The carbohydrate provide energy for body tissues and it is the primary source of energy for the brain (Holesh, et al., 2024) while protein remains the building blocks of the body system and fat serves as the major source of energy, important for hormone production, cell growth and serves as a medium through which some vitamins are absorbed into the body system (Calder, 2015)

The recommended dietary allowance (RDA) which is the average daily level of intake sufficient to meet the nutrient requirement of nearly all (97-98%) individual was presented in Table 4.5 against the parameters. These values are often used to plan nutritionally adequate diets for individuals (Murphy and Barr, 2006). The values input are values of RDA ranges for adolescents to adulthood based on age and gender. The calorific values (energy values) of both fresh and smoked catfish provide daily value of 25% and 20% for adolescents and adults respectively. According to Institute of Medicine (2005), the daily requirement for protein is between 10-35%, for fat is 20-35% and carbohydrate is 45-65%. Both fish samples contain 18-19% protein, 25% fat, and 43-47% carbohydrate translating that consuming the required serving of fish per day will adequately provide children and adolescent with recommended dietary allowance for protein, fat and carbohydrate.

4.2 Comparison of Concentration of Mineral Elements and Heavy Metals (mg/l) in the Fresh and Smoked Catfish

Table 4.2: Descriptive statistics of mineral elements and heavy metals concentration in fresh (F) and smoked (S) *C. gariepinus*

Proximate composition	**Mean**	**Standard deviation**	**Total mean ({F+S}÷2)**	**Mean square**	**F-value**	**P-value**
Zn	F=0.569 S=0.246	0.002 0.001	0.408	0.156	55233	0.000
Pb	F=0.124	0.013	0.124	0.000	0.043	0.845

	S=0.123	0.006				
Fe	F=0.730 S=0.544	0.003 0.006	0.637	0.052	2386.531	0.000
Mg	F=10.397 S=6.424	0.055 0.173	8.410	23.673	1432.723	0.000
Ca	F=9.140 S=56.230	0.259 11.770	32.685	3326.155	48.000	0.002
Cr	F=-0.638 S=-0.648	0.018 0.024	-0.643	0.000	0.351	0.586
Cd	F=0.024 S=0.025	0.001 0.000	0.025	6.67E-07	4.000	0.116
Hg	F=0.118 S=-0.009	0.075 0.161	0.055	0.024	1.525	0.284

Table 4.2 present the mean, standard deviation, mean square, f-value and p-value of mineral elements and heavy metals concentration in the fresh and smoked *C. gariepinus*, Zn, Fe and Mg were significantly higher in fresh fish with (Mean = 0.569, SD = 0.002), (Mean = 0.730, SD = 0.003) and (Mean = 10.397, SD = 0.055) respectively than in the smoked fish with (Mean = 0.246, SD = 0.001), (Mean = 0.544, SD = 0.006) and (Mean = 6.424, SD = 0.173) respectively. On the contrary, Ca is significantly higher in smoked fish (Mean = 56.230, SD = 11.770) than in fresh fish (Mean = 9.140, SD = 0.259). There was a statistically significant difference in the concentration of the mineral elements; the Zn content of the fish samples at, $F = 55233, p < 0.000$. There were also statistically significant differences in the concentration of Fe, Mg and Ca between the fresh and smoked fish, $F = 2386.531, p < 0.000$, F = 1432.723, $p < 0.000$, and $F = 48.000, p < 0.002$, respectively. On the contrary, the heavy metals shown no significant difference in their concentration in both fresh and smoked catfish at $p > 0.05$

Mineral elements are vital nutrients required by the body for growth and development; they support body structure and maintain normal function in the body. There was reduction in the concentration of Zn, Fe, and Mg in smoked fish compare to initial concentration found in fresh fish and this could be associated with the effect of the preservation technique. On the other hand, calcium content got significantly increased with smoking process, and the reason for the increased is yet to be known.

In comparison of the minerals concentration in the fish with the recommended dietary allowance (RDA) (Table 4.5) using the total mean of the mineral elements. *C. gariepinus* contains certain daily values (DV) for: calcium (2.89%); iron (female = 7.96%, male = 3.54%); magnesium (2.71%) and zinc (female = 5.1%, male = 3.7%). Minerals found in fish are extremely bioavailable (Kassebaum *et al.*, 2014), however based on these results (DV), catfish needs to be complemented with vegetables and other minerals rich foods to achieve the RDA for age and sex. The importance of mineral elements for body physiology cannot be abandoned; as calcium is major for bone formation and mineralization, calcium also regulates proper functioning of tissues and central nervous system (Sihotang *et al.*, 2019), and aid clotting of blood (*Imdad et al.*, 2011) and reduce the risk of osteoporosis in later life. Iron as components of hemoglobin, regulates oxygen across the body (*Antony et al.*, 2016). Magnesium regulates muscles, nerve function, blood sugar levels and blood pressure and also aid in making protein, bone and DNA (Volpe, 2013). Zinc is found in all body cells supporting healthiness, boost immunity, and fight against diseases (Keen and Gershwin, 1990). Zinc also plays important roles in brain functioning, growth of fetus and children (*Onyekere et al.*, 2020)

Fish as food when exposed are vulnerable to pollutants such as heavy metals that threaten their safety for human consumption. Heavy metals are naturally present in earth's crust (Hasimuna *et al.*, 2021) and have high atomic weight and a density of at least five times greater than that of water (*Hasimmna et al.*, 2021). Among the

most toxic heavy metals present in nature are mercury, lead, chromium and cadmium (Tchounwoe *et al.*, 2012). The results on the heavy metals showed that, *C. gariepinus* caught from Hadejia River contains high concentration of Hg, Lead and Cadmium.

The quality and safety of food products determine the protection of the public health. Therefore, in comparison with the maximum permissible limits of metal's intake as indicated in Table 4.6, the concentration of Hg in fresh fish, and concentration of lead and cadmium in both fresh and smoked fish exceeded the maximum permissible limit of intake. This result correlate with that of Akpanyung *et al.* (2014) who reported higher concentrations of some heavy metals including Pb, Cr and Cd above maximum recommended levels in fish organs obtained from two fishing site in Akwa-Ibom state. This result translates that the fish might not be safe for human consumption until when further processed to reduce or eliminate the toxic elements. Toxic metals such as Hg, lead and cadmium are injurious to health. Toxic metals have the ability to cause membrane and DNA damage and perturb protein function and enzyme activity (Witkowska *et al.*, 2021). Some essential metals such as zinc, iron, copper, magnesium, calcium, sodium, manganese can also cause adverse effects when the consumption exceed the tolerable upper intake level (shown in Table 9) or when their homeostasis is disturbed by some factors (Potocki *et al.*, 2012; Marchelti, 2013).

According to Gerhardson, (2022) and Garza-Lombo *et al.* (2018), exposure to xenobiotic metals can result to many chronic illnesses such as cardiovascular, reproductive, renal, gastro intestinal, respiratory and neurological disorders. Excessive level of metals intake and exposure to xenobiotic metal can also lead to cancers, lungs and kidney diseases, immune and nervous system dysfunction, lactation problems, low birth weight, muscular impairments and dermatitis. Appropriate preparation and processing are keys to reduction of heavy- metals in food products, such as fish. The mercury concentration in the fish got reduced with smoking. This correspond with the research of Perello *et al.* (2008) who affirmed that mercury in food differs between raw and cooked foods and can further be reduced by drinking green or black tea and black coffee during a meal (Oue'draogo and Amyot, 2011).

4.3 Comparison of the Vitamins Composition of the Fresh and Smoked Fish

Table 4.3. Mean and standard deviation of the vitamin composition in the fresh and smoked fish

Fish type	Statistics	Vit.A (μM)	Vit.B1 (mg/L)	Vit.B2 (mg/L)	Vit.B3 (mg/L)	Vit.B9 (mg/ml)	Vit.C (mg/ml)
Fresh	Mean	38.916	1.767	1.928	2.195	0.032	0.471
	Std Dev	0.790	0.265	0.007	2.500	1.093	0.108
Smoked	Mean	30.030	0.657	1.858	2.322	0.043	0.453
	Std Dev	0.890	0.254	0.007	2.741	1.246	0.105
Total	Mean	34.473	1.212	1.893	2.258	0.037	0.462
	Std Dev	4.923	0.259	0.014	2.620	1.169	0.106

Note: μM = micromole, mg/L = milligram per litre, mg/ml = milligram per millilitre

The result in Table 4.3 showed that fresh fish contained higher content of vitamin A and vitamin B1 with (Mean = 38.916, SD = 0.790) and (Mean = 1.767, SD = 0.265) respectively compared to that of smoked fish content (Mean = 30.030, SD = 0.870) and (Mean = 0.657, SD = 0.254) respectively. Whilst the content of vitamin B3 and B9 were higher in smoked fish with (Mean = 2.322, SD = 2.741) and (Mean = 0.043, SD = 1.246) than found in fresh fish with (Mean = 2.195, SD = 2.500) and (Mean = 0.032, SD = 1.093) respectively. And vitamin C with mean value of 0.471 in fresh fish was slightly higher than in smoked fish with mean value of 0.453.

Vitamins are organic compounds, the micronutrients that are required by the body in a smaller amount to support body growth and development. Vitamins are very important nutrients required for body's metabolic processes (Huskisson, 2007).

The concentration of the vitamins researched except for Niacin (B3) and Folate (B9) were low in smoked fish compare to that of fresh fish. These reduced values could be associated with the heat treatment during smoking process which induced loss of fat and fluid from the fish. For instance, vitamin A (retinol) is soluble in fat (oil) while vitamin B complex and vitamin C are water soluble vitamin, this condition possibly aids the loss of mentioned vitamins along with the dripped liquid during the fish preservation (smoking). According to Abraha *et al.* (2018), processing methods affect the compositional constituents of fish, such as proteins, fats, vitamins, minerals, sensory attributes and general appearance.

Generally, vitamins play important roles in human body physiology; vitamin A is highly recognized for its important functions in sight system and support eye health. The deficiency of which can lead to blindness, especially among children and younger adults (National research council (NRC), 2023). Thiamine (vitamin B1) is an essential nutrient for fetal health and lactating mothers and a key substance that support brain functions in babies and its deficiency can result to still birth (NRC, 2023).

Vitamins in fish are highly bioavailable, the constituent, of vitamins B1 and B2 in both samples of smoked and fresh catfish will adequately supply the body with recommended dietary allowance as indicated in Table 4.5. Both the smoked and fresh catfish showed excellent sources of riboflavin and thiamin, they are considerable source of vitamin C. as the fish contained 56% daily value (DV) of vitamin C. Whilst DV for vitamin B3 (Niacin) was 15%. However, both samples showed poor source of vitamin A with 4% DV. Vitamin B complex accelerates enzyme functioning which facilitate chemical processes in human body. Riboflavin (vitamin B2) is important for catalyzation of respiration and for cell differentiation while Niacin controls the level of low-density lipoprotein in the body and folate aids cell division and support the production of RNA and DNA (Ali, *et al.* 2022). Energy metabolism requires vitamin B1, B2 and B3 (Roth *et al.* 2018). Vitamin C plays key roles in disease prevention, by boosting immune health, prevent scurvy and

enables proper functioning of enzymes. According to Roth *et al.* (2018), vitamin C is essential for wound healing, maintaining tissue integrity and aid the absorption of iron in the nervous system. The importance of vitamin's functions in the body cannot be overemphasis as they are vital to the survival of infants and children.

4.4 Comparison of the Anti-Nutritrients (mg/g) and Flavonoid (mg/g) Composition the Fresh and Smoked Fish

Table 4.4: Descriptive statistics of anti-nutritrients and flavonoid concentration in fresh (F) and smoked (S) *C. gariepinus*

Flavonoid and antinutrients	Mean	Standard deviation	Total mean ({F+S}÷2)	Mean square	F-value	P-value
Flavonoids	F=4.864 S=4.854	0.023 0.002	4.859	0.000	0.560	0.496
Tannins	F=11.041 S=32.172	0.372 1.660	21.607	669.779	463.070	0.000
Oxalate	F=7.003 S=7.260	0.961 1.661	7.132	0.099	0.054	0.828
Phytate	F=4.841 S=4.181	0.504 0.505	4.511	0.653	2.566	0.184
HCN	F=1.584 1.488	0.036 0.045	1.536	0.014	8.258	0.045

The mean, standard deviation, mean square, f-value and p-value of the anti-nutritrients and flavonoid concentration in the fresh and smoked *C. gariepinus* are presented in Table 4.4 showed that HCN (mg/g) is significantly higher in fresh fish with (Mean = 1.584, SD = 0.036) than in the smoked fish with (Mean = 1.488, SD = 0.045). On the contrary, Tannins (mg/g) is significantly higher in smoked fish (Mean = 32.172, SD = 1.660) than in fresh fish (Mean = 11.041, SD = 0.371). The result showed that there was a statistically significant difference in the concentration of Tannin and HCN in the fish samples at; $F = 463.070, p = 0.000$ and $F = 8.258, p = 0.045$ respectively. And there were no significant differences in the concentration of oxalate and phytate at p. value > 0.05 while Flavonoid also exhibits no significant difference in concentration between the fresh and smoke fish at; $F = 0.560, p = 0.496$.

Antinutrients are natural compounds produced in plants and can also be synthesized, by the way of feeding and uptake, they are found in animal, and they interfere with the absorption of nutrients. Antinutrients bind with nutrients (such as calcium, magnesium, iron, protein and zinc) consumed together in same meal to form compounds that are un-absorbable by the body therefore prevent the utilization of the nutrients in the body and as a result affect the normal body growth and development (Samtiya, *et al.*, 2020). However, many traditional methods of food processing such as soaking, fermentation, spouting, malting and cooking reduce or eliminate antinutrients such as oxalate, phytate, polyphenols, etc. (Hotz, 2007). Salim *et al.* (2023) proved that the health benefits of antinutrients outweigh the toxic effects as they act as factors of antioxidant, chemo preventive, neuroprotective, anticholesterolemic, antidiabetics, anticancer and many other beneficial properties especially when consume in moderation.

The two samples of fish (fresh and smoked *C. gariepinus*) proved safe for human consumption with this factor (antinutrients) as they contain constituents far below the toxicity level when compare to their permissible daily intake for phytate; 2000-2600mg for vegetarian diets and 150-1400mg for mixed diets (Gemede, 2014), for tannins; 0-0.6mg/kg body weight (FAO/WHO, 1970), however tannins recommendation for safe and optimal amount may vary depending on individual variables such as age, health status and overall dietary intake. The UL of oxalic acid is estimated to be 200-300mg/day for most people and less than 100mg/day for those at risk for kidney stone (Shastri, *et al.*, 2023) while the hydrogen cyanide (HCN) may be dangerous to life at 50-60mg/m3 (Doman, *et al.*, 2022). However, the reviewed immediately dangerous to life health concentration (IDLH) for CN is estimated to be 25mg/m^3 and the HCN is fatal at 56mg/m^3 (Skowron and Konieczko, 2017).

4.5 Comparison of the Catfish Constituents with WHO Recommended Dietary Allowance

Table 4.5 and 4.6 depict total **mean values** of each nutrient in the examined catfish samples against the Recommended Daily Allowance (RDA) and Maximum Permissible Limit Intake (MPI)/Tolerable Upper Intake Level (UL) for the nutrients.

Table 4.5: Total mean of the catfish nutrients and their respective RDA

Nutrients	Total Mean Value of Fish	RDA
Calorie	479	1900 – 2900
Fat (%)	24.89	20 -35
Protein (%)	18.51	10 -35
Carbohydrate (%)	45.31	45 – 65
Vitamin A (mcg)	34.47	700 – 900
Vitamin B1 (mg)	1.21	1.1 – 1.2
Vitamin B2 (mg)	1.89	1.1 - 1.3
Vitamin B3 (mg)	2.26	14.16
Vitamin B9 (mg)	0.04	0.4
Vitamin C (mg)	46	75 – 90
Calcium (mg)	32.69	1000 – 1300
Iron (mg)	0.64	8 (male), 18 (female)
Chromium (mg)	-0.64	0.011-0.03
Magnesium (mg)	8.4	310-410
Zinc (mg)	0.41	8 (female), 11 (male)

Table 4.6: Maximum permissible limit of intake/tolerable upper intake level of heavy metals in milligram/gram (mg)

Metals	Mean Value	MPI/UL
Ca	32.685	2000-2500 (NIH)
Zn	0.408	40 (NIH, NRC)
Fe	0.637	48 (WHO)
Cd	0.025	0.005 (EPA), 0.003 (WHO)
Mg	8.410	350
Hg	0.055	0.002 (EPA), 0.001 (WHO)
Pb	0.124	0.025 (JECFA), 0.01 (WHO)

Cr	-0.643	0.18

Note: the maximum permissible limit of intake (MPI) for chromium (0.003mg/kg/bodyweight/day) and iron (0.8mg/kg/bodyweight/day) were converted using multiplication of average age of adult (60kg). The MPI and UL (Tolerable upper intake level) are sourced from Joint FAO/WHO Expert Committee on Food Additives (JECFA), National Institute of Health (NIH), National Research Council (US), World Health Organization, and Environmental Protection Agency (EPA).

Source: Abadin *et al.*, 2007; Gastro-Gonzalez and Mendez-Arnenta, 2008; Chunhabundit, 2016; Deka *et al.*, 2023

Conclusion

Fish is a source of quality nutrients which cannot be overemphasized for its contribution for human growth and development. The *C. gariepinus* caught Hadejia River proved excellent with its constituents of macronutrients; carbohydrates, proteins and fats. These organic compounds are responsible for production of Adenosine triphosphate (ATP) (the main body energy currency), tissue building and insulation/protection respectively.

C. gariepinus contained adequate Thiamine and Riboflavin when compared with their respective recommended dietary allowance. These vitamins are crucial to the survival of fetal and children. The fish is also a considerable source of ascorbic acid (vitamin C) that maintains and boosts immunity and acts as antioxidants.

However, the *C. gariepinus* caught from Hadejia River is considered as a poor source of some important minerals such as calcium, iron, magnesium and zinc as it contains very low amount of these elements. The heavy metals concentration was significantly higher than the stipulated safe limit for consumption which will eventually result to health problem/s via bioaccumulation.

Recommendation

This research revealed; high concentration of heavy metals in the fish caught from Hadejia river, Jigawa state, which might be as a result of their uptake and feeding from the aquatic environment. The presence of heavy metals in an aquatic ecosystem is a threat not only to the inhabitants of the ecosystem but also the wellbeing of humans, therefore, the state environmental laws enforcement agency should look into the anthropogenic/industrial activities surrounding the river and from the headwater (river source) and ensure that the aquatic environment is protected from exposure to toxic substances and from the risk associated with the use of chemicals. The state environmental laws enforcement agency should put a check/stop to any activities leading to leakage of heavy metals into the river and the activities should be regulated to ensure that heavy metals are not released either directly or indirectly into aquatic environment across the state.

The catfish from Hadejia river contained number of metals exceeding the maximum permissible level of intake, and bioaccumulation of these metals are hazardous to health, hence the regular consumers of these fish or the river products should endeavor to subject the products into combination of food preparation and processing techniques to reduce or eliminate the metal's composition of the products before consuming for safety. For this purpose, some of the processing techniques are washing, soaking, smoking, fermentation, soaking in vitamin C solution, and cooking like boiling, frying, grilling etc.

This research discovered that there are few research documentations on aquatic environment and its inhabitant in Jigawa state, specifically on Hadejia River. It is imperative to encourage the academics and researchers to start documenting their research results for knowledge acquisition, references and to aid problems solving.

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