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Determination of Crude Protein in *Limnothrissa miodon* in Lake Kivu

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Abstract

The intention of the present work is the minerals assessment and proteins found in *Limnothrissa miodon* sampled from lake kivu at kalongi shores, the concentration of Fe, Cu, Zn and protein in *Limnothrissa miodon* were determined. In total three samples of fish were collected; dried fish, fresh fish, and powdered at one site. 3 replicates from different fish samples were digested and analyzed for minerals by using DR/200 Spectrophotometer, and for protein using the Kjeldahl method. The mineral concentration found were in range 0.644 mg/g, 0.228 mg/g and 0.168 mg/g, concentration for protein was 0.52%, 0.29%, 0.164%. The results of this study showed that mineral iron (Fe) concentrated in fine-grained *Limnothrissa miodon* was terribly high compared to copper and metallic element (Zinc). Dried *Limnothrissa miodon* contain huge quantity of protein compared to contemporary *Limnothrissa miodon* and fine-grained which means that dried *Limnothrissa miodon* is good fish to health of individuals owing to our body desires protein for growth and maintenance of each reasonably cell in our body and it's concerned in defensive the body from antigens. Proteins can travel through the blood stream and are utilized the immune system to identify and defend against bacteria and viruses, Iron is required to produce red blood cells, but it is also a part of hemoglobin binding to oxygen and thus facilitating its transport from the lungs via the arteries to all cells throughout the body.

Keywords: *Limnothrissa miodon*; Proteins; Minerals; Antigens; Hemoglobin; Immune system

Introduction

The Lake Tanganyika sardine *(Limnothrissa miodon)* are species of fish in the family of Clupeidae, it is monotypic within the genus *Limnothrissa miodon*. It is found in Burundi, Democratic Republic of Congo, Mozambique, Tanzania, Zambia, and Zimbabwe. Its natural habitat is in freshwater lakes in Rwanda, is locally known as Isambaza. In the past, fishing activity on *Limnothrissa miodon* in Lake Kivu was unknown and its exploitation began regarding fifteen years once the introduction of this little malacopterygian from lake in 19590020 [1].

The piscary became vital following the UNDP/FAO piscary Development Project activities that started in 1979, on the Rwandese aspect of the lake. Since November 1979, fishing activities are focused virtually completely on *Limnothrissa miodon*, the sole species representing a crucial fish stock among the twenty-six fish species in Lake Kivu [2].

The La Kivu has traditionally supported a remarkably productive the pelagic piscary that presently provides 25%-40% of the animal Protein offer for the population of the surrounding countries. *Limnothrissa miodon* is most significant species during this piscary, however recent information shows their catch decreasing every year and Biological information are required for management of those species.

The exploitation of this new resource could be results of a combined action by each private initiative on the Zairean aspect and as an effort by Rwanda and the Netherlands Governments, the UNDP and Food and Agriculture Organization, to push fishing in Lake Kivu. Today, a complete range of 2868 fishermen area unit concerned within the artisanal piscary activities and besides this, about 3,340 ladies observe profits out of the mercantilism of the fish at the market places. A recent chemical composition analysis of *Limnothrissa miodon*, by the National Food and Nutrition Commission (NFNC) shows an excellent variation within the nutrient composition for the dry and cannon fodder, NFNC principal specialist Mr. Musonda Mofu says dry *Limnothrissa miodon*, contains 209 calories of food energy compared with eighty-five calories of food energy in recent *Limnothrissa miodon*, In addition, dry *Limnothrissa miodon* contains sixty-three grams of macromolecule and only 16 grams in Limnothrissa miodon. Overall this shows that, per portion, there are more advantages in dry *Limnothrissa miodon* than in recent *Limnothrissa miodon* [3].

In specific, *Limnothrissa miodon* could be a healthy food and wealthy in protein and because of this chemical composition, *Limnothrissa miodon*, like different fishes, may reduce the chance of malady as well as prostatic adenocarcinoma, betting on the frequency of consumption. In distinction to cereal proteins therefore fish and cereal macromolecule will supplement one another within the diet. Fish macromolecule provides an honest combination of amino acids that is very suited to man's biological process necessities and compares favorably there upon provided by meat (eggs Lates spp) [4].

In the light of that observation, rational exploitation of remarkable food reserves of Lake Kivu could be obtained only by an introduction of pelagic and plankton-eating fishes. Consequently, a set of elevens of two clupeids of Lake Tanganyika, Stolothrissa tanganicae and Isambaza (*Limnothrissa miodon*) have been discharged to Kivu Lake entre 4th June and 22nd July 1959 by [1].

The Isambaza is acclimatized itself and truly constitutes the big stock of Lake but the nutrition issues of heavy metals, deficiency and vitamin A deficiency can also be addressed by over whelming Isambaza because it incorporates a high content of those minerals, Fish could be a potential supply of minerals like Calcium, phosphorus, sodium, potassium, iron, zinc, magnesium.

It was in 1953, on which the scientific mission bank of exploration of Kivu Lake, Edouard and Albert asked about the problem of restocking of Kivu Lake could have proved the high deficiency currently in existence in the link of the ichthyological fauna of Kivu Lake, many different shores of Kivu Lake such as Rubavu, Karongi and Rusizi they produce enormously the great quantities of sardine known as Isambaza consumed as fresh, powdered and dried state.

It is difficult for the fisher men to conserve them because of poor materials and exposed a long time in the sun which can cause decreasing of the number of proteins, minerals in sardine and So far, no solution has been proposed that would be both affordable and complete for these difficulties [5].

Mineral constituents of fish muscle offer values averaged from an oversized variety of species and meant to serve solely as a rough guide, it might be unworkable in this short note, and of restricted worth, to provide an in-depth analysis for individual species.

This study aims at the chemically analyzing content in Isambaza (*Limnothrissa miodon*) obtained from Kivu Lake in different forms available on the market such us fresh, powdered, dried fish products obtained during rainy and dry seasons. A comparison between their compositions will also be made. More specifically is to determine proteins, amino acids and mineral content in "Isambaza '*Limnothrissa amiodon* [3-5].

In lake Kivu, there are two fishing areas, the first is coastal or littoral this area is complex, with some diversity of fishes and second is the pelagic zone located in the center of the lake, much simpler, there is a single fish species which is the *Limnothrissa miodon*.

Lake Kivu is mecromictic its biozone extends up to 60 meters in depth beyond which any aerobic life is impossible, only 12% of its total volume is habitable by fish. The fishing zone within which artisanal fishing units operate is found around three main fishing main centers the Rwandese Republic, this centers at Rusisizi, Rubavu and Karongi. Fishing effort is expressed in terms of fishing nights per fishing unit per fishing campaign (i.e the amount of fishing days per moon month) the typical monthly fishing effort is between twenty and twenty-two nights, the amount of fishing nights is determinate by the lunar cycle. Throughout the total moon amount, the intensity of moon light reduces the facility of the lamps to draw in the fishes, and fishing doesn't yield good results [6-10]. Alternative factors like environmental condition (rain, the roughness of waves) and the accessibility of spare components and fuel for lamps have abundant influence upon fishing effort. The quantity of protein in fish muscle is usually somewhere between fifteen and twenty percent however values under fifteen percent or as high as twenty-eight percent square measure sometimes met with in some species. All proteins, including those from fish, square measure chains of chemical units coupled along to create one long molecule. These units, of that their square measure regarding twenty sorts, are called amino acids, and sure of them square measure essential within the human diet for the maintenance of excellent health. the dietician, the cook, and the client all have a direct interest within the proteins from the *Limnothrissa miodon* [11-14]. Moreover, if a diet is to be totally and economically used, amino acids should not solely be gift however must conjointly occur within the correct proportions 2 essential amino acids referred to as essential amino acid and essential amino acid square measure typically found in high concentrations in fish proteins. Surface of lake is at Associate in Nursing altitude of 1463 m and therefore the lake covers a surface of 2370 km^2 of that forty-second percent is Rwandan body of water; it has a mean depth of 287 m with a most of 485 m within the north.

Origin of minerals found in the aquatic environment

Minerals are natural components of high density greater than 5 g per cubic centimeter (5 g/cm^3) as it was found in some publications, Minerals are present in a very compartment of the environment but usually in small quantities; they do not biodegradable, consequently, they increase their concentration with time. They can be toxic to living organisms at certain levels.

Minerals enter the aquatic environment from natural sources and anthropogenic sources their input can be the results of spills is made directly in marine and freshwater, either as an indirect route in case of dry and wet landfills and agricultural runoff, Among the important natural sources include volcanic activity, weathering of continents and forest fires.

Natural minerals came from Continental crust is the ultimate source of trace elements in hydrologic systems, Trace elements are introduced in the river basin by rock weathering, atmospheric dry, and wet deposition.

Methodology

The types of samples analyzed with chemicals area unit recent, pulverized and dried fish; they will be purchased on the market in karongi Lake Kivu shores. Fresh samples will immediately place on Ice, keep cool and transport in Ice to maintain freshness upon arrival at the Huye campus. Firstly, the sample must be washed by water to remove the ice rinsed distilled water, weigh two grams of each species then cut into slices subjected to oven, sun and smoke drying at 400°C. the encircling pressure was reduced, and enough heat can increase take away frozen water from the flesh till samples get constant weights. After preparation of samples, specified chemicals and collecting laboratory materials, we have been using different techniques based on the conversion of organic nitrogen to inorganic (digestion according to Kjeldahl method) the ammonium sulfate thus formed is diluted and made alkaline with sodium hydroxide and the ammonium distilled over and titrated by dilute sulphuric acid solution, to analyze proteins.

Mineral content in Isambaza products

The isolation of mineral (Cu^{2}+, Fe^{2+}, Zn^{2+}) content in Isambaza product was determined by using DR2000 Spectrophotometer. Samples are obtained after digestion of *Limnothrissa miodon* followed by mixing reagent such as distilled water, hydrogen peroxide, nitric acid those reagents help to destroy fish and metal complexes.

Sampling has been done on April 28, 2018 Those forms of *Limnothrissa miodon* are taken in water of Lake Kivu nearby the town of karongi; Fresh, dried powdered fish are the most consumed by the population of karongi town. All samples will immediately place on Ice, keep cool and transport in Ice to maintain freshness upon arrival at the kigali. Firstly, the sample must be washed by water to remove the ice rinsed distilled water, weigh two grams of each species then cut into slices subjected to oven, sun and smoke drying at 40°C. The surrounding pressure was reduced, and enough heat will add to remove frozen water from the flesh until samples get constant weights [15-20].

The form of sample S1, S2, S3 dried, powdered, fresh fish corresponding to *Limnothrissa miodon* (Isambaza) were dried with

the drying oven with 103°C for 24 hours to eliminate totally water; then they were crushed using a laboratory mortar. The Kjeldahl methodology will handily be divided into 3 steps: digestion together with neutralization, and volumetric analysis.

Digestion of samples

Apparatus.

Destruction-bloc with destruction tubes made of borosilicate glass.

B) Pipette model 3100 with removable tips.

Reagents, all with a low metal content.

Nitric acid, 65% HNO_3.

Hydrogen peroxide, 30% H_2O_2.

Pumice Glassware.

All rinsed with 1 ml of distilled water.

Measuring cylinder 50 ml.

Funnels with a diameter of 6 cm.

Volumetric flasks of 250 ml+Becker, spatulas.

Procedure for minerals

We weighed 1.250 g dried and, in each sample, which was transferred to Erlenmeyer.

We placed a funnel at the top of each material of digestion (Pyrex Erlenmeyer or Pyrex ballon).

By means of hotplate we carried out the heating in the following way:

Heat the tube to 100°C and maintain for 1 hour.

Heat to 125°C and maintain for 15 minutes.

Heat to125°C and maintain for 15 minutes.

Heat to175°C and maintain for 15 minutes.

Heat to 200°C and add, if necessary (if no volume is left) 5 ml HNO_3

After cooling we added to those 5 ml of solution 1 ml 30% H_2O_2 and destruct for 10 minutes; repeated once. Then after cooling, we transferred the solution in the graduated flask from 250 ml and we supplemented to the feature of the gauge with distilled water.

We agitated and let rest the solution for 15 hours. We put the filtrate in a bottle out of plastic and we transported it to the DR/2000 spectrophotometer for the analysis of minerals hereafter: Iron, Copper, and Zinc. We have been noted that all samples including the blanks are digested in the same way. After digestion, we have been used DR/2000 spectrophotometer analyzing samples. Thus, we have prepared 1 sample of Karongi powdered fish.

The procedure of analyzing protein in Isambaza product by using volumetric analysis. The Kjeldahl methodology was developed in 1883 by a brewer referred to as Johann Kjeldahl. A fish is digestible with a powerful acid, so it releases chemical element which might be determined by an acceptable volumetric analysis technique. the quantity of protein gift is then calculated from the chemical element concentration of the fish. constant basic approach continues to be used these days, though variety of enhancements are created to hurry up the method and to get a lot of correct measurements. It's sometimes thought-about to be the quality methodology of determinant protein concentration. because of the Kjeldahl methodology doesn't live the protein content directly a factor (F) is required to convert the measured chemical element concentration to a protein concentration. A factor of 6.25 is employed for several applications, however, this can be solely a mean price, and every protein contains a completely different factor looking on its amino-acid composition.

We weighed 1 g of the sample dried, powdered and fresh fish mix with 5 g of potassium sulfate, a half gram of copper (II) sulfate and 20 ml of sulfuric acid every sample was placed within the flask during an inclined position on an acceptable heating device in a fume cabinet. Heat fastidiously till foaming has ceased and the contents became liquefied. Digest by boiling gently, often rotating the flask, till the liquid is totally clear and of a light-weight blue color; boil gently for an extra 1½ hours. the results are then distilled with a tiny low amount of hydrated oxide, that converts the ammonia salt to ammonia. the quantity of ammonia gift, and therefore the quantity of Nitrogen gift within the sample. The ammonia reacts with the acid and the remainder of the acid is then titrated with a washing soda solution by approach of a methyl red pH indicator.

Total digestion time ought to be virtually four hours so organic nitrogen has been regenerating into ammonia salt fully. Then, cool the contents of the flask to regarding 40°C and cautiously add a tiny low amount of pure water. combine and permit cooling.

Transfer the contents of the flask to a 250 cubic centimeter commonplace flask, launder many times, and form up to 250 cubic centimeters. place two cubic centimeter of sample, 2 drops of phenolphthalein and 5 ml of sodium hydroxide (NaOH 30%) to create basic medium in Kjeldahl flask, then take another flask place in 20 ml of sulphuric acid and raise the flask three drops of indicator.

All those flasks are put in the Kjeldahl distiller. During this process, NH_3 will move as a vapor until reach in a flask which contains sulfuric acid. We recuperate that flask contains an ammoniac solution and start to titrate on burette, then mixing with a solution as an indicator. Repeat the analysis with the second portion of the digest. We must note that all samples including the blanks are digested in the same way. After digestion, we must use Kjeldar method analyzing samples [21-23].

All sample	Type of sample	Type of fish
S1	Dried fish	*Limnothrissa miodon*
S2	Powdered fish	*Limnothrissa miodon*
S3	Fresh fish	*Limnothrissa miodon*

Table 1: Samples taken during research.

Results

In Kalongi basins of the lake, the results of data have been obtained from one sampling site in Karongi Lake Kivu chores; that sampling site S1, S2, S3 has taken nearby The town forms, dried, powdered and fresh fish in which analyzed protein and minerals such as Fe^{2+}, Cu^{2+} and Zn^{2+}, The results in concentration (in mg per grams of solution) and protein expressed in percentage are presented in the Tables 2 and 3 below.

Minerals mg/g	Sample
	Powdered fish
Copper	0.228 mg/g
Zinc	0.168 mg/g
Iron	0.644 mg/g

Table 2: The results of minerals concentration expressed in mg/g.

Type of Sample	Protein content %
Dried	0.52%
Powdered	0.164%
Fresh	0.29%

Table 3: Protein expressed in percentage.

The concentration of minerals in 3 replicates was different (Table 2) were average (number ± SD) was 0.34 ± 0.259 and coefficient of variation 0.747, and results shown that the concentration of iron is high than other minerals for all replicates taken. Percentage of proteins in 3 different replicates Dried, powdered and fresh (Table 3) show that the coefficient of variation was 0.55 and that protein is more concentrated in dried fish, the results were repeatable.

Discussion

Variation of Fe, Cu and Zinc Content in Powdered fish from Karongi are 0.644 mg/g, 0.228 mg/g and 0.168 mg/g respectively with the average (number ± SD) was 0.34 ± 0.259. The concentration of iron for the site karongi is high, this high concentration in Fe can be natural due to the geological situation [24-26], where Fe is one of the under-sols constituent. The high concentration of Fe in fish from karongi is high than the concentration of Rubavu (0.61 mg/g) than concentration from Rusizi (0.425 mg/g) can be justified by the fact that those sites surrounded by fields where sweet potatoes are cultivated in a great amount without a buffer zone. There for, cultivation can be a favorable way of rock alteration. According to the standard for (0.644 mg/g) is beyond the permissible value. For cu, the concentration of copper in karongi isambaza fish (0.228 mg/g) is greater than concentration of Rubavu (0.21 mg/g) than Rusizi (0.072 mg/g) can derive from rock weathering.

Copper is found in nature as chalcopyrite its chemical property restricts the natural release of copper in water and fish consume that water, it's mainly presents a result of anthropogenic origin. It is used in electricity metallurgy, textile, galvanoplasty photography. There for, the main source of copper in karongi fish can be justified by the pollution from karongi city, market, which discharges in the lake. For zinc, zinc have lower accumulation that is the reason why it is has a lower concentration in *Limnothrissa miodon* of Karongi (0.168 mg/g). And is lower than Rusizi (0.184 mg/g) Greater than Rubavu (0.078 mg/g).

Our experimental show that dried *Limnothrissa miodon* have high compactness of protein (0.52%) than the concentration of recent *Limnothrissa miodon* (0.29%) and concentration of protein in powdery *Limnothrissa miodon* (0.164%) as a result of in dried fish there's no water, that's reason, why dried, have a high concentration of proteins than cannon fodder, so the upper content of water in fish related to lower protein [27-30]. Powdered fish have a lower concentration of proteins because of it's from some transformation that causes the loss of some a part of fish and therefore the concentration of Dried from karongi (0.52%) is high than Rusizi (0.369%) than Rubavu (0.244%), the concentration of recent from Rusizi (0.298%) is high than Karongi (0.29%) the Rubavu (0.105%).

Conclusion

Based on the information obtained from results, the minerals iron (Fe) is concentrated in powdered *Limnothrissa miodon* from Karongi Lake Kivu is very high compared to copper and zinc. Dried *Limnothrissa miodon* contain big amount of protein compared to fresh *Limnothrissa miodon* and powdered; it means that dried *Limnothrissa miodon* is good fish to health of people because our body needs protein for growth and maintenance of every kind of cell in our body and it is involved in defending the body from antigens. Proteins can travel through the blood stream and are utilized the immune system to identify defend against bacteria and viruses.

Iron is required to produce red blood cells, but it is also a part of haemoglobin binding to oxygen and thus facilitating its transport from the lungs via the arteries to all cells throughout the body and it recommend because *Limnothrissa miodon* contain protein and mineral which are very important in our healthy and people must consume it to have a good life. In my research project some parameters such as Amino acid, mercury, arsenic, manganese, lead, were not analyzed in *Limnothrissa miodon* and I request other researcher from The University of Rwanda and elsewhere to perform a research on it.

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