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EFFECTS OF FREEZING PERIOD ON THE PH, CRUDE PROTEIN AND SOME ESSENTIAL MINERALS IN CATFISH (CLARIAS GARIEPINUS) AND TILAPIA (OREOCHROMIS NILOTICUS) CULTIVATED IN KADUNA METROPOLIS, KADUNA STATE, NIGERIA.

Chemical Science

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ABSTRACT

This study was carried out to examine the effects of freezing period on pH, crude protein and some minerals (Calcium, Magnesium, Iron, Potassium and Sodium) levels in Catfish (*Clarias gariepinus*) and Tilapia (*Oreochromis niloticus*) fish cultivated in Kaduna Metropolis, Kaduna State. The fishes were collected from four major markets within the sampling areas. The results of the study showed that pH values ranged from 6.5 – 7.8, The Crude protein level ranged from 27.30 – 45.50% and the mineral content were as follows: Iron (0.33 – 2.52 mg/Kg), Sodium (4.55 – 7.20 mg/ Kg), Potassium (2.26 – 3.14 mg/ Kg), Magnesium (0.85 – 1.80 mg/Kg), and Calcium (0.31 – 0.87 mg/ Kg). The result indicated that pH is only affected after 30 days of freezing which resulted in increase of the value. The fish protein level decreases slightly with freezing period, however, the fishes were good in protein and can satisfactorily provide the necessary protein requirement for human consumption even after the freezing period of 30 days. The minerals were generally low (below the dietary daily value of minerals in fish as recommended by USEPA, 2016) and seem not to be affected during freezing duration.

KEYWORDS

Tilapia, Catfish, pH, Protein and Mineral Contents.

1.0. INTRODUCTION

In 2015, the Nigerian Customs Service intensified the enforcement of the ban of impoted frozen chicken, fishes and poultry products in the country (Aduge-Ani and Ofikwe, 2015). This strategy was implanted to catalyse increased agriculture investment and improve fish and poultry products production in the country. Fish is an important source of protein intake for many people, especially the people living in developing countries. In Nigeria, about 41% of the total animal protein intake is obtained from fish products and it is reported that fish is a major source of animal protein and other essential food item for Nigerians, because it is relatively cheaper than meat (Rahji and Bada, 2010; Onyia *et al.,* 2014). This makes the total demand for fish and fish products higher in Nigeria than many other West African Countries with 1.5 million tons of fish consumed annually (Ozigbo *et al.,* 2013). Yet, Nigeria imports over 900,000 metric tons of fish while its domestic catch is estimated at 450,000metric tons/year (Davies *et al.,* 2008).

Fish is nutritionally composed of protein, carbohydrate, fat and oil, vitamins, minerals and about 75-80% water. The nutritional composition of fish varies greatly from one species to another, depending on age, source, feeding habit, size, age, sex and sexual variations due to spawning, environment and season (Dana *et al.,* 1985; Silva and Chamul, 2000; Effiong and Mohammed, 2008). Apart from its food value, fish has been reported to possess medicinal values, such as, in the amelioration of asthma, arthritis, coronary heart diseases, goitre and cancer (Cobiac *et al.,* 1991). Notwithstanding the good quality associated with fishes, it is a very perishable commodity, more than cattle, sheep, and poultry, and get spoilt very easily even in temperate climates. This is due to its chemical composition, which makes its flavour and texture changes rapidly after death. So unless it is disposed off quickly after capture, it must be preserved in some way (Eyo, 2001; Emere and Dibal; 2013).

There are some preservation techniques currently used which includes chilling, freezing, drying, salting, frying, fermenting sun-drying, grilling, smoking and various combinations of these (Ayinsa and Maalekuu, 2003). In Nigeria, fish either obtained from a cultured pond or from the wild is sold to consumers as fresh, frozen, smoked or sun-dried (Akinwumi and Adegbehingbe, 2015). However, Freezing is a common practice in the meat, fish and other animal protein based industry, because it preserved the quality for an extended time and offers several advantages such as insignificant alterations in the product dimensions and minimum deterioration in products colour, flavour and texture (Obuz and Dikeman, 2003). Research has shown that the quality and nutritional aspects of frozen foods are critical and depend on the length of freezing. Adams and Moss (2000) reported that the rates of most chemical reaction are temperature dependent and so the temperature is lowered, the rate of chemical reaction decreases. When fish is frozen, ice crystals will inevitably form in the flesh of the fish, these crystals expanding during freezing and causes damage to the fish (Ukenye and Kolade, 2015). This deterioration of fish quality especially in the pH, protein and some mineral content (Iron, Potassium, Calcium and Magnesium) as a function of storage time in freezing condition was studied in this work. The study was carried out using two fish species, Tilapia and Catfish species that are cultivated within Kaduna metropolis

2.1. METHODOLOGY

2.1.1. SAMPLE COLLECTION

Fresh Tilapia and Catfish fish samples were from four major fish markets within Kaduna metropolis. The fish samples were immediately stored in an insulated freezer at a temperature of 0^0Cand transported into the laboratory for immediate analysis.

2.1.2. SAMPLE PREPARATION

Ten (10) fresh fish samples of catfish and tilapia each were washed with tap water several times rinsed with distilled water and were cut into slices. They were stored in a rubber zip seal and kept in a freezer. Samples were taken each from the front, rear and the middle of each fish, cut into pieces and mixed thoroughly to obtain a homogenized representative sample. The stored homogenized fish samples were taken for analysis at day 0, 3, 7, 10, and 30.

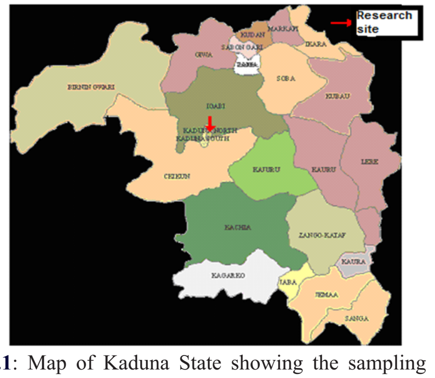

Figure 3.1: Map of Kaduna State showing the sampling location (Courtesy of Kamba and Okunade, 2016).

2.2. METHOD OF ANALYSIS

2.2.1. PH ANALYSIS

The pH of the fish samples was measured using a digital pH meter. Five grams of the representative sample was weighed in a beaker to which 45 cm^3 of distilled water was added and slurry was made using a blender. The pH was then recorded (Hur *et al.,* 2008).

2.2.2. MINERAL ANALYSIS

The homogenised fish samples (1g) was weighed using a weighing balance and transferred into a 50cm^3 digestion tube, 20cm^3 of acid mixture H_2SO_4, HNO_3, $HCLO_4$ (in the ratio of 2:1:1) was added to the sample and was digested on a hot plate until a clear colourless solution was observed. The digested sample was allowed to cool, sieved and made up to the mark with deionised water into a 50cm^3 volumetric flask. The samples were then analysed for differernt mineral elements using the Atomic Absorption Spectrophotometer (Ayeloja *et al.,* 2014).

2.2.3. CRUDE PROTEIN ANALYSIS

Simple method proposed by chow *et al.*, 1980 as cited in FAO (2015) was adapted.

The homogenised fish sample (1g) was weighed and placed in a Kjeldahl flask; 10g potassium sulphate, 0.7g mercuric oxide or copper sulphate and 20 cm^3 concentrated sulphuric acid were added to the sample in the Kjeldahl flask. The flask was tilted to the angle in the digester and boiled until the solution became clear. Heating continued for about 30 minutes, the flask was allowed to cool. After the samples had been cooled, 25 cm^3 of sodium sulphate solution was added and stirred. Glass beads (to serve as anti-bumping granules) and 80 cm^3 of 40% sodium hydroxide solution were added. The flask was then connected to the distillation unit and heated for a while. The distillate containing ammonia was collected in 50 cm^3 of the indicator solution. At the end of the distillation, the receptor flask was rinsed and the solution was titrated with standard 0.1M hydrochloric acid.

$$\text{Nitrogen in sample (\%)} = 100 \left\{\frac{A \times B \;\; 0.014}{C}\right\}$$

Crude protein (%) = % nitrogen in sample x 6.25

Where A = Hydrochloric acid used in titration (cm^3), B = Molarity of standard acid

C = Weight of sample (g)

3.0. RESULTS AND DISCUSION

TABLE 3.1: The pH readings, Crude protein and minerals level of Catfish Samples

DAY	pH	PROTEIN (%)	MINERALS (mg/Kg)				
			Fe	Na	K	Mg	Ca
1	6.7	32.20	1.48	4.55	2.66	1.03	0.31
3	6.7	30.62	0.98	5.32	2.46	1.18	0.37
7	6.5	29.75	0.33	5.06	3.16	1.19	0.45
10	6.7	29.40	0.64	5.57	3.00	1.07	0.34
30	7.8	27.30	0.83	6.33	3.31	1.10	0.43
Mean	6.88±0.52	29.85±1.79	0.85±0.42	5.36±0.65	2.91±0.35	1.11±0.06	0.38±0.05

TABLE 3.2: The pH readings, Crude protein and minerals level of Tilapia Samples

DAY	Ph	PROTEIN (%)	MINERALS (mg/Kg)				
			Fe	Na	K	Mg	Ca
1	6.7	45.50	2.52	6.14	3.14	0.85	0.44
3	6.8	43.57	0.47	7.20	2.26	1.80	0.38
7	6.6	42.52	0.44	6.49	2.75	1.19	0.69
10	6.9	41.12	0.64	6.80	2.87	1.30	0.81
30	7.5	37.62	1.72	6.31	2.50	1.65	0.87
Mean	6.90±0.35	42.06±2.95	1.15±0.92	6.58±0.42	2.70±0.33	1.35±0.37	0.63±0.21

The pH values observed in all the samples were similar for the freezing period of 1, 3, 7 and 10 days but suddenly increase after freezing for 30 days as shown in Table 3.1 and 3.2. The results showed that increase in pH values for all the fish samples could possibly start from the day 10 of freezing preservation periods or somewhere from day 10 to day 30. The result of this study is not in conformity with the one reported by Arannilewa *et al.,* 2005 in which the increase in pH on frozen fish sample was found to start from day one. Angbalagam *et al.,* 2014 reported that reduced pH (acidic condition) was evidence for the reduction of meat spoilage and The alkaline conditions as observed in the day 30 of this study could be attributed to the accumulation of metabolites of bacterial action and protein deamination on the fish samples (Angbalagam *et al.,* 2014). Ray and Bhuma, 2013 reported that a high pH in fish and meat created the platform for a more rapid spoilage process due to a more rapid bacterial growth and consumption of nutrients.

The crude protein values observed in all the fish samples showed similar characteristics of deterioration as the freezing period of 1,3,7and 10 days increases as shown in Table 3.1 and 3.2. The results showed that the Tilapia fish had more crude protein content than the Catfish in this research. This observation was also recorded by Craig Steven, 2009 who recorded a protein content of 28-32% for Catfish and 32-38% for Tilapia fish. Keremiah and Amakiri also reported a protein content value of 23.32±0.13% for adult Catfish harvested in Yenagoa, Nigeria. The reduction in the crude protein of the fish samples could be attributed to various factors as Chomnawang *et al.,* 2007 reported that reduction in crude protein content in the fish and meat content maybe as a result of the decrease in salt soluble protein and water soluble protein. Hutlman and Rustard, 2004 also reported that autolytic deterioration associated with the actions of endogenous enzymes and bacteria might be the reason for the reduction in crude protein during freezing storage. Mazrouh, 2015 also conducted a research on the protein changes of fresh *Saurida undsquamis* and its changes after been stored in a frozen condition for 21 days; reported a crude protein content of the fresh fish was 25.48±1.32 and after storage for 21 days, it decreased to a crude protein value of 13.46±1.32. This shows that further freezing in a longer period will lead to a much reduction in the crude protein content of the fish.

The mineral content values of iron, calcium, magnesium, sodium and potassium as observed between the two fishes showed similar fluctuations of values as the freezing days increases. Arannilewa *et al.,* 2005 also observed slight changes in minerals content with respect to frozen period in all the trace metals evaluated in their research. They reported that this occurrence could be attributed to drip loss and dehydration that is associated with frozen storage. Ukenye and Kolade, 2015 in their research suggested that the slight loses observed could be in the process of thawing because when fish thaws, some of the water content is lost and this water could contain some of the water soluble vitamins and minerals. Krope and Bowers, 1992 also suggested that freezing has a negative effect on the nutritional values (mineral contents) of freshwater fish species. The reduction and increase in their values can be likened to the negative effects of freezing and storage period which including freezer burn, product dehydration, rancidity, drip loss and product bleaching which can have an overall negative effect on the quality of the frozen foods. The recommended dietary allocation of iron intake from foods and supplements is 13.7 – 15.1 mg/day in children aged 2-11 years, 16.3 mg/day in children and teens aged 12-19 years, 19.3 – 20.5 mg/day in men and 17.0-18.9 mg/day in women older than 19. The median dietary intake in pregnant women is 14.7 mg/day (Institute of Medicine, 2001). The low iron mineral content in the Catfish and Tilapia fish samples as recorded in Table 3.1-3.2 were also observed and reported by Sani *et al.,* 2016 and Arannilewa *et al*., 2005. Lack of iron in diets can lead to irregular heartbeats called arrhythmias, a heart murmur, an enlarged heart, or even heart failure (NIH, 2014). The sodium values were not adequate to meet the sodium daily value of 56mg/100g in Tilapia fish recommended by USDA, 2016. Adefemi, 2011 also reported low sodium values for Tilapia fish samples obtained from four major dams (Ureje, Ero, Egbe and Itapaji) in Ekiti State. The inadequate addition of sodium chloride (salt) into the fish feeds might also be a cause in the low amount of sodium analysed in the fish samples. Inadequate sodium mineral content could lead to diarrhoea, vomiting, diuretics, and sweating (Lee *et al.,* 2014).

The Catfish samples had a high potassium content than the Tilapia fish and this was supported by USDA, 2016 which stated that the daily value of potassium in Catfish was 358 mg/100g and that the Tilapia fish had a daily value of potassium at 302 mg/100g; this values concludes that the Catfish has more potassium content than the Tilapia fish. Still yet, the Catfish and Tilapia potassium content were short of the daily value as recommended by USDA, 2016 for human consumption. Axe, 2016 reported that one of the biggest problems with having a low potassium intake is that the body isn't able to neutralize acids as well. Non-carbonic acids are generated during digestion and metabolism of both plant and animal proteins, including meats, dairy and grains. Its potassium's job to balance these acids in order to keep the body at a proper pH, as low potassium can mean the body becomes too acidic. The daily value of magnesium recommended by USDA, 2016 for Tilapia fish is 34 mg/100g and catfish is 23 mg/100g. These facts when compared with the amount of magnesium observed in the fish samples as shown in Table 3.1 – 3.2, it shows that the fish samples didn't have the right amount of magnesium and thus was inadequate for daily human consumption in diets. The low fish magnesium mineral content in the Catfish and Tilapia fish samples were also observed and reported by Sani *et al.,* 2016. Gaal *et al.,* 2004 also reported in their research that Magnesium (Mg) supplementation given to fishes remarkably improves the digestibility of feed; it increased weight gain and improved the quality of egg production. Symptoms of magnesium deficiency include hyper excitability, muscular symptoms (cramps, tremor, fasciculations, spasms, tetany, and weakness), fatigue, loss of appetite, apathy, confusion, insomnia, irritability, poor memory, and reduced ability to learn (Rude and Shils, 2006). The low amount of calcium observed in the fish sample shows that the samples were not properly fed well with calcium fortified feeds; as the value was a far cry from calcium values obtained by Emorutu *et al.,* 2014 which reported a calcium value of 18.1±7.3 mg/Kg for Tilapia fish and 23.9 mg/Kg for Catfish. This deficiency if calcium could have been corrected by adding sufficient calcium supplements in the feeds of the fish samples. Low calcium content in fish could lead to dietary calcium deficiency. Dietary calcium deficiency is a condition in which there is an inadequate calcium intake from diets or meals, which can lead to depleted calcium stores in the bones, thinning and weakening of the bones and osteoporosis (Haines, 2013).

4.0. CONCLUSION.

The result of the investigation showed that the Catfish and Tilapia fish were very good source of protein. Although the minerals analysed were below the recommended dietary value for human consumption; they can still play active part in replenishing the body with secondary addition of mineral supplements. The investigation also showed that fishes could be stored in a frozen state for a long period of time but most specifically, they shouldn't be stored more than the 30th day frozen period analysed. It is better fishes are best eaten when fresh and if this can't be achieved, it shouldn't extend the first ten days of storage because deterioration increases as the duration of storage increases.

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