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Proximate and Mineral Quality Changes in Fillets of Three Fish Species (*Mugil cephalus*, *Chrysichthys nigrodigitatus* and *Oreochrumis niloticus*) at Frozen Storage (Sub 0°C)

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Abstract: The proximate and mineral composition of fillets from two brackish water fish species (*Mugil cephalus and Chrysichthys nigrodigitatus*) obtained from the wild were compared with fillets from farmed fresh water fish species (*Orechromus niloticus*) fed commercial diet. The study also investigated the effect of frozen storage on the proximate and mineral composition of the fillets of these fishes. Analysis was carried before and after frozen storage using standard methods recommended by AOAC. Comparisons among sample means were made by one-way analysis of variance (ANOVA) at 5% confidence level. The mean ash content in brackish water fish species was 1.71±0.03% and 0.86±0.02% in fresh water fish species. Similarly, dry matter content was 23.84±0.05% in brackish water fish species and 8.81±0.24% fresh water fish species. The concentration of calcium ranged from 70.14±2.30 in brackish water fish species to 94.86±0.43 mg/100g in fresh water species, while the level of magnesium was 34.80±1.30 in brackish water species and 32.00±2.30 mg/100g in fresh water species. The level of potassium in brackish water fishes was 254.66±0.04 and 150.06±0.40 mg/100g in fresh water species. The concentration of sodium was 141.20±57 mg/100g in brackish water fish species and 57.20±0.20 mg/100g in fresh water fish species, concentration of phosphorus was 150.93±0.31 in brackish water fish species and 94.67±0.43 mg/100g in fresh water fish species. The percentage protein in *Mugil cephalus* was 36.14±0.39% before freezing and 36.05±1.81% after freezing while in *Chrysichthys nigrodigitatus* it was 36.85±0.20% in fresh samples and 36.63±0.20% after frozen storage. The protein content in *O. niloticus* varied between 41.50±0.40% before and 41.42±0.40% after frozen storage. The concentration of sodium in fillets of M. cephalus varied from 142.67±0.24mg/100g before storage to 140.33±0.11mg/100g after storage. The mean concentration of sodium in the fillets of *C. nigrodigitatus* before freezing was 139.73±0.90mg/100g and 136.67±0.23mg/100g after freezing. The concentration of sodium in *O. niloticus* was 57.20±0.24 mg/100g before and 56.07±0.11mg/100g after freezing. In conclusion, all the fish under study belonged to high protein low fat category. Under the experimental conditions, frozen can be effectively used to preserve protein, dry matter, sodium, magnesium and potassium.

Keywords: Nutritional, Proximate, Quality, Brackish, Fresh Water, Fish Species

1. Introduction

Fish is an important component in human diet because it is a rich source of animal protein (1). Fish and fish products are consumed as food all over the world. It has been shown that fish products have high nutritional value based on studies from fin fishes (2, 3, 4). In Nigeria, fish constitute 40% of protein intake (5) where per capita fish consumption has been estimated at 8.8kg/caput/annum (6). According to FAO (7), Nigeria a major importer of fish in the world produced as much as 43, 950 and 84 578 metric tonnes of fish in 2004 and 2006 respectively. Post-production losses in Nigeria have been estimated at 30-40% (8, 9, 10, 11, 12 and 13). In Nigeria, freezing is one of the most common preservation

methods for all food materials including fish. Freezing is known to prevent microbial spoilage, minimize chemical deterioration which mainly involves lipid oxidation and protein denaturation. Freezing greatly reduces or halts the biochemical reactions in fish flesh, freezing does not improve the quality of fish, at best the technique maintains the fish in much the same condition as it was before storage. The objectives of this study were,

- To evaluate the proximate and nutritional quality of two species of fish from brackish water environment and one species of fresh water fish species and to determine if there are any environmentally induced differences in quality.
- To access possible changes in the nutritional quality of fish stored under frozen conditions for thirty days.

2. Materials and Methods

A total of 75 fish samples each of *Mugil cephalus* (with a mean wet body weight of 158.15±15.35g and mean total length 26±1.17cm) and *Chrysichthys nigrodigitatus* (with a mean wet body weight of 185.15±12.39g and mean total length 27±1.18cm) were used for the study. These fishes were obtained from artisan fishers in Bonny River, Rivers State, Nigeria a brackish water environment. Seventy five (75) samples of *Orechromus niloticus* (with mean weight of 195.65±18.75g and mean total length of 23±2.32cm) was obtained from a local fish farm in Umuahia, Abia State, Nigeria. All samples were collected in the first week of June 2013.

In each case, the fishes were transported in ice packs to the Central laboratory of National Root Crop Research Institute, Umudike, Abia State, Nigeria. Before analysis, samples were thawed at room temperature of 25-280C.

2.1. Sample Preparation

In the laboratory, fish samples were weighed to the nearest 0.1gm on a Metler balance. Their total length (to the nearest 0.1mm) was also obtained from a measuring board. The fish samples were cleaned, de-scaled, eviscerated and cut length–wise along the vertebral column. The head, bones and visceral parts were discarded. To obtain fish fillets, each fish was cut along its full length. Each of the three fish species was replicated four times to yield a total of 12 samples. All samples (each weighing 300± 2.35gms) were blended into a smooth paste in a 3.8 L kitchen-type blender (Warning Products, New Hartford, CT) which was thoroughly cleaned and dried between samples.

2.2. Nutritional Evaluation

Fresh blends of fish paste were analyzed for proximate composition. Moisture, crude protein, crude lipid and ash contents were determined according to AOAC (14).

The major elements, comprising calcium, magnesium, sodium, potassium and trace elements (iron and zinc) were determined according to the method of James (15) and Shahidi et al. (16).

2.3. Statistical Analysis

The results are presented as the mean ± SD (standard deviation). All data were analyzed by one-way analysis of variance (ANOVA) using the software of the SPSS 11.0 for Windows. Student's t-test was performed to separate differences among means. The differences are considered significant at P≤0.05.

3. Results and Discussion

Nutritional composition of brackish and fresh water fish species: The nutritional composition of fish obtained from both brackish water species (*M. cephalus* and *C. nigrodigitatus*) and fresh water species (*O. niloticus*) is presented on Table 1.

Table 1. *Nutritional Composition of brackish water fish (M. cephalus and C. nigrodigitatus) and fresh water fish (O. niloticus).*

Parameters (%)	Aquatic Habitats	
	Brackish Water Species	**Freshwater Species**
Ash	$1.71^{a} \pm 0.03$	$0.86^{b} \pm 0.02$
Protein	$20.47^{a} \pm 0.29$	$23.50^{b} \pm 0.01$
Moisture Content	$66.16^{a} \pm 0.06$	$70.13^{b} \pm 0.22$
Dry Matter	$9.84^{a} \pm 0.05$	$4.81^{b} \pm 0.24$
Fat	$1.82^{a} \pm 0.08$	$0.72^{b} \pm 0.06$

Values are means of triplicate determinations. Means with different superscripts along the same row are significantly different (p≤0.05).

The ash content of brackish water fish was 1.71±0.03 and 0.86±0.02 for fresh water fish. Dry matter content was 9.84±0.05 for brackish water fish species and 4.81±0.24 fresh water fish species. Table 1 shows that ash content and dry matter levels in brackish water fish species were significantly higher (p≤0.05) than fresh water fish species. Brackish water systems characteristically have a higher concentration of minerals than fresh water systems and this may account for the significantly higher ash content in fish from brackish water systems. Ikeme, (17); Suleiman and Abdullahi, (18), Mba et. al (19) and Olagunju et al., (20) had similarly observed that habitat and species differences are some of the factors that account for the difference in ash content.

Table 1 shows that protein content was 20.47±0.29% in brackish water fish species and 23.50±0.01% in fresh water fish species, moisture was 66.16±0.06% in brackish water and 70.13±0.02% in fresh water fish species and fat content varied between 1.82±0.08% in brackish water fish species and 0.72±0.06% in fresh water fish species. Significantly higher (p≤0.05) levels of protein, fat and moisture were recorded in fresh water fish species. Protein content in the three species of fish here studied were relatively higher than was recorded for different species in earlier reports (21, 22, 23, 24) but however it is comparable with records of protein content in *Clarias gariepinus* (25). Two reasons may account for the observed higher protein content. Firstly fish species in

this study may have benefitted from a higher nutritional status of the environment, a pre-requisite for increased protein content in fish fillets. Secondly, protein content has been shown to vary with species (19). The significantly higher ($p \leq 0.05$) fat content in *O. niloticus*, a farmed fresh water fish in comparison with brackish water fish species is in agreement with earlier findings in which cultivated catfish had five times as much as wild catfish (26). However, Alam et al., (27) observed that wild species of *Labeo rohita* were richer in fat than farmed counterparts. Fat content may also be influenced by low energy utilization, limited tendency to migration for foraging for food and spawning as was the case in farmed *O. niloticus* in this study.

Table 2. *Concentration of minerals in fillets of fish from both fresh water and brackish water.*

Parameter (mg/100g)	Aquatic Habitats	
	Brackish Water	Freshwater
Calcium	70.14^{a}±2.30	94.86^{b}±0.43
Magnesium	34.80^{a}±1.30	32.00^{b}±2.30
Potassium	254.66^{a}±0.40	150.00^{b}±0.40
Sodium	141.20^{a}±0.57	57.20^{b}±0.2
Phosphorus	150.93^{a}±0.31	94.67^{a}±0.43

Values are means of triplicate determinations. Means with different superscripts along the same row are significantly different ($P<0.05$).

Mineral content of brackish and fresh water fish species: The mineral composition of brackish and fresh water fish is presented in Table 2

The concentration of calcium ranged from 70.14±2.30 in brackish water fish species to 94.86±0.43 mg/100g in fresh water species, while the level of magnesium was 34.80±1.30 in brackish water species and 32.00±2.30 mg/100g in fresh water species. The level of potassium in brackish water fishes was 254.66±0.04 and 150.06±0.40 mg/100g in fresh water species. The concentration of sodium was 141.20±57 mg/100g in brackish water species and 57.20±0.20 mg/100g in fresh water fish, concentration for phosphorus was 150.93±0.31 to 94.67±0.43 mg/100g. The calcium level in fresh water species under study compare favorably with those of pond raised catfish (28). The concentration of calcium in fresh water fish was significantly higher ($p \leq 0.05$) than in brackish water fish and was similarly observed by Robinson et. al. (29). Brackish water fish species contained significantly higher ($p \leq 0.05$) concentrations of magnesium, potassium, sodium and phosphorus and this may be due to the relatively higher mineral content in brackish water environments. The results indicate that these species of fish are good sources of macro-minerals for man.

Proximate quality of frozen and unfrozen fish fillets: The nutrient composition of *M. cephalus*, *C. nigrodigitatus*, *O. niloticus* before and after storage is presented in Table 3.

Table 3. *Nutritional composition of M. cephalus, C. nigrodigitatus O. niloticus before and after storage.*

Parameters (mean±SD) (%)	*M. cephalus*			*C. nigrodigitatus*			*O. niloticus*		
	Before storage	After storage	LSD	Before storage	After storage	LSD	Before storage	After storage	LSD
Ash	3.51^{a}±0.001	1.28^{b}±0.02	0.03	1.57^{a} ± 0.05	1.01^{b} ± 0.02	0.05	3.83^{a} ± 0.02	2.81^{b} ±0.01	0.03
Protein	20.14^{a}±0.39	19.17^{a}±1.81	0	20.85^{a}±0.20	20.47^{a}±0.20	0.01	23.50^{a}±0.06	23.41^{a}±0.40	0.02
Fat	3.41^{a} ±0.03	1.71^{b} ±0.11	0.35	2.33^{a} ± 0.03	1.25^{b} ± 0.28	0.04	2.75^{a} ± 0.01	1.42^{b} ± 0.09	0.22
Moisture	63.29^{a}±0.01	68.33^{b}±0.12	0.31	66.03^{a}±0.12	68.21^{b}±0.09	0.4	68.13^{a}±0.22	71.30^{b}±0.12	0.25
Dry Matter	9.65^{a}±0.23	9.53^{a}±0.77	0.78	9.25^{a}±0.88	9.96^{a}±0.21	0.03	1.82^{a}±0.97	1.06^{a}±1.26	0.88

Values are means of triplicate determinations. Means with different superscripts along the same row are significantly different ($P \leq 0.05$). SD is Standard deviation.

Ash content in M. cephalus was 3.51±0.001% before storage and 1.28±0.02% after freezing. In *C. nigrodigitatus* ash content was 1.57± 0.05% before and 1.01±0.02% after storage. Ash content in *O. niloticus* was 3.83±0.02% before storage and 2.81±0.01% after storage. These values indicate a significant decline ($P \leq 0.05$) in ash content during storage and this may result from the leaching of ash content during thawing. The percentage protein in M. cephalus was 20.14±0.39% before freezing and 19.71±1.81% after freezing while in *C. nigrodigitatus* it was 20.85±0.20% in fresh samples and 20.47±0.20% after frozen storage. The protein content in *O. niloticus* varied between 23.53±0.40% before and 23.41±0.40% after frozen storage. These data show that protein levels were not significantly reduced ($p \leq 0.05$) after frozen storage. On the contrary, several workers have observed that protein decreased with frozen storage (30, and 31). Reduction in percentage protein is explained by denaturation and aggregation (32, 33). In this study, it is possible that the temperature in which the fish was stored was effective in preserving protein over the time frame. Possibly too, the duration of the study was not sufficient to agitate a decline in protein content. Sharaf (34) reported that significant decline in protein content of Tilapia muscles occurred after eight weeks of frozen storage.

Fat content in M. cephalus before freezing was 3.41±0.03% and 1.71±0.11% after freezing. In fillets of *C. nigrodigitatus*, fat levels decreased significantly ($p \leq 0.05$) from 2.33±0.03% before to 1.25±0.28% after frozen storage. Similarly, fat levels in fillets of *O. niloticus* significantly declined ($p \leq 0.05$) from 2.75±0.01% before to 1.42±0.09% after frozen storage. In all the fish species under study, there was significant depression ($p \leq 0.05$) in fat content with frozen storage. Omotosho and Olu

(30) similarly observed that in all the species studied, the concentration of fat decreased after each succeeding period of frozen storage. Thus the observed reduction in fat content in this study appears to be associated with oxidation of the fat (35, 36). Many lipid degradation processes and products capable of either denaturing protein or cross-linking polypeptides that can aggregate into insoluble proteins (37). The later may have contributed to the relatively stable protein content in the fishes under the conditions of the study.

Percentage moisture in *M. cephalus* was 63.29±0.01% and 68.33±0.12% before and after frozen storage respectively. In *C. nigrodigitatus* this variable was 66.03±0.12% before and 68.21±0.09% after frozen storage. Percentage moisture in *O. niloticus* was 68.13±0.22% before frozen storage and 71.30±0.12% after that. These data indicate significant increases ($p \leq 0.05$) in percentage moisture in the three species of fish under study and is in compliance with observations made by Omotosho and Olu (30). In contrast however, Ruff et. al. (38), Keyvan et. al., (39) have shown that fillets subjected to frozen storage had lower moisture content compared with fresh fillets. Drip loss during the thawing process might be the reason for the decrease in moisture levels after frozen storage (40). Protein denaturation during frozen storage can cause loss of water-holding capacity in thawed fillets (41). In this study, protein levels did not depreciate over the study period and this is an indication that protein was not denatured under the study conditions and as such the fillets were able to sustain their moisture holding capacity.

It is worthy to note that in all the species under study, percentage fat in fillets depreciated as the moisture levels appreciated. This observation is in agreement with studies on salmonid fishes where fat and moisture levels have been shown to be inversely related (37).

Mineral content of frozen and unfrozen fish fillets: The mineral concentration in fillets of *M. cephalus*, *C. nigrodigitatus* and *O. niloticus* before and after storage is presented in Table 4. The mineral compositions in all three species of fish were comparable to values reported by Nettleton et al. (42). The concentration of sodium in fillets of M. cephalus varied from 142.67± 0.24mg/100g before storage to 140.33±0.11mg/100g after storage. The mean concentration of sodium in the fillets of *C. nigrodigitatus* before freezing was 139.73±0.90mg/100g and 136.67±0.23mg/100g after freezing. The concentration of sodium in *O. niloticus* was 57.20±0.24mg/100g before and 56.07±0.11mg/100g after freezing. These results show that there is significant decline ($p \leq 0.05$) in the sodium content of fish after storage.

Phosphorous concentration in the fish fillets decreased significantly ($p \leq 0.05$) during the study period and study conditions. The concentration of this mineral in M. cephalus was 185.70±0.40 mg/100g before and 174.75±0.25mg/100g after frozen storage and in *C. nigrodigitatus* it was 116.17±0.23mg/100g before and 108.42±0.23mg/100g after frozen storage. In fillets of *O. niloticus*, the concentration of phosphorous decreased significantly ($p \leq 0.05$) from 94.67±0.43 mg/100g to 88.68±0.46 mg/100g.

Calcium in the tissue of M. cephalus was 65.46±2.30mg/100g before storage and 62.79±2.30 mg/100g after freezing. The level of calcium in the flesh of *C. nigrodigitatus* was 74.82±2.30 mg/100g and 69.47±4.50 mg/100g before and after storage respectively. In fillets of *O. niloticus*, calcium concentration was 94.86±2.30 mg/100g before storage and 96.19±2.20 mg/100g after storage. These results do not indicate any significant decreases ($p \leq 0.05$) in the level of calcium in fish flesh during storage. Freeze storage however did not similarly impact on the level of potassium in the flesh of fishes under study conditions.

Magnesium content in flesh of M. cephalus was 34.40±1.30 (before storage) and 35.20 ±1.30 (after storage). In the flesh of *C. nigrodigitatus*, the concentration of magnesium was 35.20±1.30 and 34.40 ±1.30 before and after storage respectively. This variable was 32.00±1.30 (before storage) 31.20±2.40 (after storage) in the flesh of *O. niloticus*. These results also imply that freezing at sub 00C for 30 days did not diminish the level of magnesium in flesh of M. cephalus, *C. nigrodigitatus* and *O. niloticus*. Magnesium plays an important role in cellular metabolism especially in binding to ligands such as Adenosine Triphosphate (ATP) in ATP-requiring enzymes.

The concentration of potassium was 248.13±4.2mg/100g (before storage) and 247.60±3.40 (after storage) in fillets of M. cephalus, 261.20 ±1.30 mg/100g and 259.47±8.46mg/100g before and after storage respectively in *C. nigrodigitatus* and 150.00±3.40 mg/100g (before storage) 149.20±2.40 (after storage) in the *O. niloticus*.

All the studied fish species contained an appreciable amount of sodium, phosphorous, calcium, magnesium and potassium that are capable of meeting the nutritional requirements of human beings. The recommended daily allowance (RDA) for magnesium (and other metals) is 440.0mg per day (43, 44). These results also imply that freezing at sub 00C for 30 days did not diminished the level of sodium and phosphorous but the levels of calcium, magnesium and potassium in flesh of M. cephalus, *C. nigrodigitatus* and *O. niloticus* were diminished. These elements are required at various doses (Table 4) for the many metabolic processes that occur in humans. Potassium was observed to dominate other minerals in both the brackish water fishes (*M. cephalus and C. nigrodigitatus*) and in fresh water fish species (*O. niloticus*). This observation was supported by the findings of Adeniyi et. al. (45).

Table 4. *Minerals in fillets of brackish water fish species M. cephalus, C. nigrodigitatus) and O.niloticus before and after storage.*

	M. cephalus			*C. nigrodigitatus*			*O. niloticus*			**Recommended Daily Intake (FAO/WHO, 43, Lentech, 44)**
Minerals (mg/100g)	**Before storage**	**After storage**	**LSD**	**Before storage**	**After storage**	**LSD**	**Before storage**	**After storage**	**LSD**	
Sodium	142.67^{a}±0.24	140.33^{b}±0.11	0.29	139.73^{a}±0.90	136.67^{b}±0.23	2.07	57.20^{a}±0.24	56.07^{b}±0.11	0.76	2400-2500
Phosphorus	185.70^{a}±0.40	174.75^{b}±0.25	1.19	116.17^{a}±0.23	108.42^{b}±0.23	0.02	94.67^{a}±0.43	88.68^{b} ± .46	2.14	1000-2000
Calcium	65.46^{a} ± 2.30	62.79^{a}±2.30	0.00	74.82^{a} ± 2.30	69.47^{a}±4.50	0.00	94.86^{a}±2.30	96.19^{a}±2.20	0.00	1000
Magnesium	34.40^{a} ± 1.30	35.20^{a}±1.30	0.00	35.20^{a} ±1.30	34.40^{a}±1.30	0.00	32.00^{a}±1.30	31.20^{a}±2.40	0.00	350
Potassium	248.13^{a}±4.20	247.60^{a}±3.40	0.00	261.20^{a}±6.60	259.47^{a}±8.46	0.00	150.00^{a}±3.40	149.20^{a}±5.40	0.00	3500

Values are means of triplicate determinations. Means with different superscripts along the same row are significantly different ($p \leq 0.05$).

4. Conclusion

The species investigated in the present study: *M. cephalus, C. nigrodigitatus, and O. niloticus* are popular table fishes in Nigeria. Proteins, fats, ash and moisture which were considered in evaluating the nutritional value of the species studied. These fish species were also assessed for the level of magnesium, potassium, sodium and phosphorus in their fillets. The nutritional elements showed variable values in the species analyzed; with moisture recording the highest values and lipid recording the lowest. All the fish species that were studied belonged to high-protein (18-23%) low-oil (<5%) category. Ash content and dry matter levels in brackish water fish species were significantly higher than fresh water fish species while significantly higher levels of protein, fat and moisture were recorded in fresh water fish species. Magnesium, potassium, sodium and phosphorus were recorded at various levels in the species that were studied. The concentrations of magnesium, potassium, sodium were relatively higher in brackish water fish species while calcium level was higher in fresh water fish species. Observed variations in proximate composition and mineral content of studied fish species may result from habitat and species differences, seasonal and biological differences (size, age and sexual maturity), food source and environment (water chemistry, salinity, temperature and contaminants). Frozen storage minimized the level of ash, fat, moisture, sodium and phosphorous over a period of thirty days. In this time frame and experimental condition the level of protein, dry matter, sodium, magnesium and potassium did not decline.

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