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EFFECT OF STORAGE TIME ON THE QUALITY OF FROZEN *OREOCHROMIS NILOTICUS*

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108 fish samples of *Oreochromis niloticus* (average weight 210 ± 15g) was used. Analysis carried out include: proximate, mineral composition, biochemical, amino acid and sensory evaluation. Data obtained was subjected to Analysis of Variance (ANOVA) while the sensory data was subjected to nonparametric test (Kruskal Wallis test). Proximate quality, mineral, amino acid, biochemical and sensory quality of frozen *O. niloticus* content reduced with increase in storage period. Glutamic acid was the most concentrated and highest non-essential amino acid, lysine was the most abundant EAA. The EAA/NEAA ratio and predicted protein efficiency ratio (P-PER) increased with increased storage time while the essential amino acid index and biological value decreased with increased storage time. It is recommended that *O. niloticus* should be consumed as early as possible so as to maximize its nutrients.

Key word: Storage time, quality, frozen, *Oreochromis niloticus*

Fish is one of the most important foods in the world as its flesh is a source of high-quality protein, and it represents a significant proportion of the animal protein in the diet of many people as it is either taken fresh or cured in a variety of ways such as smoking, salting, drying, charring, icing, and chilling (Ayeloja, 2019). Roopma *et al.* (2012) stated that fish play important role in addressing nutritional and livelihood security of many people in the developing countries such as Nigeria as supply good quality polyunsaturated fatty acids (PUFA's), protein, minerals and vitamins which are essential for good health of man. Ayeloja *et al.* (2017) also submitted that fish is a much-cherished delicacy among many people in Nigeria because it is a highly proteinous, it is available, palatable and it has little or no religious taboo. As important as fish is, yet it is one of the most rapid perishable foods because of its short shelf life (Roopma *et al.*, 2012). Akinola *et al.* (2006) reported that some of the different types of preservation methods employed to reduce fish spoilage include: drying, smoking, freezing, chilling and brining. Roopma *et al.* (2012) also reported that the extension of fish shelf life can be achieved by freezing, chilling, salting, smoking, glazing etc. Freezing is one of the common fish preservation method employed by many countries of the world as consumers usually buy fish in bulk and store in refrigerator (Ali, 2013). However, deterioration of fish quality still continues during freezing. Changes in chemical composition and nutritional quality of fried sardine (*Clupea pilchardus*) produced by frozen storage and microwave reheating were reported by Castrillon *et al.* (1997). Kropf and Bowers (1992) stated that some of the disadvantages of freezing food products include product dehydration, rancidity, drip loss and product bleaching which have an overall effect on the quality of frozen food. Arannilewa *et al*. (2005) also observed decrease in protein quality with increasing duration of frozen storage. Whittle (1997) stated that storage time and temperature play important role in fish quality and shelf life, while Ryder *et al.* (1993) linked the availability of vital nutrients in fish to the method of storage. Quality deterioration of fish is characterized by the initial loss of the fresh fish flavour (sweet, seaweedy) followed by the development of a neutral odour/flavour (Helene *et al.*, 2010), leading to the

detection of off-odours and -flavours. Therefore, sensory attributes related to spoilage such as sour, pungent odour and flavour are used indicators of fish spoilage and thus used to determine fish shelf life (Martinsdottir *et al*., 2001). However, there is limited information on the effect of storage time on the quality of frozen *Oreochromis niloticus* which is one of the commercially important fish species in the study area as reported by Ayeloja *et al.* (2013) thus the need for this research.

MATERIALS AND METHODS

Sample Collection: 36 replicates each of 3 commercially important fish species namely *Clarias gariepinus,Oreochromis niloticus and* Heteroclarias (average weight 210 ± 15g) were collected at a commercial fish farm within Ilorin metropolies, Kwara state North-Central Nigeria. They were taken to laboratory where they were gutted, washed after which the following analysis were carried out.

PROXIMATE NUTRIENT ANALYSIS

Proximate compositions of fish were determined by conventional method of (AOAC, 2000).

Estimate of moisture: Petri dish was cleaned and weighed (W1). Then 1.0g of each of the grounded fish samples was measured in each petridish and then weighed (W2). They were each transferred into the oven at 105˚C for 3hours nonstop. After the first 3hours, the petridish was removed from the oven, allowed to cool and weighed. The petridish was returned into the oven and was brought out after an hour and weighed again; this process was repeated till a constant weight was achieved (W3). The percentage moisture was calculated by using this formula;

The % Moisture Content = (W2– W3/weight of sample used) × 100

Estimation of crude protein: The crude protein was determined by using Kjeldahl method). The fish sample (either smoked or frozen) will be grinded into a fine or smooth texture. A known weight (5.0g) of the fish sample is then weighed into a long necked Kjedahl flask along with 5g of coppersulphate anhydrous and 5g of sodium sulphate anhydrous. Then, 25ml of concentrated sulphuric acid (H_2 SO_4) was added. The flask was gently placed and the content was heated, the heating continued strongly until a clear solution was obtained. The digestion lasted between 3 to 5 hours. The clear hot solution obtained was allowed to cool after which the solution was filtered using filter paper. Then, 5ml of the filtered digested sample wass poured into the protein determination sector and add 10ml of 40% NaOH and then, distillation occur in the reactor. The steam being passed in the reactor condenses and drops into a conical flask containing boric acid (5ml) till the mixture changes color. After changing color, 50ml of the liquid was collected and titrated with 0.01M of HCl till the color (green) changes to deep blue.

Estimation of crude lipid: For the estimation of fat content, the dried samples left after moisture determination were finely grinded and the fat was extracted with a non polar solvent, ethyl ether. After extraction, the solvent was evaporated and the extracted materials were weighed.

The percentage of fat content was calculated as:

Fat of (%) = (weight of extract/weight of sample) x 100

Estimation ash: Crucible was cleaned and weighed (W1). Then 1.0g of each of the grounded fish samples was placed in each crucible and weighed (W2). They were each transferred into the furnace at 550˚C for 4 hours until each turned into ash. Thereafter, the crucible and the content were removed and placed in the desiccator to avoid moisture absorption, allowed to cool in the desiccator and weighed (W3). The difference in weights before and after ashing gives the total ash content and is expressed in terms of the total percentage of ash in the respective sample as thus:

The % Ash content = (W2– W3/weight of sample used)× 100

Carbohydrate (CHO): The total carbohydrate content was determined by subtracting the sum of the percentage moisture, ash, crude lipid, crude protein and crude fiber from 100%, that is:

Carbohydrate (%) = 100-(moisture+ash+protein+lipids+fiber %)

Mineral composition

Crucible was cleaned and weighed and then 5.0g of each of the ground fish samples was measured into each crucible. They were each transferred into the oven at 60˚C for the duration of 45minutes to 1hour. After oven drying, the sample was weighed into a conical flask and was digested using Nitric acid and HCl. After digestion, the concentration of the minerals was determined using Atomic Absorption Spectrophotometer (AAS).

Biochemical Test

The Total Volatile Base Nitrogen (TVBN), trimethyl amine (TMA), pH, peroxide value (PV) and free fatty acid (FFA) were determined following the method of Pearson (1982).

Amino acid analysis

The preparation of the fish samples was adapted from the procedure described by Benitez (1989). The fish samples were taken through the procedures of drying to constant weight, defatting, hydrolyzing (Bligh and Dyer, 1959), evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

Nitrogen determination

One hundred-fifteen milligram of ground sample was weighed, wrapped in whatman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10ml) was added. Catalyst mixture of 0.5gram containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Six pieces of anti-bumping granules were added. The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100ml in standard volumetric flask. Aliquot (10m1) of the diluted solution with 10ml of 45% sodium hydroxide was put into the Markham distillation apparatus and distilled into 10ml of 2% boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70ml of distillate was collected. The distillate was then titrated with standardize 0.01 N hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:

a = Titre value of the digested sample

b = Titre value of blank sample

v = Volume after dilution (100ml)

W = Weight of dried sample (mg)

C = Aliquot of the sample used (10ml)

14 = Nitrogen constant in mg.

Hydrolysis of the sample

A known weight (2.0g) 0f the defatted sample was weighed into glass ampoule and 7ml of 6N HCL was added. In order to avoid possible oxidation of some amino acids during hydrolysis such as methionine and cystine, nitrogen was passed into the ampoule to expel oxygen. The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^0C \pm 5^0C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer. It is noteworthy that with this hydrolysis procedure tryptophan content of the sample was not determined as it is chemically decomposed by 6N HCl during acid hydrolysis.

Determination of tryptophan

To identify tryptophan, a separate sample of the defatted tissue was hydrolysed using antioxidants such as dodecanethiol to replace 6N hydrochloric acid (HCl), thereby preserving tryptophan. The tryptophan in the known sample was hydrolyzed with 4.2 M Sodium hydroxide (Maria *et al.*, 2004). The known sample was dried to constant weight, defatted and hydrolyzed by taking a known weight (2.0g) of the defatted sample was weighed into glass ampoule. The reason being that alkaline hydrolysis has been shown to produce higher tryptophan recovery than acid hydrolysis. Sodium hydroxide was used instead of barium hydroxide to avoid problems of precipitation

and adsorption of tryptophan (Maria *et al*, 2004). Nitrogen was passed into the ampoule to expel oxygen and it was then sealed with Bunsen burner flame and put in an oven preset at $105^0C \pm 5^0C$ for 4 hours. The ampoule was allowed to cool and the content was filtered to remove the humins. The filtrate was then neutralized to pH 7.00 and evaporated to dryness at 40^0C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of borate buffer (pH 9.0) and store in plastic specimen bottles, which were kept in the freezer.

Loading of the Hydrolysate into the PTH analyser

Sixty microlitre of the hydrolysate obtained from the procedures was loaded in the analyzer. This was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of the analysis lasted for 45 minutes.

To calculate amino acid values, an integrator attached to the Analyzer calculates the peak area proportional to the concentration of each of the amino acids. The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and the width of the peak on the half-height was accurately measured and recorded. Approximate area of each peak was then obtained by multiplying the height with the width at half-height.

The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{\text{Area of norleucine peak}}{\text{Area of each amino acid}}$$

NB :- Norleucine is an internal standard

A constant S was calculated in g/100g protein using the following formula:

Sstd =NEstd × Mol. Weight ×µMAAstd

Finally, the amount of each amino acid present in the sample was calculated in g/100g protein using the formula:

Concentration (g/100g protein) = NH × W@ NH/2 × Sstd × C

Where: C= Dilution × 16/Sample wt (g) × N% × 10. Vol. Loaded ÷NH × W (nleu)

NH = Net height

W = Width @ half height

Nleu = Norleucine

The period of analysis lasted for 45 minutes. To determine nitrogen in the separated sample for analysing tryptophan, a 200mg ground sample was weighed, wrapped in whatman filter paper (No.1) and the procedure for nitrogen determination for amino acids as described was repeated. Percentage nitrogen was calculated.

Estimation of dietary protein quality

Predicted Protein Efficiency Ratio (P-PER)

The predicted protein efficiency ratio (P-PER) was estimated by using the equation given by Alsmeyer *et al.* (1974)

P-PER = -0.468 + 0.454(Leu) – 0.105(Tyr).

Amino Acid Score (AAS)

The essential amino acid score was calculated based on the whole hen's egg amino acid profiles (Paul and Southgate, 1976).

$$\text{Amino acid score} = \frac{\text{Amount of amino acid per test protein (g/100g)}}{\text{Amount of amino acid per protein in reference (g/100g)}}$$

Essential Amino Acid Index (EAAI)

The essential amino acid index (EAAI) was calculated by using the ratio of test protein to the reference protein for each ten essential amino acids (Oser, 1959)

Essential amino acid index =

$$\sqrt{\frac{\text{lysine P} \times \text{Tryptophan T} \ldots \text{threonine P}}{\text{lysine S} \times \text{Tryptophan S} \ldots \text{threonine S}}}$$

P = test protein

S = standard whole egg protein

Biological value (BV)

The Biological Value (BV) was calculated by the method of Oser (1959)

BV= 1.09 (EAAI) – 11.73

Organoleptic assessment

The various smoked fish species were subjected to sensory quality evaluation using descriptive test based on 5-point hedonic scale modified from Tobor (1994) and Eyo (2001). Odour, flavour and texture were sensory attributes examined, the following grades were allotted depending on their qualities: $8 \leq 10$ = Excellent, $6 \leq 8$ = Very good, $4 \leq 6$ = good, $2 \leq 4$ = bad and ≤ 2 = worst. Thirty semi-trained panelists from Department of Aquaculture and Fisheries, Faculty of Agriculture University of Ilorin

Kwara State Nigeria were used for the assessment.

Statistical analysis

SPSS 16.0 version was used for the statistical analysis. Data collected on descriptive organoleptic assessment using hedonic scale were subjected to nonparametric test (Kruskal Wallis test). While other data were subjected to Analysis of variance (ANOVA) using F-test to determine the treatments level of significance. Means of the significantly different treatments were separated using Duncan multiple range test at 95% confidence value.

RESULTS

The result of the proximate composition of frozen *Oreochromis niloticus* as presented on Table 1 indicates that the moisture content of the fish ranged between 80.5% ± 0.10 to 81.83% ± 0.15 within a storage period of 56 days. There was no significant difference ($p>0.05$) in the moisture content of the fish during the first 2 weeks of storage. However, the moisture content of the fish significantly increased ($p\leq0.05$) from day 28 through the remaining days of storage (56 days). The percentage crude protein of frozen *O. niloticus* ranged between 11.41% ± 0.31 to 15.23% ± 0.01. There was no significant difference ($p>0.05$) in the percentage crude protein of the fish during the first 2weeks of storage but later decreased significantly ($p\leq0.05$) from day 28 through the remaining days of storage (56 days). The highest crude protein value of 15.23% ± 0.01 was recorded at the onset (day 0) of the experiment which decreased significantly ($p\leq0.05$) to 11.41% ± 0.31 with increase in duration of frozen storage. Similar trend as observed for crude protein was also observed for the crude lipid which ranged between 1.44% ± 0.02 to 2.17 ± 0.06 and ash content which ranged between 1.19% ± 0.04 to 1.30% ± 0.02. However, the percentage carbohydrate content of the frozen *O. niloticus* increased significantly ($p\leq0.05$) with increase storage time.

The mineral composition of frozen *O. niloticus* (Table 2) indicates that the calcium (Ca) calcium decreased significantly ($p\leq0.05$) through the period of the storage and it ranges between 0.33mg/l - 0.55mg/l. Similar trend was observed for sodium (Na), iron (Fe), magnesium (Mg) and magnesium (Mg).

TABLE 1: Proximate composition of frozen *Oreochromis niloticus* with increased storage time

	DAY 0 (%)	**DAY 14 (%)**	**DAY 28 (%)**	**DAY 42 (%)**	**DAY 56(%)**
Moisture	80.50 ± 0.10^{a}	80.63 ± 0.06^{a}	81.25 ± 0.13^{b}	81.20 ± 0.52^{b}	81.83 ± 0.15^{c}
Crude protein	15.23 ± 0.01^{d}	15.10 ± 0.02^{d}	13.26 ± 0.16^{c}	12.95 ± 0.01^{b}	11.14 ± 0.31^{a}
Crude lipid	2.17 ± 0.06^{c}	2.15 ± 0.05^{c}	1.80 ± 0.18^{b}	1.83 ± 0.03^{b}	1.44 ± 0.02^{a}
Ash	1.30 ± 0.02^{bc}	1.41 ± 0.11^{c}	1.17 ± 0.07^{a}	1.24 ± 0.06^{ab}	1.19 ± 0.04^{ab}
CHO	0.80 ± 0.07^{a}	0.71 ± 0.10^{a}	2.52 ± 0.20^{b}	2.77 ± 0.55^{b}	4.39 ± 0.14^{c}

*Mean with different superscript in the row indicates significant difference at $p< 0.05$

TABLE 2: Mineral composition of frozen *Oreochromis niloticus* with increased storage time

	DAY0 (mg/l)	**DAY 14 (mg/l)**	**DAY 28 (mg/l)**	**DAY 42 (mg/l)**	**DAY 56 (mg/l)**
Calcium(Ca)	0.55 ± 0.01^{e}	0.50 ± 0.01^{d}	0.43 ± 0.01^{c}	0.38 ± 0.01^{b}	0.33 ± 0.04^{a}
Sodium (Na)	3.55 ± 0.07^{d}	3.10 ± 0.14^{c}	2.95 ± 0.21^{bc}	2.75 ± 0.07^{ab}	2.45 ± 0.07^{a}
Iron (Fe)	0.06 ± 0.01^{b}	0.05 ± 0.01^{b}	0.03 ± 0.00^{a}	0.03 ± 0.01^{a}	0.02 ± 0.00^{a}
Magnesium (Mg)	1.20 ± 0.00^{c}	1.05 ± 0.07^{b}	0.94 ± 0.06^{ab}	0.94 ± 0.01^{ab}	0.87 ± 0.02^{a}

*Mean with different superscript in the row indicates significant difference at $p< 0.05$

The result of the effect of storage time on biochemical quality of frozen *O. niloticus* (Table 3) indicates that significant difference ($p \leq 0.05$) exist in the TMA value of the fish which increase with increase in storage time. Similar trend was observed in TVBN and PV of the fish.

Table 4 shows the amino acid profile of *O. niloticus* during frozen storage. The result indicates that lysine and glutamic acid were recorded as the most abundant EAA and NEAA with values ranging between 2.20 to 2.79 g/100g cp and 3.03 to 4.29 g/100g cp respectively. Lysine abundance was followed by leucine (2.20 to 2.79 g/100g p) and arginine ranked third highest EAA with values ranging between 2.11 to 2.24 g/100g cp. Aspartic acid was the second highest/dominating AA after glutamic acid with values ranging between 2.48 to 3.51 g/100g cp while proline is the third highest NEEA (1.47 to 2.03 g/100g cp). The least EAA mean value was tryptophan (0.17 to 0.26 g/100g cp) followed by histidine (0.43 to 0.56 g/100g cp) and methionine (0.60 to 0.83 g/100g cp) while cystine (0.20 to 0.30 g/100g cp), serine (1.31 to 1.72 g/100g cp) and glycine (1.35 to 1.98 g/100g cp) were the least NEAAs respectively. Glutamic was the most abundant amino acid throughout the period of the study. A significant decrease ($p \leq 0.05$) was observed in lysine, tryptophan, aspartic acid, proline, threonine and cystine with increase in storage time

Table 3: Effect of biochemical quality on frozen *Oreochromis niloticus*

	TMA	TVBN	pH	PV	FFA
Day 0	1.07 ± 0.01^{a}	14.82 ± 0.01^{a}	7.17 ± 0.06^{a}	23.42 ± 0.02^{a}	6.27 ± 0.01^{d}
Day 14	1.22 ± 0.01^{b}	16.04 ± 0.01^{b}	7.27 ± 0.06^{ab}	25.05 ± 0.02^{b}	6.21 ± 0.01^{a}
Day 28	1.27 ± 0.02^{c}	16.10 ± 0.01^{c}	7.27 ± 0.06^{ab}	25.10 ± 0.01^{c}	6.21 ± 0.01^{a}
Day 42	1.30 ± 0.01^{d}	16.16 ± 0.02^{d}	7.33 ± 0.06^{b}	25.21 ± 0.01^{d}	6.23 ± 0.01^{b}
Day 56	1.34 ± 0.02^{e}	16.24 ± 0.01^{e}	7.37 ± 0.06^{b}	25.30 ± 0.01^{e}	6.25 ± 0.01^{c}

Means $\pm$ SD with different superscript in the same column indicating significant differences at $p \leq 0.05$

Table 4: Effect of storage time on amino acid profile of *O. niloticus*

AMINO ACID (g/100g)	DAY 1	DAY 28	DAY 56
ESSENTIALS			
LEUCINE	2.70 ± 0.06^{b}	2.96 ± 0.04^{ab}	3.01 ± 0.13^{a}
LYSINE	2.79 ± 0.04^{a}	2.61 ± 0.00^{b}	2.20 ± 0.04^{c}
ISOLEUCINE	1.79 ± 0.02^{a}	1.55 ± 0.04^{b}	1.59 ± 0.02^{b}
PHENYLALANINE	1.06 ± 0.00^{a}	1.07 ± 0.01^{a}	1.03 ± 0.04^{a}
TRYPTOPHAN	0.26 ± 0.00^{a}	0.22 ± 0.01^{b}	0.17 ± 0.01^{c}
VALINE	2.04 ± 0.06^{a}	1.98 ± 0.01^{a}	1.68 ± 0.03^{b}
METHIONINE	0.83 ± 0.04^{a}	0.74 ± 0.06^{a}	0.60 ± 0.00^{b}
ARGININE	2.24 ± 0.12^{a}	2.16 ± 0.11^{a}	2.11 ± 0.06^{a}
HISTIDINE	0.45 ± 0.09^{a}	0.53 ± 0.03^{a}	0.43 ± 0.04^{a}
THREONINE	1.91 ± 0.02^{a}	1.59 ± 0.11^{b}	1.21 ± 0.01^{c}
NON-ESSENTIALS			
PROLINE	2.03 ± 0.00^{a}	1.63 ± 0.00^{b}	1.47 ± 0.07^{c}
TYROSINE	1.03 ± 0.00^{a}	0.95 ± 0.13^{a}	0.78 ± 0.11^{a}
CYSTINE	0.30 ± 0.00^{a}	0.25 ± 0.01^{b}	0.20 ± 0.00^{c}
ALANINE	2.19 ± 0.13^{a}	2.15 ± 0.07^{a}	2.20 ± 0.00^{a}
GLUTAMIC ACID	4.29 ± 0.06^{a}	3.72 ± 0.74^{a}	3.03 ± 0.00^{a}
GLYCINE	1.98 ± 0.04^{a}	1.75 ± 0.11^{a}	1.35 ± 0.07^{b}
SERINE	1.72 ± 0.06^{a}	1.44 ± 0.04^{b}	1.31 ± 0.01^{b}
ASPARTIC ACID	3.51 ± 0.13^{a}	3.11 ± 0.01^{b}	2.48 ± 0.08^{c}

Data is expressed as mean (g/100g protein) ± SD of two replicate. Values in the same column with different superscript are significantly different ($p \leq 0.05$).

while phenylalanine, arginine, tyrosine, histidine, alanine and glutamic acid showed no significant variation (p> 0.05) with increase in storage time.

The result of protein quality parameters of frozen *O. niloticus* with increase storage time (Table 5) indicates that *O. niloticus* have higher concentration of non-essential amino acids than essential amino acids. The EAA/NEAA ratio increased with increased storage time. Its chemical scores ranges between 85.36 to 90.56 g/100g cp. The essential amino acid index decreased with increased storage time and ranged between

Table 5: Changes in quality parameters of Nile Tilapia (*O. niloticus*) with increased storage time

	DAY 1	DAY 28	DAY 56
TAA (g/100g CP)	33.12	30.41	26.85
TEAA (g/100g CP)	16.07	15.0	14.03
TNEAA (g/100gCP)	17.05	15.41	12.82
EAA/NEAA	0.94	0.97	1.09
EAAI	0.28	0.26	0.23
CS (g/100g CP)	85.56	87.78	90.56
P-PER (g/100g CP)	0.65	0.78	0.82
BV	18.37	16.97	13.47

TAA= total amino acids; TEAA= total essential amino acid; TNEAA= total non-essential amino acid; CS= chemical score; EAAI= Essential amino acid index; P-PER= predicted protein efficiency ratio; BV= biological value.

Table 6: Amino acid score and Indispensable Amino acid Index (IAAI) of *O. niloticus*

Essential amino acids	Amino acid scores (g/100g crude protein)			Whole hen's egg protein	FAO/WHO provisional amino acid scoring pattern
	Day 1	Day 28	Day 56		
LEUCINE	0.33	0.36	0.36	8.3	7.0
LYSINE	0.45	0.42	0.35	6.2	5.5
ISOLEUCINE	0.32	0.28	0.28	5.6	4.0
PHENYLALANINE	0.21	0.21	0.20	5.1	+tyr 6.0
TRYPTOPHAN	0.14	0.12	0.09	1.8	1.0
VALINE	0.27	0.26	0.22	1.5	5.0
METHIONINE	0.26	0.23	0.19	3.2	+cys 3.5
ARGININE	0.37	0.35	0.35	6.1	
HISTIDINE	0.19	0.22	0.18	2.4	
THREONINE	0.37	0.31	0.24	5.1	4.0
EAAI	0.28	0.26	0.23		

EAAI = Essential amino acid index. Whole hen's egg protein: Adopted from Paul and Southgate (1976). FAO / WHO provisional amino acid scoring pattern of whole hen egg (FAO/WHO, 1991).

Table 7: Effect of storage time on organoleptic quality of frozen *Oreochromis niloticus*.

	Day 0	**Day 14**	**Day 28**	**Day 42**	**Day 56**	χ^2	**P- value**
Eye	8.067	7.000	7.400	7.533	7.267	6.021	0.20
Gill	8.267	6.133	7.333	7.400	7.133	12.892**	0.01
Skin	7.933	7.400	8.000	7.600	7.067	5.323	0.26
Odor	7.933	6.600	7.667	7.800	7.467	5.583	0.23
Flesh	7.533	6.000	7.867	7.933	7.067	12.298*	0.02

Kruskal Wallis test (χ^2) is significant along the row $P \leq 0.05$.

23.09 to 27.59 g/100g cp. The predicted protein efficiency ratio (P-PER) ranged between 0.65 to 0.82 g/100g cp. The highest and least biological values were recorded in day 1 and day 56 respectively.

The amino acid scores of *O.niloticus* in relation to the amino acid scoring pattern of whole hen's egg protein as presented on Table 6 indicates that lysine had the highest amino acid score ranging from 35.48 to 45.00 g/100g cp while tryptophan had the least amino acid score with values ranging between 9.44 to 14.44 g/100g cp

The result of the effect of storage time on organoleptic quality of frozen *O. niloticus* (Table 7) showed that there was significant difference (χ^2 = 12.892, $p \leq 0.01$, χ^2 = 12.298, $p \leq 0.01$) in the values allotted by taste panelist for gill and flesh of frozen *O. niloticus* with increase in storage time while the eye, skin and odor had no significant differences (χ^2= 6.021, 5.323, 5.583, $p > 0.05$ respectively) with increase in storage time.

DISCUSSION

The result on Table 1 which indicates that significant ($p < 0.05$) increase was observed in the moisture content of frozen *O. niloticus* during the period of storage could be due to the medium in the fish was stored. The observed decrease in percentage crude protein and crude lipid with increase storage time was similar to that observed by other researchers such as Marwa (2015) who also observed significant decrease ($p < 0.05$) in the crude protein of *Saurida undosquamis* during freezing storage. Siddique *et al.*(2011) also observed significant decrease in crude protein of *Puntius sp*. and *Tilapia nilotica* during frozen storage. The decrease in the crude protein of frozen *O. niloticus* could be attributed to protein denaturation and loss in gelatin resulting from extended frozen storage (Marwa, 2015). The observed decrease in the crude lipid in this study is similar to that reported by Arannilewa *et al*. (2005) during frozen storage of tilapia fish (Sarotherodun galiaenus). However, carbohydrate of frozen *O. niloticus* increased significantly with increased storage time. This study also established that *O. niloticus* is a good source of minerals required for normal tissue metabolism and for maintenance of human health and development as it is rich in Ca, Na, Fe, Mg and Mg (Table 2). The observed decrease in the mineral content of *O. niloticus* with increased storage time in this study is similar to the findings of (Pawar *et al*., 2013) who observed a decrease in the mineral content of *Catla catla* during frozen storage which was attributed to the drip loss and the dehydration associated with frozen storage. Marwa (2015) stated that calcium is necessary to maintain an optimal bone development, with more of Ca being required during childhood and growing stages to prevent rickets and osteomalacia while magnesium is required in the plasma and extra cellular fluid, where it helps in maintaining osmotic equilibrium. It is also required in many enzyme catalyzed reactions. The biochemical quality (TMA, TVBN, pH and PV) of frozen *O. niloticus* (Table 3) increased significantly ($p \leq 0.05$) with increase storage time. Similar result was observed by Stamatia *et al.* (1997). Increase in pH of frozen *O. niloticus* could be due to enzymatic degradation of fish muscle leading to an increase in volatile bases. Similar result was reported by Obemeata *et al.* (2011) who observed an increase in pH of Tilapia fish during frozen storage. Pawar *et al.* (2013) also observed increase in the pH of *Catla catla* during frozen storage. The EAA and NEAA observed in *O. niloticus* (Table 4) is similar to that reported by Shi *et al.* (2013) who reported that EAA and NEAA detected in Juvenile Paddlefish (*Polyodon spathula*) include: Threonine, Valine, Metheionine, Phenyl alanine, Leucine, Lysine and Aspartic acid, Glutamic acid, Serine, Glycine, Alanine, Tyrosine, Cystine, Proline respectively. The result (Table 4) also indicate that lysine and glutamic acid were the most abundant EAA and NEAA with values ranging between 2.20 - 2.79 g/100g cp and 3.03 - 4.29 g/100g cp respectively while lysine abundance (EAA) was followed by leucine (2.20 - 2.79 g/100g p) and arginine (2.11-2.24 g/100g cp). Aspartic acid was the second highest AA after glutamic acid with values ranging between 2.48 to 3.51 g/100g cp while proline is the third highest NEEA (1.47 to 2.03 g/100g

cp). Adeyeye *et al.* (2014) reported similar finding that lysine was the most concentrated essential amino acid in *Acanthurus monronviae* while glutamic acid was reported to be the most concentrated amino acid amino acid and the highest Non Essential amino acid (NEAA). The least EAA in this study was tryptophan (0.17 to 0.26 g/100g cp) (Table 4) followed by histidine (0.43 to 0.56 g/100g cp) and methionine (0.60 to 0.83 g/100g cp) while cystine (0.20 to 0.30 g/100g cp), serine (1.31 to 1.72 g/100g cp) and glycine (1.35 to 1.98 g/100g cp) were the least NEAAs respectively. A significant decrease ($p \leq 0.05$) was observed in lysine, tryptophan, aspartic acid, proline, threonine and cystine with increase in storage time while phenylalanine, arginine, tyrosine, histidine, alanine and glutamic acid showed no significant variation ($p > 0.05$) with increase in storage time. This is in contrast with the report of El Lahamy (2018) in their study of Effect of frozen storage and cooking method on amino acid composition of mullet fish (*Mugil cephalus*) where it was reported that tyrosine, serine and argnine increased during frozen storage Bergendal *et al.* (2008) also reported that frozen storage did not have influence on the amino acid values of fish species examined except cysteine that have the highest amino acid loss during frozen storage. Somayeh *et al.* (2012) also reported that the amino acid that was most damaged during the process of freezing storage and defrosting was cysteine. Bergendal *et al.* (2008) attributed the change in some amino acids during frozen storage to transition of one kind of amino acid to another through oxidation and deamination. The result on Table 5 indicates that *O. niloticus* have higher concentration of non-essential amino acids than essential amino acids. The EAA/NEAA ratio increased with increased storage time. Its chemical scores ranges between 85.36 to 90.56 g/100g cp. The essential amino acid index decreased with increased storage time and ranged between 23.09 to 27.59 g/100g cp. The predicted protein efficiency ratio (P-PER) ranged between 0.65 to 0.82 g/100g cp which is lower than the standard value of 2.7, which is the standard value of casin protein. The protein efficiency ratio (PER) determines the effectiveness of a protein through the measurement of animal growth (Adeyeye, 2014). The highest biological value was recorded on the first day (day 1) of the experiment (18.37) and it decreased with increase storage time indicating that the longer the fish stay in freezer, the lesser the efficient utilization of its nutrients by human body. The amino acid scores of *O.niloticus* in relation to the amino acid scoring pattern of whole hen's egg protein as presented on Table 6 indicates that lysine had the highest amino acid score ranging from 35.48 to 45.00 g/100g cp while tryptophan had the least amino acid score with values ranging between 9.44 to 14.44 g/100g cp. The result of the effect of storage time on organoleptic quality of frozen *O. niloticus* (Table 7) showed that there was significant difference ($\chi^2 = 12.892$, $p \leq 0.01$, $\chi^2 = 12.298$, $p \leq 0.01$) in the values allotted by taste panelist for gill and flesh of frozen *O. niloticus* with increase in storage time while the eye, skin and odor had no significant differences ($\chi^2 = 6.021$, 5.323, 5.583, $p > 0.05$ respectively) with increase in storage time.

CONCLUSION

This study revealed that the quality (proximate, minerals, biochemical, amino acid and sensory qualities) of frozen *O. niloticus* reduced with increase storage time. Lysine and glutamic acid were the most abundant EAA and NEAA in the fish. The longer the fish stay in freezer, the lesser the efficient utilization of its nutrients by human body as its biological value decrease with increase storage time. It is therefore recommended that *O. niloticus* should be consumed as early as possible so as to maximize its nutrients.

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