Effect of storage time on the quality of smoked *Oreochromis niloticus*

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**ABSTRACT**

Effect of storage time on the quality of smoked *Oreochromis niloticus* was studied. One hundred fish samples of *O. niloticus* (average weight 210 ± 15g) was used. Analysis carried out include: proximate, mineral composition (Ca, Na, Fe and Mg), 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. Na and Mg were the most abundant mineral while glutamic acid and lysine were the most concentrated non-essential amino acid (NEEA) and essential amino acid (EAA) respectively. The total volatile base nitrogen (TVBN), trimethyl amine (TMA) and peroxide values (PV) obtained are within the recommended level for good quality food while NEAA and EAA are within recommended values by WHO. The amino acid scores of the fish in relation to the amino acid scoring pattern of whole hen's egg protein indicate that lysine had the highest amino acid score. No significant (χ² = 6.624, p > 0.05) variation was observed in the score allotted for the flavor of smoked *O. niloticus* during storage. Smoked *O. niloticus* being a good source of protein, minerals and amino acids, and the quality is within good quality protein recommended by world health organization (WHO), is therefore recommended for consumption.

1. **Introduction**

Fish is consumed in many parts of the world because of its good nutritional quality such as proteins, essential amino acids, vitamins, minerals, fats, and it is highly digestible (Geoffroy et al., 2018). Fagbenro et al. (2005) also reported that fish protein is more consumed than others because it is available at a lower cost than other animal proteins. The protein content of fish has immense nutritional importance to pregnant women for proper development of the foetus (Isangedighi et al., 2017); it will also enhance the proper mental and immunity development against disease among growing children (NAFDAC, 2003). However, fish is a highly perishable food due to its high water activity, protein content and activities of autolytic enzymes, which cause spoilage (Masgaw et al., 2014). Okeyo et al. (2009) also reported that the more nutrient rich food is delayed prior to processing, the lesser the quality of the resultant product. Smoking is the major fish preservation method employed in Nigeria (Ayeloja et al., 2015). Fish smoking is a very relevant activity in the fisheries sector, as a method of fish processing and preservation because it enhances flavour and increases the utilization of fish thereby reducing wastage that might result from fish spoilage which will in turn influence protein availability in the country (Kumolu-Johnson and Ndimele, 2011). Kiin-Kabari et al. (2011) opined that smoke-dried fish is an important ingredient in the Nigerian traditional diet as it is known for its appetizing taste and flavour. *Oreochromis niloticus* is one of the most valued fish species in Nigeria as it is rich in vitamins, proteins and minerals (Geoffroy et al., 2018). However, there limited information on its quality when smoked and stored on shelf thus the need for this research. This study therefore examines effect of storage time on the quality of smoked *Oreochromis niloticus*.

1.1. **Ethical approval**

Approval for the use of *Oreochromis niloticus* for this study was obtained from the Ethical Review Committee of the University of Ilorin, Ilorin Nigeria.

2. **Materials and methods**

Standard operating procedures in accordance with University of Ilorin guidelines and regulations for the care and use of laboratory animals were followed during transportation of fish, welfare, housing prior to commencement, at the beginning and during the experiment. The
reportage was inline with ARRIVE (Animal Research: Reporting of In-vivo Experiments) guideline.

2.1. Sample collection

100 samples of Oreochromis niloticus (average weight 210 ± 15g) were collected at a commercial fish farm within Ilorin metropolis, Kwara state North-Central Nigeria. They were taken to laboratory where they were prepared following the method modified from the method of Ayeloja et al. (2015) (Figure 1) as the fish were euthanized, gutted, washed and smoked using NIOMR (Nigeria Institute of Oceanography and Marine Research) designed smoking kiln which is known to regulate Polycyclic Aromatic Hydrocarbons (PAH) including benzo pyrene in smoked fish products to acceptable limits for human consumption (Ayeloja et al., 2019) after which the following analysis were carried out.

3. Proximate nutrient analysis

Proximate compositions of fish were determined by conventional method of (AOAC, 2000). Moisture contents was determined via loss on drying at 65 °C for 72 h. The crude protein was determined by using Kjeldahl method. Fat content was estimated using the Soxhlet method. The ash was determined by heating in furnace at 550 °C for 24 h. The total carbohydrate content was determined by subtracting the sum of the percentage moisture, ash, crude lipid, crude protein and crude fiber from 100%. Water activity was calculated using the method of Doe et al. (1982).

3.1. 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 1 hour. 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).

3.2. 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).

3.3. 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.

3.4. 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 °C ± 5 °C 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 °C 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.

3.5. Estimation of dietary protein quality

3.5.1. 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)

\[
P\text{-PER} = \frac{0.468 + 0.454\text{[Leu]} - 0.105\text{[Tyr]}}{}
\]

(1)

3.5.2. Amino acid score (AAS)

The essential amino acid score was calculated based on the whole hen’s egg amino acid profiles (Paul et al., 1976).

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

(2)

3.5.3. 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)

\[
\text{EAAI} = \sqrt{\frac{\text{Lysine P} \times \text{Tryptophan P} \times ... \times \text{Threonine P}}{\text{Lysine S} \times \text{Tryptophan S} \times ... \times \text{Threonine S}}}
\]

(3)

\[
P = \text{test protein}
\]

\[
S = \text{standard whole egg protein}
\]

3.5.4. Biological value (BV)

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

\[
\text{BV} = 1.09\times(\text{EAAI}) - 11.73
\]

(4)

Figure 1. Flow chart for the production of smoked catfish, Oreochromis niloticus.
### 3.6. Organoleptic assessment

The various smoked fish species were subjected to sensory quality evaluation using descriptive test based on 5-point hedonic scale modified from Tobar (1994) and Eyo (2001). Odour, flavour and texture were sensory attributes examined, the following grades were allotted depending on their qualities: 8 ≤ 10 = Excellent, 6 ≤ 8 = Very good, 4 ≤ 6 = good, 2 ≤ 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.

### 3.7. 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.

### 4. Results

The proximate composition of smoked *Oreochromis niloticus* is presented on Table 1. The result indicates that moisture content of smoked *Oreochromis niloticus* ranged between 16.12% and 17.17%. The highest moisture content (17.17%) of the fish was recorded on the 56th day of storage while the lowest moisture content (16.12%) was recorded on the 28th day of storage. However, the moisture content of the fish increased significantly (p < 0.05) from one another.

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Table 1. Proximate composition of smoked *Oreochromis niloticus* with increased storage time.

| Proximate        | DAY 0     | DAY 14    | DAY 28    | DAY 42    | DAY 56    |
|------------------|-----------|-----------|-----------|-----------|-----------|
| Moisture (%)     | 16.30 ± 0.01<sup>a</sup> | 16.15 ± 0.06<sup>b</sup> | 16.12 ± 0.02<sup>c</sup> | 16.22 ± 0.02<sup>d</sup> | 17.17 ± 0.06<sup>e</sup> |
| Crude protein (%)| 60.07 ± 0.03<sup>a</sup> | 59.42 ± 0.20<sup>b</sup> | 58.84 ± 0.06<sup>c</sup> | 56.62 ± 0.23<sup>d</sup> | 55.67 ± 0.12<sup>e</sup> |
| Crude lipid (%)  | 11.48 ± 0.01<sup>a</sup> | 11.42 ± 0.03<sup>b</sup> | 11.27 ± 0.06<sup>c</sup> | 11.24 ± 0.02<sup>d</sup> | 10.10 ± 0.10<sup>e</sup> |
| Ash (%)          | 4.62 ± 0.01<sup>a</sup> | 4.53 ± 0.09<sup>b</sup> | 4.14 ± 0.05<sup>c</sup> | 4.52 ± 0.08<sup>d</sup> | 4.21 ± 0.01<sup>e</sup> |
| CHO (%)          | 7.54 ± 0.01<sup>a</sup> | 8.48 ± 0.08<sup>b</sup> | 9.63 ± 0.08<sup>c</sup> | 11.41 ± 0.19<sup>d</sup> | 12.85 ± 0.21<sup>e</sup> |
| Salt (%)         | 21.50 ± 0.20<sup>a</sup> | 22.33 ± 0.22<sup>b</sup> | 22.97 ± 0.07<sup>c</sup> | 25.34 ± 0.20<sup>d</sup> | 26.40 ± 0.14<sup>e</sup> |
| Water activity   | 0.79 ± 0.01<sup>a</sup> | 0.78 ± 0.01<sup>b</sup> | 0.77 ± 0.01<sup>c</sup> | 0.75 ± 0.01<sup>d</sup> | 0.74 ± 0.01<sup>e</sup> |

<sup>a</sup>Mean with different superscript in the row are significant different (p < 0.05) from one another.

The mineral composition of smoked *Oreochromis niloticus* with increase in storage time is presented on Table 2. The calcium (Ca) composition of the fish ranged between 0.43 mg/l to 0.63 mg/l within 56 days. There was no significant difference (p > 0.05) in the calcium composition of the fish during the first 28 days while the calcium (Ca) constituent significantly reduced (p ≤ 0.05) at day 42. However, there was no significant difference (p > 0.05) in the calcium (Ca) composition of the fish at day 42 and day 56. The sodium (Na) composition of the smoked *Oreochromis niloticus* ranged between 4.05 mg/l to 4.88 mg/l. The sodium (Na) composition of the smoked *Oreochromis niloticus* decreased significantly (p ≤ 0.05) through the storage period. The iron (Fe) composition of the fish ranged between 0.06 mg/l to 0.10 mg/l. However, there was no significant difference (p > 0.05) in the iron composition of the fish in the first 28 days of storage. Also, there was no significant different (p > 0.05) in the iron composition of the fish at day 42 and day 56.

Table 2. Mineral composition of smoked *Oreochromis niloticus* with increased storage time.

| Mineral       | DAY0     | DAY 14    | DAY 28    | DAY 42    | DAY 56    |
|---------------|----------|-----------|-----------|-----------|-----------|
| Calcium (Ca) (mg/l) | 0.63 ± 0.04<sup>b</sup> | 0.62 ± 0.00<sup>b</sup> | 0.58 ± 0.01<sup>b</sup> | 0.48 ± 0.04<sup>c</sup> | 0.43 ± 0.04<sup>d</sup> |
| Sodium (Na) (mg/l) | 4.88 ± 0.04<sup>a</sup> | 4.65 ± 0.07<sup>b</sup> | 4.45 ± 0.07<sup>c</sup> | 4.26 ± 0.06<sup>d</sup> | 4.05 ± 0.07<sup>e</sup> |
| Iron (Fe) (mg/l)  | 0.10 ± 0.01<sup>a</sup> | 0.09 ± 0.02<sup>b</sup> | 0.08 ± 0.01<sup>c</sup> | 0.06 ± 0.01<sup>d</sup> | 0.06 ± 0.01<sup>e</sup> |
| Magnesium (Mg) (mg/l) | 1.90 ± 0.06<sup>a</sup> | 1.87 ± 0.01<sup>b</sup> | 1.77 ± 0.02<sup>c</sup> | 1.68 ± 0.03<sup>d</sup> | 1.53 ± 0.04<sup>e</sup> |

Mean with different superscript in the row are significant different (p < 0.05) from one another.
significant difference (p > 0.05) in the iron composition of the fish from day 14 through to day 56 of storage. The magnesium (Mg) composition of the smoked *Oreochromis niloticus* ranged between 1.53 mg/l to 1.90 mg/l. There was no significant difference (p > 0.05) in the magnesium composition of the fish during the first 14 days. Thereafter, the magnesium composition decreased (p < 0.05) significantly through the remaining period of storage (56 days).

Effect of storage time on biochemical quality of smoked *Oreochromis niloticus*.

Table 3 presents the effect of storage time on biochemical quality of smoked *Oreochromis niloticus*. The result shows that significant difference (p ≤ 0.05) exist in the TMA value of the fish with increase in storage time (day 0 to day 56) having the highest TMA value of (22.62) and the lowest value (18.10) was recorded with values increasing with increase in storage time. Similar trend was observed for the TVBN value of the fish where the highest value recorded was (26.62) and the lowest value was (16.50). However, the pH had no significant differences (p > 0.05) with storage time. Similarly, the PV of the fish had no significant differences respectively. There was no significant differences (p > 0.05) in the FFA of the fish with increase in storage time.

### 4.1. The Amino Acid profile of *Oreochromis niloticus* across storage time

The amino acid profile of *Oreochromis niloticus* muscles at different storage time (day 0, day 28 and day 56) are presented in Table 5.

| Essential Amino Acid | Day 0        | Day 28        | Day 56        |
|----------------------|--------------|--------------|--------------|
| Leucine              | 7.97 ± 0.42a | 6.25 ± 0.37b | 5.41 ± 0.13c |
| Lysine               | 9.18 ± 0.08a | 7.68 ± 0.05b | 6.94 ± 0.16c |
| Isoleucine           | 4.00 ± 0.05a | 3.58 ± 0.04b | 3.52 ± 0.03b |
| Phenylalanine        | 3.76 ± 0.05a | 3.73 ± 0.11a | 3.34 ± 0.05b |
| Tryptophan           | 0.88 ± 0.01a | 0.81 ± 0.03ab| 0.74 ± 0.04ab|
| Valine               | 4.53 ± 0.04a | 4.04 ± 0.07b | 3.50 ± 0.14a |
| Methionine           | 3.07 ± 0.11a | 2.31 ± 0.01b | 1.91 ± 0.13ab|
| Threonine            | 4.32 ± 0.62a | 3.89 ± 0.17a | 3.70 ± 0.00ab|
| Arginine             | 5.77 ± 0.12a | 5.77 ± 0.11a | 4.73 ± 0.12a |
| Histidine            | 2.40 ± 0.05a | 1.94 ± 0.04b | 1.80 ± 0.00ab|

Non-Essential Amino Acid (g/100g)

|            | Day 0     | Day 28    | Day 56    |
|------------|-----------|-----------|-----------|
| Cystine    | 0.55 ± 0.09a | 0.86 ± 0.01a | 0.72 ± 0.00a |
| Alanine    | 5.39 ± 0.01a | 5.43 ± 0.16a | 4.95 ± 0.07a |
| Glutamic Acid | 12.64 ± 2.99a | 13.11 ± 0.09a | 12.06 ± 0.28a |
| Glycine    | 4.06 ± 0.17a | 7.01 ± 0.44a | 6.26 ± 0.08a |
| Serine     | 3.73 ± 0.08a | 3.72 ± 0.05b | 3.52 ± 0.03b |
| Aspartic Acid | 8.75 ± 0.26a | 8.88 ± 0.16ab | 8.25 ± 0.07ab |
| Proline    | 3.28 ± 0.31a | 4.98 ± 0.14a | 4.36 ± 0.14ab |
| Tyrosine   | 3.27 ± 0.00a | 2.84 ± 0.11b | 2.68 ± 0.11ab |

Values in the same row with different superscript are significantly different.

Eighteen amino acids (10 essential, 8 non-essential) were observed in the fish with their mean values as shown in the table above. The highest and lowest mean value at day 0 was observed in glutamic acid (12.64) and cystine (0.55) respectively. The highest and lowest mean value at day 28 was observed in glutamic acid (13.11) and tryptophan (0.81) respectively. The highest and lowest mean value at day 56 was observed in glutamic acid (12.06) and cystine (0.72) respectively. Decrease in content is observed across storage time in primarily some amino acid. In the one-way ANOVA test of the samples, significant difference (p < 0.05) was observed in leucine, lysine, valine, methionine and histidine content of the sample throughout the storage period. No significant difference (p > 0.05) was observed in glutamic acid content of sample throughout the storage period. No significant difference was observed in tryptophan between the periods of 0–28 days and 28–56 days of storage (p > 0.05), however a significant difference was noticed between 0 and 56 days of storage (p < 0.05). No significant difference was observed in aspartic acid between the periods of 0–28 days and 0–56 days of storage (p > 0.05), however a significant difference was noticed between 28 and 56 days of storage (p < 0.05). Phenylalanine, arginine, alanine and serine showed similar trend of no significant difference (p > 0.05) at day 0 and 28 days, but a statistical variation (p < 0.05) is noted at 56 days of storage. A significant difference was observed in isoleucine, proline, tyrosine, cystine, glycine and threonine between the periods of 0–28 days and 0–56 days of storage (p < 0.05), however no significant difference was noticed between 28 and 56 days of storage (p > 0.05).

The Table 5 showed the protein quality parameters of *Oreochromis niloticus* as storage time increases. A decrease in total amino acid is observed with increased storage time. A higher concentration of total non-essential amino acid was recorded than total essential amino acid at day 28 and 56 while at day 0, higher concentration of total essential amino acid was recorded than total non-essential amino acid. The table also records the ratio of EAA to NEAA, which was 1.10 at the beginning of the storage period and slightly increased at day 56 to 0.83. Tryptophan was recorded as the limiting amino acid in the sample with chemical scores of 0.49, 0.45 and 0.41 at day 0, day 28 and day 56 respectively. The highest EAA value of 0.84 was recorded at day 0 and decreased slightly to 0.73 and decreased again to 0.65 at day 28 and day 56 respectively. The predicted protein efficiency ratio (P-PER) ranged between 2.64 to 3.74 g/100g cp. The highest and least biological values were recorded in day 0 and day 56 respectively.

Table 6 indicates the amino acid scores of the fish in relation to the amino acid scoring pattern of whole hen’s egg protein. This value is found to be favourable in fish sample at day 0 of smoking and decreased at the end of storage period. Lysine had the highest amino acid score ranging from 1.12 to 1.48 g/100g cp. Tryptophan was observed as the limiting amino acid with values ranging between 1.41 to 0.49 g/100g cp.

The taste panelist scores allotted for the odor and texture of smoked *Oreochromis niloticus* increased significantly (χ² = 13.403 p ≤ 0.01, χ² = 4.14).
increased as the duration of storage at ambient temperatures increases. Also indicate that TMA, TVBN, PV and FFA of smoked *O. niloticus* (Daramola et al., 2007 and Adeyeye et al., 2015). This study (Table 3) showed that with increase storage time at ambient, Mosarrat et al. (2016) observed similar result in their study. The result obtained for proximate composition in this study is similar to that obtained for some smoked fishes in Lagos state Nigeria by Adeyeye et al. (2015). The percentage moisture and carbohydrate increased with increase storage time (Table 1) while the percentage crude protein, crude lipid and ash reduced with increase storage time. Mosarrat et al. (2016) observed similar trend in their study of shelf-life quality of smoke-dried freshwater SIS fish stored at laboratory condition (26’–31°C) where it was observed that during storage period, the percentage of moisture increased whereas protein, fat and ash contents considerably decreased. The observed reduction in crude protein and lipid content during storage may be attributed to leaching out of some extractable soluble protein fraction and hydrolysis of some of the lipid fractions (Daramola et al., 2007). The result obtained for water activity (Aw) in this is (0.74–0.79) is similar to that reported by Sajib et al. (2015) who reported a water activity of 0.69–0.74 for smoked *Laubuka dadiburjori* during storage at room temperature. Brewer (1999) stated that it is difficult for bacteria to survive at Aw less than or equal to 0.70; however, values obtained for Aw in this study (0.74–0.79) is higher than 0.70 indicating the possibility of microbial spoilage in the fish with increase storage time.

This study (Table 2) also indicate that Sodium (Na) was the most dominant mineral in smoked *O. niloticus* (ranged between 4.05–4.88 mg/l) followed by Magnesium (1.53–1.90 mg/l) while iron (Fe) composition was least (0.06–0.10 mg/l). Adeyemi et al. (2013) and Shady et al. (2016) also observed that Mg and Ca were the most dominant minerals in smoked fish samples studied. The mineral content showed that the fish is a good source of mineral needed for human development. Soetan et al. (2010) stated that calcium must be constantly eaten to build bone and maintain the blood level of calcium while sodium is needed to maintain normal blood pressure and normal function of muscles and nerves all of which are present in smoked *O. niloticus*. The mineral content of smoked *O. niloticus* in this study decreased with increase in duration of storage at ambient, Mosarrat et al. (2016) observed similar result in their study.

The TMA values (22.62–18.10mg/100g), TVBN values (26.62–16.50 mg/100g) and PV values (7.74–8.18 mg/100g) obtained in this study (Table 3) are within the recommended level for good quality food (Daramola et al., 2007 and Adeyeye et al., 2015). This study (Table 3) also indicate that TMA, TVBN, PV and FFA of smoked *O. niloticus* increased as the duration of storage at ambient temperatures increases. This is similar to the findings of Mosarrat et al. (2016) who observed continuous increase in the TVB-N value of all smoke-dried fish sampled throughout the period of storage which was attributed to gradual degradation of the initial protein to more volatile product such as total volatile base nitrogen while Sajib et al. (2015) attributed increase in TVB-N of *Laubuka dadiburjori* during storage at room temperature to microbial activity, storage temperature, absorption of moisture and relative decrease in salt content. Daramola et al. (2007) stated that there is gradual degradation in smoked fish thereby leading to more volatile products like Total Volatile Bases (TVB), Hydrogen sulphide and Ammonia. Reduction in lipid content of Oxidation of smoked fish lipid usually lead to product like peroxides, aldehydes, ketones and the free fatty acids due to high content of poly-unsaturated fatty acids (PUFA), (Horner, 1992). Increase in the peroxide value with increase storage time observed in this study is inline with that observed by Daramola et al. (2007) for smoked catfish which was attributed to fat oxidation and break down is to other components.

Eighteen amino acids (10 essential, 8 non-essential) were obtained in smoked *O. niloticus* (Table 4). The highest concentrated amino acid and non-essential amino acid (NEAA) was glutamic acid (12.06–13.11) and cystine (0.55–0.86) was the least concentrated NEAA while lysine (6.94–9.18) was the highest concentrated essential amino acid (EAA) while tryptophan (0.74–0.88) was the least concentrated EAA. This is similar to the observation of Jannat-Alipour et al. (2010) who reported that lysine was the most dominant essential amino acid in Persian sturgeon fish and glutamic acid was the most dominant non essential amino acid. Decrease in amino acid content of smoked *O. niloticus* was observed across storage time in many of the amino acids (Table 4). Atowa et al. (2014) observed similar result that essential amino acids and non essential amino acids in smoked *Trachurus trachurus* reduced with increase in duration of storage. Decrease in total amino acid with increased storage time at ambient observed in this study (Table 5) is inline with that reported by Atowa et al. (2014). A higher concentration of total non-essential amino acid was recorded than total essential amino acid in this study. The EAA/NEAA ratio decreased from 1.10 at the onset of the experiment to 0.83 at the end of the storage period. The total essential amino acid (ranged 35.59–45.88 g/100g) recorded in this study is higher than 33.9 g/100g WHO standard protein. Similarly the total non essential amino acids (ranged 41.67–46.85) is higher than WHO standard of 32g/100g (Atowa et al., 2014). TAA in this study decreased from 87.55 to 78.39 g/100g; similar decrease in protein quality was observed in TEAA which decreased from 45.88 to 35.59 g/100g, EAA decreased from 79.60 to 59.68, PV decreased from 79.60 to 59.68 (mEq.peroxide/kg) and the predicted protein efficiency ratio (P-PER) decreased from 3.74 (g/100) to 2.64 (g/100g) while the chemical score (CS) increased with increase storage time whereas no significant (\(\chi^2=6.624, p>0.05\)) decrease in the quality of flavor observed during storage.

### Table 6. Amino acid scores and Indispensable Amino acid index (IAAI) of *Oreochromis niloticus*.

| Essential amino acids | Amino acid scores (g/100g) | Whole hen's egg protein (g/100g) | FAO/WHO provisional amino acid scoring pattern (g/100g) |
|-----------------------|---------------------------|-------------------------------|---------------------------------------------------|
| Leucine               | Day 1 0.96 Day 28 0.75 Day 56 0.65 | 8.3                           | 7.0                                               |
| Lysine                | 1.48 1.24 1.12            | 6.2                           | 5.5                                               |
| Isoleucine            | 0.71 0.64 0.63            | 5.6                           | 4.0                                               |
| Phenylalanine         | 0.74 0.73 0.65            | 5.1                           | +tyr 6.0                                          |
| Tryptophan            | 0.49 0.45 0.41            | 1.8                           | 1.0                                               |
| Valine                | 0.60 0.54 0.47            | 1.5                           | 1.5                                               |
| Methionine            | 0.96 0.72 0.60            | 3.2                           | +cys 3.5                                          |
| Arginine              | 0.95 0.95 0.78            | 6.1                           |                                                   |
| Histidine             | 1.00 0.81 0.75            | 2.4                           |                                                   |
| Threonine             | 0.85 0.76 0.73            | 5.1                           | 4.0                                               |
| EAAI                  | 0.84 0.73 0.65            |                                |                                                   |

EAAI = essential amino acid index; Whole hen’s egg protein: adopted from Paul et al. (1976); FAO/WHO provisional amino acid scoring pattern: FAO/WHO (1973).
Table 7. Effect of storage time on organoleptic quality of smoked Oreochromis niloticus.

|            | Day 0 | Day 14 | Day 28 | Day 42 | Day 56 | χ²   | P-value |
|------------|-------|--------|--------|--------|--------|-------|---------|
| Odor       | 8.733 | 7.600  | 8.533  | 8.000  | 7.867  | 13.403* | 0.01    |
| Flavor     | 8.800 | 7.600  | 8.533  | 8.333  | 8.333  | 6.624  | 0.16    |
| Texture    | 8.733 | 7.600  | 8.467  | 8.000  | 7.500  | 10.638* | 0.03    |

Kruskal Wallis test (χ²) is significant along the row p < 0.05.

from 51.11 (g/100g) to 58.89 (g/100g). Similar report was given by Atowa et al. (2014) who stated that essential amino acids, the non essential amino acids, lysine, methionine and proline decreased with increase in duration of storage at ambient. The decrease with storage time was attributed to complex chemical reactions, such as protein–peptide interaction, protein-fat interaction, and maillard type reactions (El and Kavas, 1996).

The amino acid scores of the fish in relation to the amino acid scoring pattern of whole hen’s egg protein (Table 6) indicates that lysine had the highest amino acid score which decreased from 1.48 to 12 g/100g cp. The scores allotted by taste panel for the odor and texture of smoked O. niloticus increased significantly (χ² = 13.403 p < 0.01, χ² = 10.638 p < 0.01) with increase in storage time (Table 7) whereas no significant (χ² = 6.624, p > 0.05) variation was observed in the score allotted by taste panel for the flavor of smoked O. niloticus during storage.

6. Conclusion

The percentage moisture and carbohydrate increased with increase storage time while the percentage crude protein, crude lipid and ash reduced with increase storage time. This study also indicate that Sodium (Na) was the most dominant mineral in smoked O. niloticus. The TMA, TVBN and PV values obtained in this study are within the recommended level for good quality food. The highest concentrated amino acid and non-essential amino acid (NEAA) was glutamic acid and cystine TAA, TEAA, EAAI, BV and P-PER recorded in this study decreased with increase in duration of storage at ambient. The decrease with storage time is an indication of occurrence of isoelectric precipitation, which is the reason for the decrease in amino acid score (PASI). This is also corroborated by the decrease in TAA and TEAA, which is the major source of protein, minerals and amino acids, and the quality is within good quality protein recommended by WHO throughout the period of this study.

Declarations

Author contribution statement

Ahmed A. Ayeloja: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Wasiu A. Jimoh: Analyzed and interpreted the data.

Mary B. Adetayo: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Adam Abdullahi: Contributed reagents, materials, analysis tools or data.

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