

RESEARCH ARTICLE

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STUDY OF QUALITY CHANGES IN *Clarias gariepinus* (BURCHELL 1822) STORED IN MODIFIED ICE FISH BOX™

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ABSTRACT

Fish is a very perishable commodity that deteriorates after harvest without proper handling, transportation, and storage. Nigerian Stored Products Research Institute Ice Fish Box™ has been improved to have a greater capacity for more storage of fish as well as the development of a trolley to ease movement and a change in position of the drain spot increasing the performance of the drainage system. Thirty (30) kilograms of freshly harvested *Clarias gariepinus* and thirty (30) kilograms of ice were layered alternately at a ratio 1:1 in the modified box for pH, total volatile base nitrogen (TVBN), trimethylamine (TMA), total viable count (TVC) and freshness evaluation using Quality Index (QI) at a sampling period of 24 hours and sensory evaluation of processed fish using Likert scale. *C. gariepinus*, stored for a maximum of 48 hours with pH between 6.70 and 7.17, TVBN, 5.52 - 8.25 mg/100 g, TMA, 0.84- 2.33 mg/100g, TVC, 1.0×10^2 - 3.8×10^3 cfu/g, QI, 7 and sensory evaluations were above 4. The quality indices were within acceptable limits. It is anticipated that this would lower the amount of loss that occurs after harvest thus boosting revenues in the fish value chain and improving food and nutritional security.

Keywords: Earnings, Ice Fish Box, Post-harvest loss, Modified, Quality, Sensory

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INTRODUCTION

Many people across the world rely on fish as a primary or secondary food source and/or source of income. It contains beneficial nutrients including protein, vitamins, and minerals (Tsado *et al.*, 2012). African catfish (*Clarias gariepinus*) is one of the fastest-growing production volumes of freshwater fishes (Kucharczyk *et al.*, 2019). The total production of this fish species is increasing steadily and spreading worldwide (Dauda *et al.*, 2018; Onwuteaka and Prince, 2015). Fish and fish products are important sources of protein in Nigeria, but the country's consumption patterns are below the worldwide norm (FAO, 2022). The world's average annual per capita consumption of fish is roughly 21 kilograms, while Nigeria is believed to be about 12 kilograms (Nukpezah *et al.*, 2020). Fresh fish deteriorates rapidly after harvest due to improper handling, transportation, and storage (Akinneye *et al.*, 2007; Pigott, 2015). This portends the loss of nutrition and income in the fish value chains across the world (Lucy, 2011) as an estimate has it that about 10–12 million tonnes are lost annually due to rotting, according to an evaluation of post-harvest losses, while an estimated 20 million tonnes are dumped at sea (Kumolu-Johnson and Ndimele, 2011; FAO, 2010).

Fish defenses stop working and enzymatic, oxidative, and bacterial spoiling starts in, drastically decreasing the fish's quality (Diei-Ouadi and Mgawe, 2011). Fresh fish can be stored for a longer period with proper handling as soon as it is on board in a cooled or frozen state (Brigitte *et al.*, 2004). Without ice, fish spoils quickly, so it's important to get them to land as soon as possible while keeping them in a clean, shaded boat (Brigitte *et al.*, 2004). Insulated boxes are used as one technique of storing fish to keep it from going bad. Nonabsorbent, long-lasting polymers are used to construct insulated boxes, which are then sandwiched between two layers of materials that keep the contents at a constant temperature for a considerable length of time (lagging material). Fish are kept at temperatures below room temperature by placing them in an ice bath. The insulated container slows the rate at which ice melts. The Nigerian Stored Products Research Institute created an insulated box for the storage, handling, shipping, and sale of fresh fish in 2014. *Oreochromis niloticus* and *C. gariepinus* were kept fresh for 40 and 36 hours, respectively, in an insulated box known as Ice Fish Box™. In 2019, adjustments were made to Ice Fish Box™ to improve the capacity of the plastic box that allows for more storage of fish, development of a trolley to ease movement, and change in the position of the drain spot increasing the performance of the drainage system.

Volatile amines together with sensory measurements are the most prevalent criterion for assessing fish quality since they are the molecules responsible for the fishy odour and flavour that persist in fish for several days after being caught. Multiple writers have stressed the importance of using this criterion, and Oehlenschläger (1997a, b) and FAO Fisheries Technical Paper 348 provide a summary of the evidence for and against using volatile amines as freshness/spoilage of seafood (Huss, 1995). Therefore, the objective of this research was to assess the microbiological, biochemical, and gustatory qualities of *C. gariepinus* kept in a customised Ice Fish Box™.

MATERIALS AND METHODS

Sampling

The modified Ice Fish Box was used to transport 30 kg of freshly harvested *C. gariepinus* from a fish pond in Yaba, Lagos State, to the NSPRI Fish Processing Centre. Fish averaged 25 cm in length and 450 g in weight were sampled. Fish were stacked alternately in the boxes with 30 kg of ice while a control treatment containing fish in a non-insulated box without ice was added. These setups were carried out in triplicate.

Temperature and Relative Humidity

A Temlog 20H data logger digitally calibrated using Elitelog V4.2.1 software was used to determine the environment's temperature and relative humidity. The ambient temperature and humidity gauges were immediately taken. Subsequently, readings were taken until the ice melted.

pH

pH was determined using Abelti's method with slight modification. Ten grams (10 g) of *C. gariepinus* meat from upper, middle, and lower regions were homogenised in 50 mL of distilled water, blended for 5 minutes, and allowed to cool at room temperature, and filtered using Whatman Filter Paper Number 1. After calibrating with pH 7 and pH 4 standard buffers, a Jenway 3310 pH metre was used to determine the pH of the homogenate at room temperature. The samples were taken both immediately after sampling and 24 hours afterward in triplicates.

Total Volatile Base Nitrogen (TVB-N)

The modified Jinadasa (2014) method was used to determine TVB-N. Ten grams of *C. gariepinus* meat with 20 mL was blended into a paste with 7.5% trichloroacetic acid (TCA) (BDH England) for 2 minutes. It was filtered using Whatman Number 1 filter paper to get the crude extract. An alkaline solution of 6 mL of 10% NaOH (BDH England) solution and 20 mL of distilled water was added to 25 mL of the crude extract which was quantitatively transferred into a semi-automatic distillation apparatus's distillation tube connected to a flask containing 25 mL of 4% (w/v) boric acid (Analar, England) and a few drops of a mixed indicator (methyl red/methylene blue 2:1 (Merck USA). The steam entrapment and distillation process were completed after the content of the flask was almost 100 mL which was titrated against 0.05M H₂SO₄ until the colour changed from green to pink. After correcting for blank using 25 mL distilled water. TVB-N was calculated using the expression below in milligrams per one hundred grams.

$$TVB - N \left(\frac{mg}{100g} \right) = 14 \text{ mg mol} * a * b * \frac{300}{25} \text{ mL}$$

Where a = volume of sulphuric acid used (mL) and b = molarity of sulphuric acid

Trimethylamine (TMA)

The TMA was performed using a modified version of the procedure described by Stockemer and Kruse (1985). 10 grams of fish muscle was homogenized with 20 mL of 7.5% trichloroacetic acid (TCA) (BDH England) solution for 2 minutes followed by filtration using Whatman filter paper (No 1). 25 mL of the filtrate was quantitatively transferred into the Struer automated distillation apparatus followed by the addition of 6 mL 10% NaOH (BDH England). A beaker beneath the condenser with 15 mL of 4% boric acid (Analar, England) and 0.04 mL each of methyl red and bromocresol green (Merck USA) indicator was used to collect the distillate. Primary and secondary amines were prevented from entering the distillation tube by the addition of 20 mL of 35% formaldehyde (Merck, USA). The steam entrapment and distillation process ended when the volume of the flask was 100 mL. The distilled TVBN was titrated with 0.01 mL of a 0.03 N sulphuric acid (Sigma Aldrich Switzerland) solution in a graduated micro burette, causing the boric acid solution to become green. Validation of the titration process (equivalence point) was affirmed by the addition of a single drop of 0.03 N H₂SO₄ which turned from green to crimson. Steam distillation was used to rectify the blank with a sample size of 25 mL of distilled water. TMA concentration (in milligrams/ 100g) was calculated from the titre value (mL) and concentration (N) of sulphuric acid using the expression below.

$$TMA \left(mg - \frac{N}{100g} \right) = \text{volume of titration} \times \text{Normality of acid} \times 14 \times 10$$

Microbial analysis

The fresh (muscle with skin) and control, cultured using serial dilution, were taken in a sterile environment. Total viable counts (TVC) of bacteria were assessed by incubating a sample in Nutrient agar at 37 degrees Celsius for 18 to 24 hours, whereas Coliform counts were done using MacConkey agar. Oxoid Grade England Reagents were used throughout. The colony-forming units per gram (cfu/g) were used to quantify the microbiological data.

Statistical analysis

The statistical analysis was carried out with SPSSV20 (IBM) for the test of significance, using One Way Analysis of variance, and utilising a simple average for the triplicate determination.

RESULTS AND DISCUSSION

pH

pH increased from 6.70 to 7.17 as shown in Figure 1. After harvest, a fish's pH falls because lactic acid is formed in a chain reaction brought on by the significant amount of stress the fish has passed through (reference). Due to the mild post-mortem glycolysis seen in this investigation, there was no decrease in pH. This could be attributed to transporting and storing freshly harvested fish in a cool storage system-fish ice box. Results from this study were consistent with reports by Bosworth *et al.*, (2007); this Castillo-Yanez *et al.*, (2014). However, this was contrary to a report by Pessu *et al.*, (2016) that pH value of *C gariepinus* did not fall from its initial value of 7.10 to 6.40 across all treatments by the sixth hour. Although it is not a reliable gauge of freshness, pH can be taken into account with other quality indices when deciding on a product's quality.

Total Volatile Base Nitrogen

TVB-N level increased from 5.52 to 8.25 mg/100 g as shown in Figure 1. This was due to low amine synthesis, notably of trimethylamine, via autolytic processes during the storage period. The TVB-N content of freshly harvested fish is typically greater than 10 mg/100 g and does not exceed 15 mg/100 g, except for pelagic fish, which can have as much as 30 mg/100 g of TVB-N (Pérez-Villarreal and Pozo, 1990; El Marrakchi *et al.*, 1990; Malle *et al.*, 1983; sardines; 16-18 mg/100 g;). The lower TVBN content in *C. gariepinus* in this study may be a result of the absence of trimethylamine which is the major component of volatile bases in fish and meat (Pessu *et al.* (2016)). End-of-storage TVB-N levels were often lower than the 30-35mg TVB-N/100g threshold considered acceptable for fresh fish (Huss, 1988, Connell, 1995). These findings are consistent with those of Pessu *et al.* (2016) where the TVB-N of *C. gariepinus* stored in NSPRI Ice Fish Box™ increased from an initial value of 5.29 mg/100g to 7.22 mg/100g. TVB-N is one of the most popular measures in determining fish quality. Ammonia, dimethylamine (DMA), trimethylamine (TMA), and other volatile basic nitrogenous chemicals make up this total. TVB-N is insensitive to freshness, as stated by Horner (1997), hence it is not an indicator of freshness, however, it is a reliable sign of spoiling and hence an excellent indicator of unfitness for human consumption.

TMA

The initial value was 0.84 which increased to a final value of 2.33 mg/100g changes TMA values are shown in Figure 1. The change was a result of the activity of microorganisms breaking down a compound called trimethylamine oxide (Malle *et al.*, 1986). The characteristic "fishy" odour of rotting seafood is caused by this strong volatile amine (Gill, 1992). TMA-N levels in fresh fish are usually low: virtually nil in albacore (Pérez-Villareal and Pozo, 1990), 2 mg/100g wet weight in cod (Dyer and Mounsey, 1945), and an average of 2 mg/100g for several fish species from the North-East Atlantic (Oehlenschläger, 1996). The quality of many different types of fish has been said to be well reflected by this indicator (Gill, 1990; Baixas-Nogueras *et al.*, 2002, 2003). One major drawback of TMA-N analysis is that it can only be relied upon when dealing with certain fish species (Oehlenschläger, 1997 a, b; Huss, 1995; Pedrosa-Menabrito and Regenstein, 1990). Low levels of TMA during storage cause TVB-N readings to be t lower. Despite being a reliable sign of deterioration in frozen fish (MACÉ *et al.*, 2012), TMA levels are not regulated.

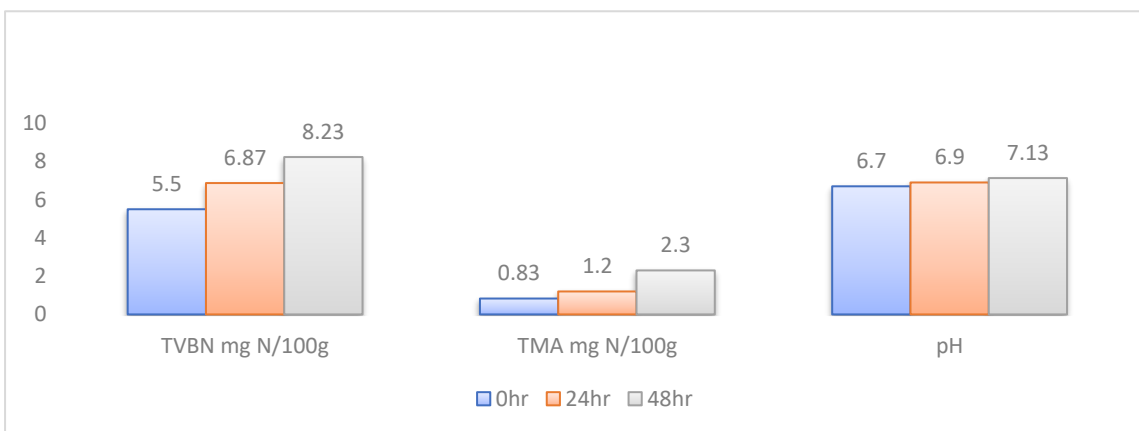


Figure 1: Biochemical characteristics of freshly harvested *C.gariepinus* during storage in modified NSPRI Iced Fish

Microbial analysis

The total viable count increased from 1.0×10^2 to 3.8×10^3 cfu/g in comparison to the control at 24 hours in water with a count of 5.5×10^7 cfu/g. At the end of the storage period, the TVC was below the permissible threshold of 1.0×10^6 as specified by International Commission on Microbiological Specifications for Food (ICMSF, 1986). Ihuahi *et al.* (2016) and Pessu *et al.* (2016) reported the same finding. Several studies have shown that TVC is a significant indicator of the quality of fish kept in the freezer; TVC is the primary cause of rotting in fish kept in the freezer (Castillo-Yanez *et al.*; 2014; Gram, 1992; Huss, 1997). Channel catfish held on ice for 7 days has a limit of 1.0×10^7 cfu/g, according to research by Castillo-Yanez *et al.* (2014), making it safe for human consumption. After being stored for 48 hours, the coliform count rose from an initial value of 0.6×10^2 cfu/g to 1.5×10^3 cfu/g. As shown in Table 1, this number is significantly lower than the maximum allowable value of 1.0×10^6 .

Table 1: Values of microbial load in *C. gariepinus* stored in modified Ice Fish Box®

Time (hours)	TVC (cfu/g)	Coliform count (cfu/g)
0	1.0×10^2	0.6×10^2
24	2.3×10^3	1.0×10^3
48	3.8×10^3	1.5×10^3

Sensory Evaluation (raw and processed fish)

Quality Index Method (QIM), as described by Bernardo *et al.*, 2020 was used to conduct a sensory evaluation of the raw fish. Physical characteristics such as skin colour, flesh firmness, gill colour/shape, and eye colour and shape were observed in whole fish samples. . Quality Index Method (QIM) was used in the evaluation, with 7 experts serving as the panel. The rating was from 0 to 7, with lower numbers indicating greater freshness and higher numbers indicating greater degradation. Using multiple factors and a scoring system ranging from 0 to 4 points off, QIM is based on the most important sensory characteristics of raw fish. The sensory score, which serves as a quality measure, is the sum of the ratings given to each of the attributes.

There was a sensory evaluation of the finished products (cooked and smoked). Ten panelists who are familiar with sensory evaluation were selected and 5 points (Values 5-1) Likert's Scale (Scale of Summated Rating) was used for the scoring procedure. A cut-off point of 3 was established, above the cut-off point was regarded as good, and below was regarded as poor. Ten trained panelists assessed all of the treatments during the storage time, making notes on factors including flavour, odour, taste texture, and appearance. Likert's Scale (Scale of Summated Rating) with values ranging from five to one. The threshold was set at 3, over which was considered good, and below which was considered bad (Pimentel, 2010).

Sensory assessment (raw, cooked, and smoked)

Table 2 shows the results of a sensory evaluation with lower numbers indicating greater freshness and higher numbers indicating greater degradation. With longer periods of storage, the values were found to rise. There is a correlation between the microbiological assessment and an increasingly big number, suggesting worsening. The longer the fish is kept in the ice, the more microbiological and physicochemical processes occur, altering the fish in ways that are immediately perceptible to the senses. For instance, red tilapia scored 21 points after 17 days (Gutiérrez *et al.*, 2015), but ungutted red tilapia scored 29 points after 16 days. Fish hardness is measured by pressing down on a muscle with your finger and seeing how quickly it springs back to its original shape. The average texture penalty points were near zero at first because of the muscle's inherent stiffness, but this shifted as proteolysis loosened the muscle. Curiously, researchers found that the smell and colour changed the most and reached their worst possible state during storage. The fundamental reason for fish's declining popularity is its unpleasant odour (dos Santos *et al.*, 2014). Large quantities of non-protein nitrogen, a high quantity of fat, and autolytic enzymes in the fish tissues may be responsible for the noticeable difference in odour during storage. These results suggest that particular quality measures may degrade rapidly or slowly after storage, depending on microbiological and chemical activity, even though the overall decline in fish quality tends to grow linearly. The QI values for appearance (colour, size, etc.), smell, taste, and gill health (healthiness) were all satisfactory.

Table 2: Quality Index Method (QIM) score sheet for *C. gariepinus* in Modified Ice Fish Box™

Storage time (hr)	0	24	48
<u>Attributes:</u>			
Skin	0	0	0
Blood spot on gill cover	0	0	0
Stiffness	0	1	2
Belly	0	1	1
Smell	1	0	1
Eyes			
Clarity	0	0	0
Shape	0	1	1
Gills			
Colour			
Smell	0	0	1
	0	0	1
Sum of scores (0 min, 20 max)	0	3	7

Sensory assessment of prepared samples (cooked and smoked) from *C. gariepinus* stored in modified box are presented in Tables 3 and 4. It can be observed that as the storage hours increases the scores decreases however, there was no significant difference between the raw fish and prepared fish from the box during storage ($p>0.05$) during the period of storage. This is closely related to Pessu *et al.*, (2016); Osibona and Ezekiel (2014). Overall, the fish was acceptable by the panel and therefore fit for consumption.

Table 3: Mean values of sensory assessment for cooked *C. gariepinus* from modified box

Storage (hours)	Taste	Texture	Appearance	Colour	Aroma
0	4.9 ± 0.03 ^a	5.0 ± 0.00 ^a	4.9 ± 0.01 ^a	4.8 ± 0.03 ^a	4.9 ± 0.09 ^a
24	4.7 ± 0.98 ^a	4.5 ± 0.55 ^b	4.4 ± 0.96 ^b	4.7 ± 0.72 ^a	4.5 ± 0.75 ^b
48	4.4 ± 1.04 ^b	4.0 ± 0.73 ^c	4.0 ± 0.18 ^c	4.4 ± 0.61 ^b	4.2 ± 0.27 ^c

*Mean values within the same column with different alphabet are significantly different (P<0.05)

Table 4: Mean values of sensory assessment for smoked *C. gariepinus* from modified box

Storage time (hours)	Taste	Texture	Appearance	Colour	Aroma
0	4.8 ± 0.04 ^a	4.8 ± 0.05 ^a	4.8 ± 0.90 ^a	4.9 ± 0.03 ^a	4.8 ± 0.02 ^a
24	4.7 ± 0.08 ^a	4.5 ± 0.81 ^b	4.6 ± 1.34 ^b	4.7 ± 0.77 ^b	4.7 ± 0.03 ^a
48	4.1 ± 0.19 ^c	4.0 ± 0.025 ^c	4.0 ± 0.22 ^c	4.1 ± 0.33 ^c	4.7 ± 0.80 ^a

*Mean values within the same column with different alphabet are significantly different (P<0.05)

CONCLUSION

Fish is an extremely perishable commodity therefore; proper preservation methods are essential for extending its shelf life and quality. This study aims to access the effectiveness of the modified ice fish box in maintaining the quality *C. gariepinus*. The results of the studies showed that *C. gariepinus* qualities were considered good for 48 hours using the modified Ice Fish Box™. This model is an upgrade over the previous one, which kept *C. gariepinus* fresh for thirty-six hours. All of the quality criteria were well within the acceptable range. It is anticipated that this will minimize the difficulties involved with the handling, shipping, storage, and marketing of fresh fish.

CONFLICT OF INTEREST

We declare that the work described was original research. All authors contributed significantly to this study, and there are no conflicts of interest.

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