

**CHEMICAL, SENSORY AND MICROBIOLOGICAL CHANGES OF
SPOTTED GRUNTER (*Pomadasys commersonnii*) UNDER ICE STORAGE**

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ABSTRACT

The effects of two types of ice on the quality of *Pomadasys commersonnii* with storage time were conducted. The overall sensory evaluation otherwise known as quality index (QI) ranged from 3 – 0, the scores of 3 was for very fresh fish while zero implied deterioration. The QI for fish stored in ice block ranged from 2.9 – 1.0, 2.0 – 0.0, 2.2 – 0.0, 3.0 – 0.0, 2.8 – 0.2 and 3.0 – 1.0 for skin, gill, belly, odour, eyes and colour, respectively and for fish samples stored in ice cube were 2.9-1.0, 2.5 -0.0, 2.2-0.0, 2.9-0.0, 2.2-0.0 and 3.0-1.0 for skin, gill, belly, odour, eyes and colour respectively from the 4th to the 22nd day. The QI of the fish stored in ice block at 18th day which is the shelf life were 1.6, 1.4, 1.2, 1.1, 1.4 and 2.0 while that of fish samples stored in cube ice were 1.6, 1.3, 1.0, 0.6, 1.5 and 2.0 for texture of skin, gill, belly, odour, eyes and colour along vertebra column. The odour was fresh until after the 8 days; the belly/ gut has the least QI scores and this corresponded with the microbial load that has the highest count. The shelflife of the fish species was estimated to be 18 days. At this time, the fish was still acceptable by the panel members. The microbial load of the skin, flesh, gut and gill at day zero was less than 0.5×10^2 and $8.5 \times 10^2 - 1.56 \times 10^5$, $5.5 \times 10^2 - 1.25 \times 10^7$, $8.5 \times 10^2 - 3.78 \times 10^7$ and $1.3 \times 10^2 - 1.89 \times 10^7$ cfu/g and 1.22×10^5 , 1.82×10^5 , 2.48×10^7 and 2.22×10^7 cfu/g, respectively for samples stored in block and cube ice. Trimethylamine for fish samples stored in block ice ranged from 0.6 -15.1 mg/100g while that of cube ice was from 0.6 to 16.3 mg/100g. Total volatile nitrogen ranged from 19.5- 42.1 mg/100g and 19.5 - 43.7 mg/100g for samples stored in block and cube ice, respectively. The pH increased steadily throughout storage from an initial pH of 7.0 to 7.48 and 7.56 for block and cube ice, respectively. Moisture and protein range for samples stored in block ice were 81.3-80.6%, and 19.5-17.02% while for samples stored in cube ice were 81.3-83.9% and 19.5-15.5%, respectively. Ash remained constant at 1% for all the samples. Results showed a non-significant difference ($p = 0.05$), which is an indication that they have the same storage or preservative capacity.

Key words: Ice, grunter, sensory, microbiological, proximate

INTRODUCTION

Spotted grunter *Pomadasys commersonnii* is an important fish species in Nigerian waters. It is an abundant demersal fishery resource in Nigeria continental shelves. Spotted grunter is an estuarine-dependent species [1]. It has a high quality fish flesh and is consumed mostly as fresh fish in the country.

Fish is one of the most perishable staple foods, recognised as being highly perishable, having a relatively short shelf life defined as the length of time from the day of catch that fresh fish can be in the market place unspoiled [2]. The high ambient temperature in the tropics hastens fish spoilage by accelerating the activities of bacteria, enzymes and chemical oxidation of fat in fresh fish [3].

Presently, there are numerous problems facing the field of fisheries, some of which are related to the keeping quality of the fish. Because of its high perishability, preserving fish has faced major obstacles. The quality of fresh fish is an important concern to the fish industry and consumers. Freshness is the single most important attribute when assessing fish quality.

Freezing and storing at low temperatures slow down bacterial growth and deterioration of fish through some enzymatic and chemical changes that progress slowly [4]. Using good fishing techniques to ensure the fish is not damaged and cooling the fish, with the help of ice on board, can increase the storage life of fresh fish [5].

Different species of fish have different shelflife depending on the oil levels, catch area, season, and duration of rigor mortis, intrinsic conditions of the fish and way it was captured and handled [6].

Fish spoilage is defined as a change in fish or fish products that renders them less acceptable, unacceptable or unsafe for human consumption [7]. Fish undergoing spoilage has one or more of the following signs: slime formation; discolouration; changes in texture; off-odours; off-flavours and production of volatile compounds. The development of these spoilage indicators in fish and fish products is due to a combination of microbiological, chemical and enzymatic and physical phenomena [8].

Various types of analyses have been developed to estimate the loss of fish freshness and to detect spoilage. The two main methods of assessing fish quality are sensory and non-sensory methods. Sensory methods rely mostly on appearance, odour, texture and taste of the fish while non-sensory methods use physical, biochemical, chemical and microbiological means [6].

The aim of microbiological examination of fish is to evaluate the possible presence of bacteria of public health significance. Microbial measurements can be used to evaluate the degree of fish freshness. When such microbiological measurements are

needed, it is recommended to use the numbers of specific spoilage organisms (SSO) or total viable counts (TVC) measurements [9].

Trimethylamine (TMA) can be used as a spoilage indicator since it appears after 3 or 4 days of storage. The fish is considered as stale when the amount of TMA is higher than 30mg/100g cod [10], but the levels between 10-15mg of TMA-N 100g⁻¹ were considered as the limit for fresh fish [11].

Man has preferred to consume fresh fish rather than other types of fish products [12]. Considering that consumers are the ultimate judges of the quality, realistic determination and accurate prediction of shelflife of fresh and lightly preserved seafood are important to meet consumer demands and to comply with legislative requirements [12]. The post-harvest losses are high due to several reasons, including poor handling of the catch such as rough handling or unhygienic handling, lack of or delayed icing, which all contribute to accelerated spoilage.

It is important to quantify the effect of different post-harvest treatments on the quality of fish to evaluate how effective they are on the shelf life and the quality of fresh fish [13].

Previous work revealed that the principal spoilage organisms during iced storage were *Pseudomonas* spp. while at ambient storage was *Micrococcus* and *Pseudomonas* spp [14].

There are only a few reports on postmortem changes in biochemical properties of ice-stored spotted grunter *P. commersonnii* and information on changes in quality indices in this fish species during storage in ice is lacking. This knowledge is necessary for adequate post-harvest management and processing.

Thus, the absence of adequate information on this species has raised the need for more research on the commercially important tropical fish *P. commersonnii*.

The objectives of this study were, to determine the chemical composition, proximate composition, sensory evaluation and microbial load of *P. commersonnii* stored in blocks and cube ice.

MATERIALS AND METHODS

Sample collection and storage

The fish samples were purchased at a landing site known as Better Life Fish Market Makoko, Lagos Mainland Yaba, Lagos State, Nigeria. The samples were then taken to the laboratory for proper identification and analysis. The weight of the purchased samples ranged between 50-180g, and these were divided into three batches. The first and the second batches were iced immediately in crushed ice block and cubed ice, respectively in insulated boxes before taking to the laboratory, at the ratio of 1:1 ice to fish. The block and the cube ice were made from the same source of water. The third

batch was kept in insulated box at ambient temperature. Water from the melted ice was drained from a tap at the base of the insulated box. The iced samples were maintained in alternate layers of ice until spoilage was noticed. Chemical, proximate, sensory and microbiological analyses were carried out to determine the overall quality.

Chemical analysis

Determination of Trimethyl -Amine-Nitrogen (TMA-N)

Trimethyl-amine nitrogen (TMA-N) was determined by a slight modification of Conway Microdiffusion Method [15]. Twenty five grams of the fish samples were chopped and mixed thoroughly with 75 ml distilled water in a 250 ml beaker. The pH was adjusted to 5.2 by addition of few drops of 2N HCl, followed by heating at 70°C and cooling to room temperature. After cooling, the sample was filtered into a conical flask. Two ml of 0.025N HCl was transferred to the central compartment of the micro diffusion dish with the aid of a pipette; this was followed by the addition of 2ml of the extract and 0.5ml of 35% formaldehyde with 1 ml of saturated K₂CO₃ solution into the outer ring. The dish was covered immediately with a glass plate and the set-up was left at room temperature (28±2°C) for 24h. Thereafter, the HCl in the inner compartment was titrated with 0.025N NaOH using 2-3 drops of methyl red/methylene blue indicator. The result was expressed in mg, TMA-N/100g of fish.

Determination of Total Volatile Bases (TVN)

Total Volatile Bases (TVB-N) was determined by a slight modification of Conway Microdiffusion Method [15]. About 25g of the fish samples were chopped and mixed thoroughly with 75 ml distilled water in a 250 ml. beaker. The pH was adjusted to 5.2 by addition of few drops of 2N HCl, followed by heating at 70°C and cooling to room temperature. After cooling, the sample was filtered into a conical flask with the aid of filter paper. Two ml of 0.025N HCl was transferred to the central compartment of the microdiffusion dish with the aid of a pipette; followed by the addition of 2ml of the extract and 1ml of saturated K₂CO₃ solution into the outer ring. The dish was covered immediately with a glass plate and the set up was left at room temperature for 24h. Thereafter, the HCl in the inner compartment was titrated with 0.025N NaOH using 2-3 drops of methyl red/methylene blue indicator. The result was expressed in mg, TVB-N/100g of fish.

Proximate analysis

Proximate composition of the sample was determined using modified method described by AOAC [16]. Protein determination was performed using modified method of Kjeldahl as described by AOAC [16]. A gram of the fish sample was weighed into the digestion flask, and then a tablet of selenium was added after which 12ml of sulphuric acid were added. Digestion was carried out for 1h, after which it was allowed to cool for 15min, 75ml of distilled water was added and then distilled for 5min. in 25ml of boric acid with sodium hydroxide. The distillates were titrated with 0.1N HCl using methyl orange as indicator.

The moisture content of each fish sample was determined using the oven drying method, which involved determination of the percentage of water in the sample by drying the sample to constant weight and expressed the water content as percentage by weight of the dry sample. Thereafter, the samples were placed in a muffle furnace (Carbolite Sheffied LMF 3 model) and heated first at a temperature of 150⁰C until smoking ceased. The temperature was then set to 500⁰C to burn off as CO₂ leaving a white ash.

pH measurement

About 10g of each sample was homogenised with 20 ml distilled water for 30s and pH value of fish homogenate were measured by means of a pH-meter Pioneer, Ohaus model PA124, USA which was previously standardized.

Sensory assessment

The quality of sensory attributes of cooked and fresh fish stored in different ice was evaluated by Quality Index method [17] using a 10 to 0, and 3 to 0 hedonic scales for cooked and fresh fish, respectively by a panel of 10 semi-trained panellists. The characteristic features of the fish such as texture of skin, gill, gut, odour, eyes and colour of the muscle were observed during the storage period.

Microbial analysis

Total viable counts of organisms in the samples stored in two different ice (ice cube and ice block) were estimated by plating serial dilution of fish samples prepared by homogenising 1g each of fish muscle and gut in 9ml of sterile peptone water (0.1% w/v), respectively. Skin surface and gill counts were obtained by swabbing, after which the swab was transferred into 10ml distilled water and the mixture shaken together. Serial dilution of the suspension was made and plated in duplicate on standard plate count agar for total viable count. The standard plate agar used were Mannitol Salt agar for *Staphylococcus spp*, *Salmonella shigella* agar for *Salmonella spp* and triple sugar iron agar for hydrogen sulphide producing organisms. The plates were incubated at 37⁰C for 18-24h. The total numbers of cells per gram of sample were estimated after counting the colonies on the plates. The colonies were noted and sub-cultured. The pure isolates were identified using biochemical and morphological characteristic [18].

Statistical analyses

The data obtained were analysed using clustered column, scatter diagram (excel) and Analysis of Variance (ANOVA) [19]. Where significant differences occurred, Tukey's simultaneous test was used to separate the mean at P<0.05 using SPSS 18 windows.

RESULTS

The quality assessment of *Pomadasys commersonii* stored in block and ice cube was carried out using trimethylamine (TMA), total volatile nitrogen (TVN), pH, proximate composition overall sensory evaluation, and total viable count.

The values of TMA were 13.75mg/100g (block) and 14.0 mg/100g (cube) on day 18 and TVN values 39.36mg/100g (block) and 41.5mg/100g (cube) on day 18 as shown in Figures 1 and 2, respectively. The TMA and TVN contents increased as spoilage advanced. The values increased moderately during the initial part of spoilage. After 14 days on ice, the TMA and TVN contents had increased significantly.

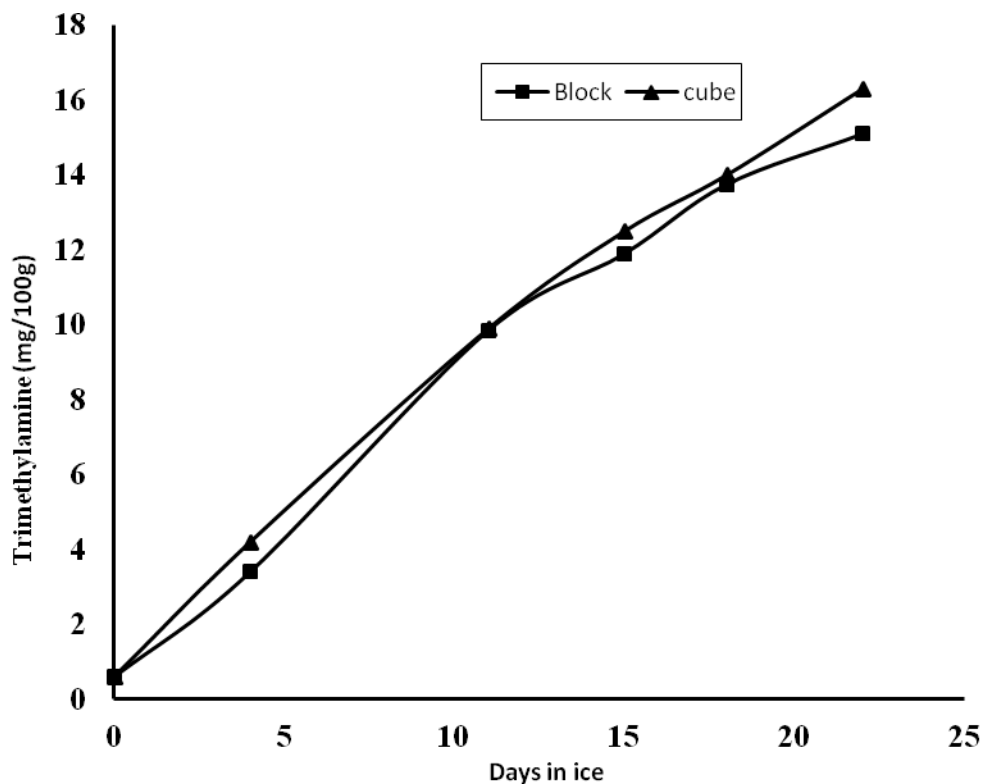


Figure 1: Trimethylamine compounds of *Pomadasy commersonii* stored in block and cube ice

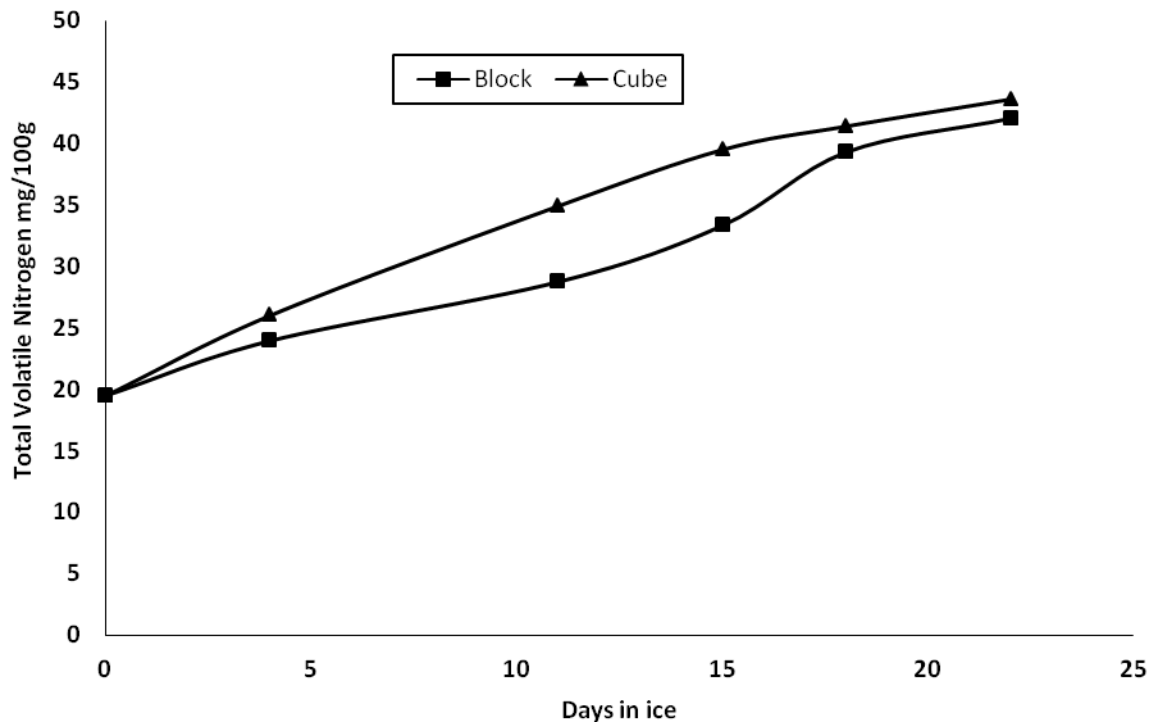


Figure 2: Total Volatile Nitrogen (TVN) Compounds (mg/100g) of *P. commersonii* stored in block and cube ice

Pomadasy commersonii had 81.3% moisture, 19.5% protein and 1% ash. At rejection by sensory panels 83% moisture, 15.5% protein (ice cube) and 80.6% moisture, 17.02% protein for fish stored in ice block were obtained. Although there were decreases in protein and moisture contents of the fish as spoilage progressed, this was, however, not significantly different while ash remained constant (Table 1). There were greater reductions in protein and moisture of fish samples stored in cube than those in block ice (Fig 3).

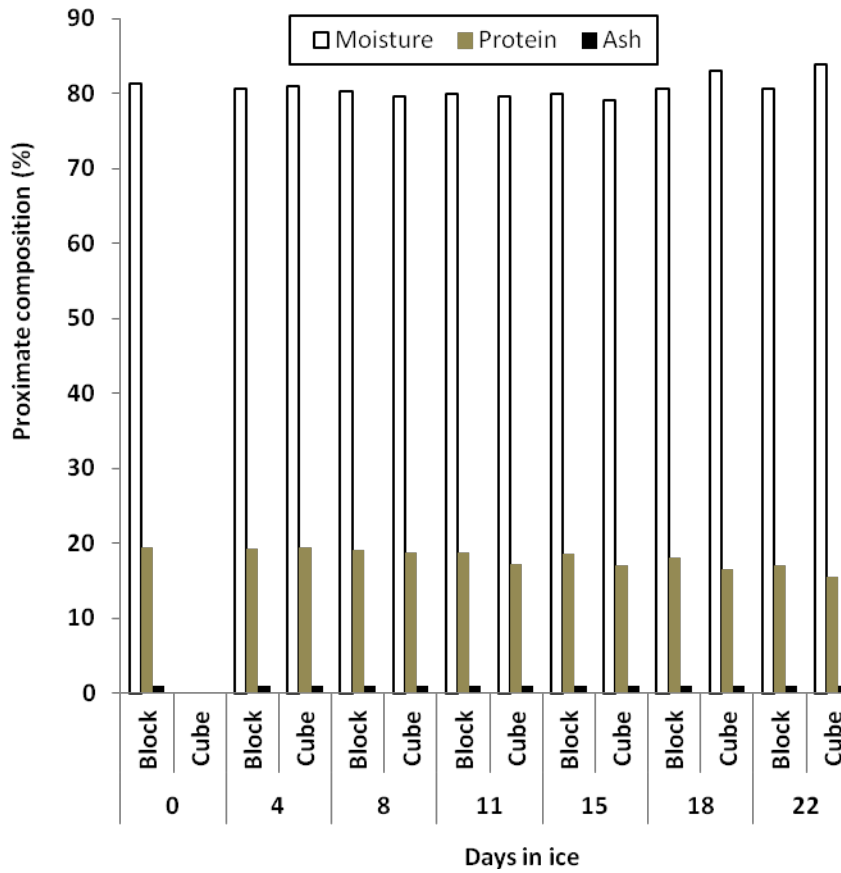


Figure 3: Changes in protein, moisture and ash contents of *Pomadasys commersonnii* stored in block and ice

The pH increased steadily throughout the storage period from an initial pH of 7.0 to 7.54 and 7.80 for block and cube, respectively (Table 2 & Figure 4).

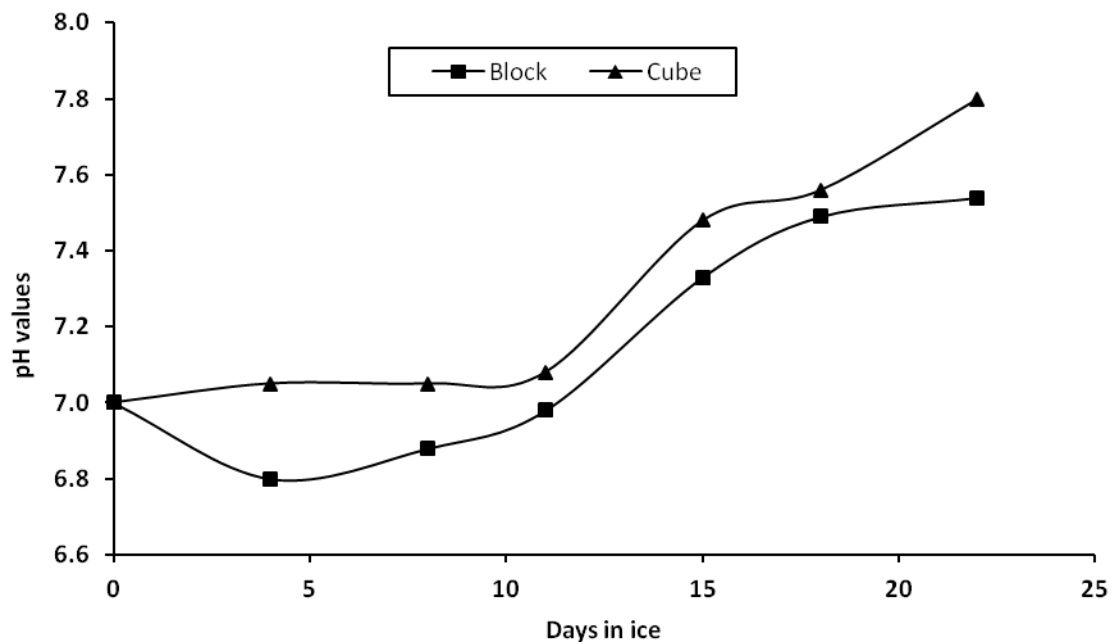


Figure 4: Changes in pH values of *Pomadasys commersonnii* stored in block and ice cubes as time of storage progressed

Sensory assessment on the quality of spotted grunter stored in block and cube ice are shown in Figures 5 and 6. A high positive correlation was found between Quality Index (QI) and storage time 0.972 and 0.962 for block and cube, respectively (Figure5). It was generally observed that both the population and types of bacterial flora increased as spoilage progresses.

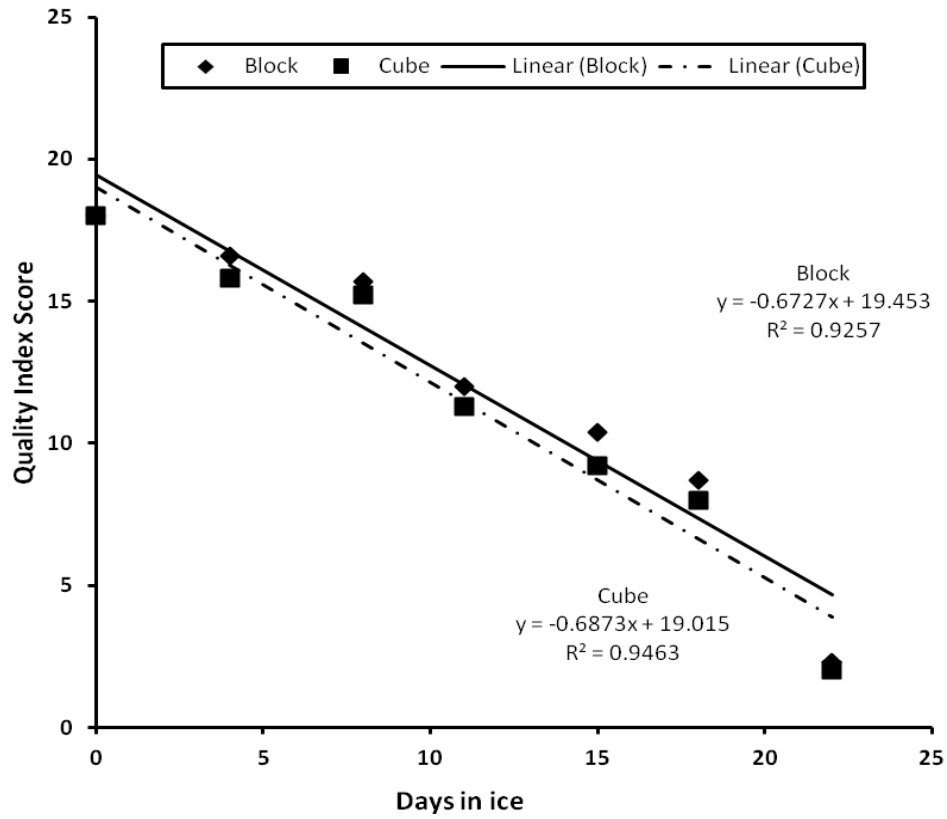


Figure 5: The sensory evaluation (QI) of raw *Pomadasys commersonnii* stored in block and cube ice

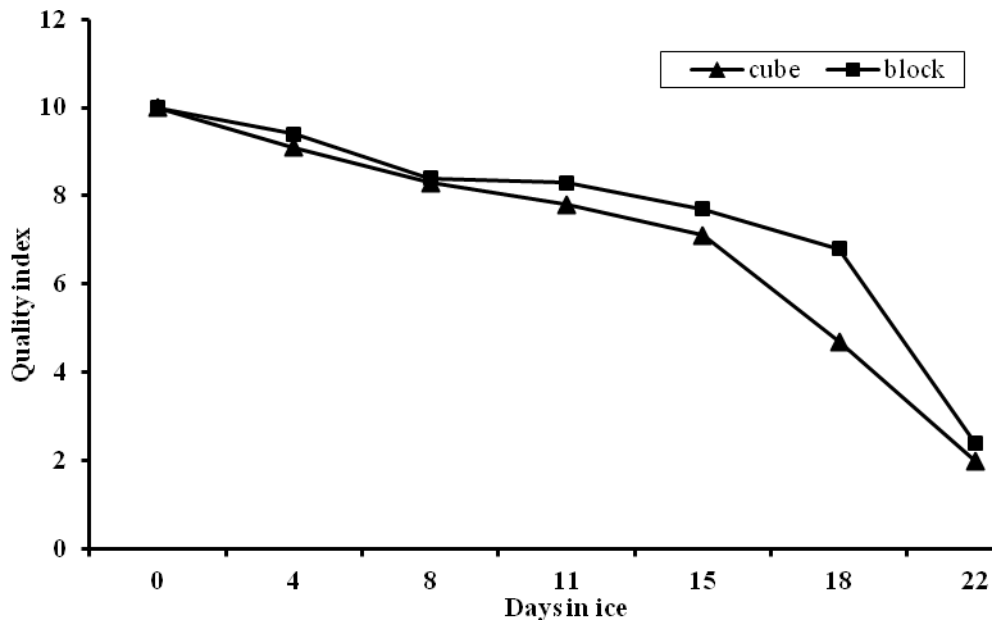


Figure 6: Effect of storage duration on Quality indices (QI) of cooked *Pomadasys commersonii* stored in cube and block ice

The microbial loads on the muscle of the sample at day 18 were 1.82×10^5 and 4.1×10^7 cfu/g for block and cube ice, respectively (Figures 7 and 8).

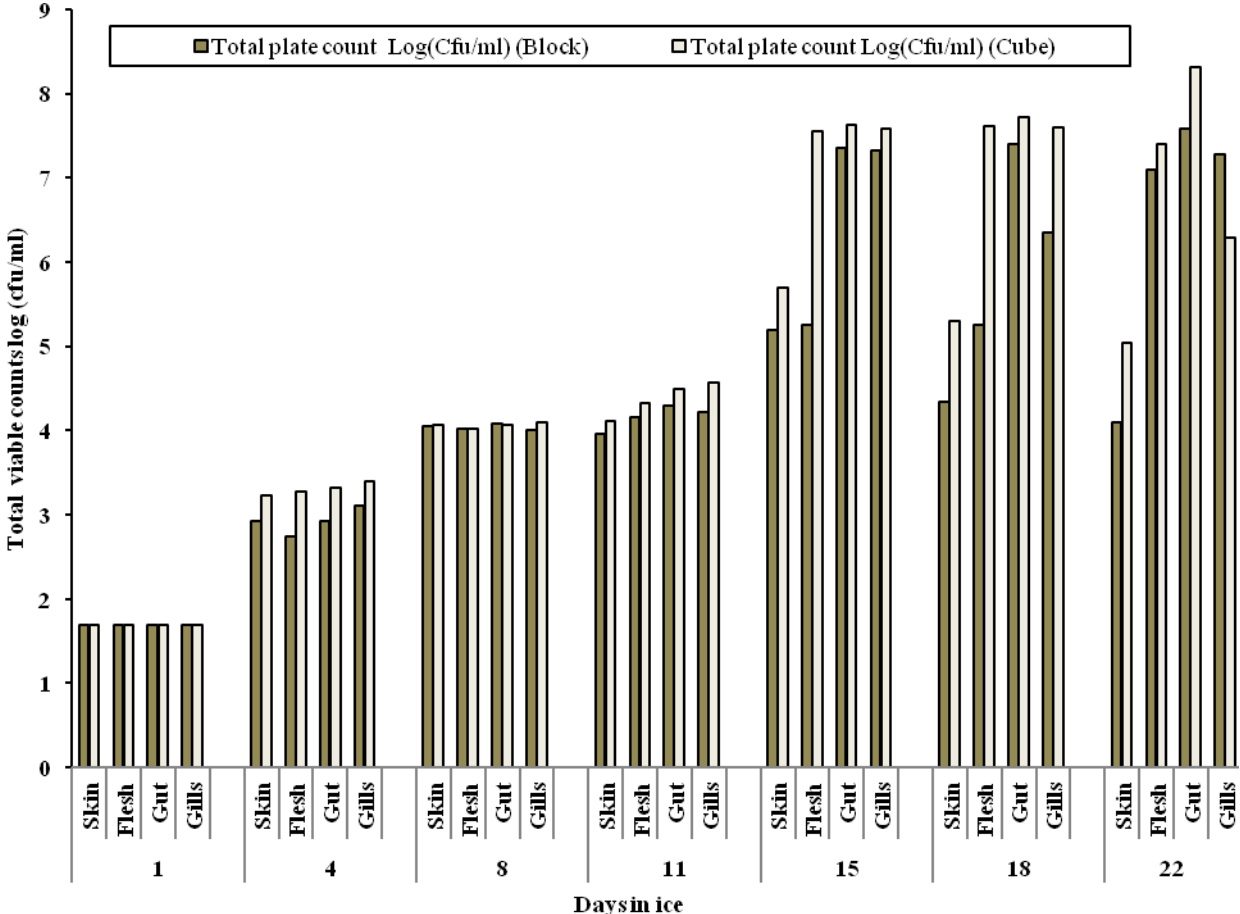


Figure 7: Changes in microbial load in *Pomadasys commersonii* under storage with time

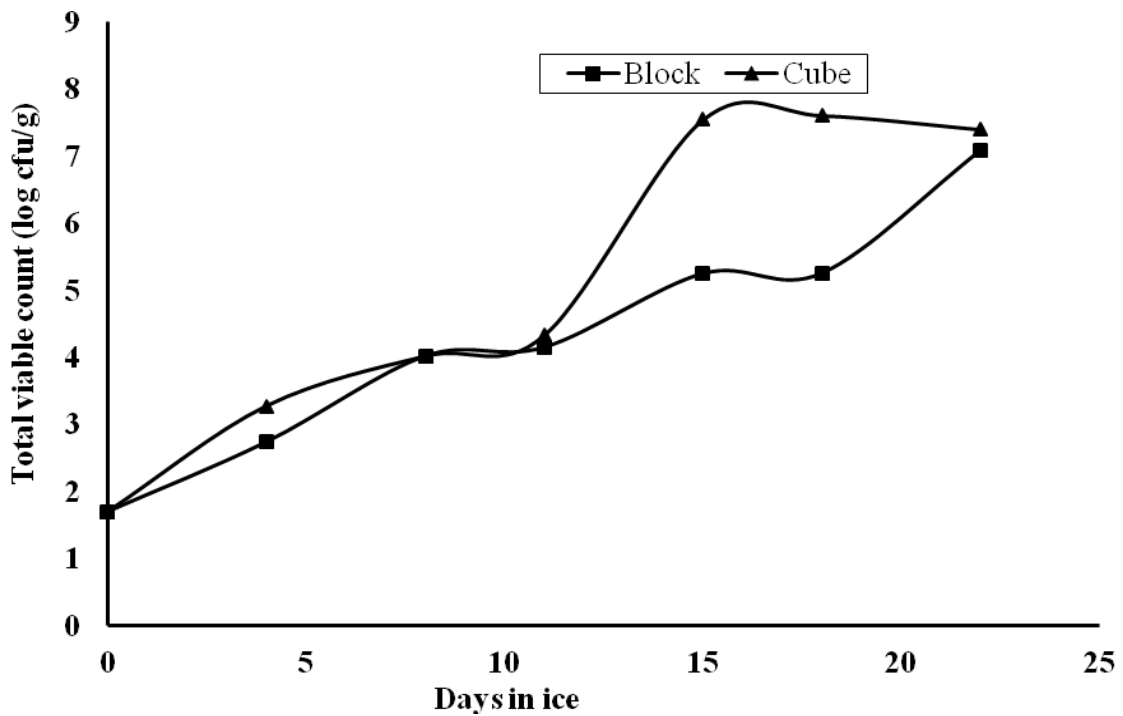


Figure 8: Total viable counts of bacteria in the muscle of *Pomadasys commersonnii* stored in block and cube ice

The values of TMA and TVN compounds were positively correlated with microbial counts at 0.966 and 0.988, respectively. This increase corresponded to the total viable count and general observations on quality (Figures 9a and 9b).

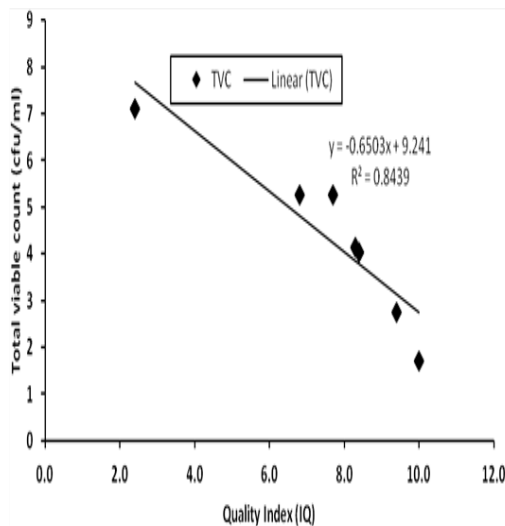


Figure 9a

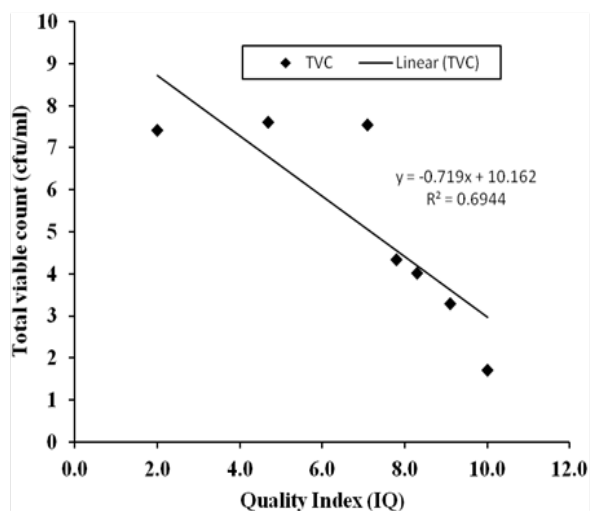


Figure 9b

Figures 9a and 9b: Correlation between Total viable counts and quality index of cooked *Pomadasys commersonnii* stored in block and cube ice

There were positive correlations between sensory evaluation of cooked fish and total viable count, with correlation coefficients r : 0.976 and 0.964 for fish stored in block and cube ice.

DISCUSSION

The quality assessment of a common Nigerian marine/ brackish fish, (spotted grunter) was carried out in order to determine the shelflife of the fish sample under different storage conditions using chemical, sensory, and microbiological methods of evaluation.

The values of trimethylamine (TMA) at 18 days for fish stored in block and cube ice were 13.75 and 14mg/100g, while those of TVN were 39.36 and 41.5mg/100g, respectively. Total volatile nitrogen at 18 days of shelf life fell within the range 30 – 40 mg/100g limit of acceptability as reported previously [20], with the values increasing moderately during the initial part of storage. A study on the storage life of tilapia (*Oreochromis niloticus*) in ice showed that the total volatile nitrogen (TVN) of the samples increased with storage time [13].

According to Connell [20], TMA and TVN values in freshly caught fish ranged from 0.5 – 2.0 and 5 – 20mg/100g, respectively and these values increased as spoilage progressed. Ola and Oladipo [21] also observed shelf life of tropical species *Pseudolithus senegalensis* to be 12h using sensory, microbial and chemical approach.

Moisture and protein contents of the stored fish decreased as spoilage progressed though not significantly. The decrease in the protein content can be attributed to leaching out of the soluble components, especially water soluble proteins and urea [22].

pH determination showed a gradual increase with storage life. The trend (increase in pH) coincided with increase in TVC in the currently presented research findings. The pH of live fish muscle tissue was close to neutrality as reported by Huss [6]. Pedrosa-Menabrito and Regenstein [23] stated that pH depended on fish species and was usually between 6.2 and 6.5 immediately after *rigor mortis*. The lactate formed from glycolysis in the post-mortem muscle lowers the pH on the first day after death even at a temperature just below 0°C. The amount of lactic acid produced is related to the amount of stored carbohydrate (glycogen) in the living tissue. Physiological conditions or degree of ante-mortem activity or stress, or both, may have significantly contributed to the rate and extent of post-mortem autolytic changes and consequently to the last post-mortem pH [24]. The pH observed by Hiltz and Dyer [24] (7.27-6.63) did not agree with the findings of this work (7.0 to 7.54 and 7.80 for block and cube ice, respectively)

There was a strong correlation between the total viable counts and the TMA and TVN values. Results of chemical analysis for TMA, TVN, and pH showed that fish samples stored in block ice performed slightly better than those stored in cube ice. Muscle from fish stored in cube ice had a higher microbial count compared to that of block ice. Fish samples in cube ice had higher TMA and TVN content including their pH.

Sensory evaluation of the fish flavour showed notable changes during the period of ice storage (Table 3) where it was observed that during the first 8 days of icing, the fish from the two treatments (block and cube ice) still had bulging and transparent eyes, the gills were bright red, the flesh and scale were firm and elastic, while the values obtained for odour were very high indicating a high degree of freshness. The fish received low score values after 18 days in ice, developing distinctive off-odours and flavours (Fig. 6) at this storage period when the bacterial counts increased dramatically. A positive correlation was found between sensory evaluation of cooked fish and microbiological development in the fish.

At the 18th day, fish stored in blocked ice falls within grade one while the fish stored in cube ice fall within grade II based on structured scaling method for quality determination and shelf life studies [25]. At day 22, the fish in block and cube ice were rejected since they fall under grade III. Based on this scale, score 4 is the limit of acceptability while scores below 4 are rejected. Thus, whole spotted grunters kept in ice were organoleptically acceptable to the taste panel until the 18th day of storage, Jonsdottir [17]. A high positive correlation was found between Q1 and stored days; these values increased proportionately especially during the latter stages of storage.

The initial lag phase of micro-organisms in the stored fish was followed by an increase in population on the 22nd day of storage, the total viable amount was 2.54×10^7 and 1.25×10^7 for cube and block ice, respectively. These values fall within the $5 \times 10^5 - 5 \times 10^7$ marginally accepted quality as pointed by ICMSF [26], while the quality levels were based on the plate counts for acceptance or rejection of fishery products for human consumption. At ambient temperature, the total viable count for fresh fish was 2.1×10^3 and 1.7×10^{10} at 6h and 24h, respectively. This means that TVC for fish left at ambient temperature for 24h exceeded the proposed international limits for the evaluation of the shelf life of fish and fishery products. Study on the quality changes in iced African Catfish (*Clarias gariepinus*) showed that *C. gariepinus* was in good condition for 27 days and 15h when stored in ice and at ambient temperature, respectively [14].

The tissue was considered as a reference point for bacterial spoilage because every other part of the fish harbours normal bacterial flora even while alive. The tissue of a healthy fish is normally considered sterile until bacterial invasion that leads to spoilage.

The initial total bacterial count of the fish was less than 10^2 cfu/g. On rejection by taste panellists, the level rose to 10^7 cfu/g for both treatments. This was also the maximum microbiological limit for fresh fish recommended by Anon [27]. Results from this

study agree with previous report on the quality changes in iced African Catfish *Clarias gariepinus* that the initial total bacterial load ranged from 100- 1000cfu/g of fish with hydrogen sulphide producers and enterobacteriaeae counts of $10^7 - 10^9$ cfu/g and $28 - 100$ cfu/g, respectively. At the point of rejection, the total viable counts, hydrogen sulphide producers and enterobacteriaeae were $10^7 - 10^9$ cfu/g, $10^7 - 10^8$ cfu/g and $10^6 - 10^8$ cfu/g, respectively [14].

Spotted grunter maintained good quality for 18 days in ice and this was within the range obtained for other tropical species [3, 14, 28].

The sensory and microbiological analyses were found to be more reliable than the chemical approach. The quality assessment of Nile perch under different storage conditions showed sensory and microbiological methods to be more reliable than the chemical methods [29].

CONCLUSION

Spotted grunter maintained good quality for 18 days in ice. Crushed block and cube ice can be used to extend the shelf life and quality of fresh fish. Block ice storage was a better means of storage than ice cubes. Tissue from fish stored in cube ice had a higher microbial count compared to that of block ice. Fish samples in cube ice had higher TMA and TVN content including their pH.

Table 1: Proximate composition of spotted grunter stored in block ice and cube ice

Day	Block ice		
	Moisture (%)	Protein (%)	Ash (%)
0	81.30	19.43	1.00
4	80.77	19.23	1.00
8	80.37	19.06	1.00
11	80.03	18.69	1.00
15	80.00	18.54	1.00
18	80.64	17.93	1.00
22	80.62	17.04	1.00
Day	Cube ice		
	Moisture (%)	Protein (%)	Ash (%)
0	81.30	19.43	1.00
4	81.00	19.35	1.00
8	79.79	18.76	1.00
11	79.60	17.28	1.00
15	79.01	17.01	1.00
18	83.00	16.58	1.00
22	83.93	15.51	1.00

Data presented as Mean \pm SD. Means were not significantly different at ($P < 0.05$)

Table 2: Sensory evaluation of *Pomadasys commersonnii* stored in block and cube ice

Day	Texture of skin		Gill		Belly		Odour		Eyes		Colour along vertebral column
	Block	Cube	Block	Cube	Block	Cube	Block	Cube	Block	cube	block
0	3.0±0d	-	3.00±0.00f	-	3.00±0.00e	-	3.00±0.00d	-	3.00±0.00e	-	3.00±0.00d
4	2.90±0.32d	2.90±0.32	2.40±0.52df	2.50±0.53	2.20±0.42d	2.20±0.42	3.00±0.00d	2.90±0.32	2.80±0.42e	2.20±0.42	3.00±0.00d
8	2.80±0.42d	2.80±0.42	2.60±0.52e	2.60±0.52	2.00±0.00cd	1.90±0.32	3.00±0.00d	2.80±0.42	2.30±0.48d	2.00±0.00	3.00±0.00d
11	2.10±0.32c	2.00±0.00	2.10±0.32cd	2.00±0.00	1.7±0.48c	1.7±0.48	1.50±0.85c	1.20±0.63	1.90±0.32c	1.50±0.53	2.80±0.42cd
15	1.70±0.48b	1.60±0.52	2.00±0.00c	1.80±0.42	1.30±0.48b	1.00±0.00	1.10±0.32b	1.00±0.00	1.70±0.48c	1.70±0.48	2.60±0.52c
18	1.60±0.52b	1.60±0.52	1.40±0.52b	1.3±0.48	1.2±0.42b	1.00±0.00	1.10±0.32b	0.60±0.52	1.40±0.52b	1.50±0.53	2.00±0.00b
22	1.00±0.00a	1.00±0.00	0.00±0.00a	0.00±0.00	0.00±0.00a	0.00±0.00	0.00±0.00a	0.00±0.00	0.20±0.42a	0.00±0.00	1.00±0.00a

Data presented as Mean ±SD. Mean values followed by different letters in the same column were significantly different (P < 0.05)

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