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# Physicochemical, microbial and sensory changes in iced Threadfin bream (*Nemipterus japonicus*) stored in indigenously developed fish vending and display unit

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**Abstract**
This work was conducted to study the physicochemical, microbial and sensory changes in iced threadfin bream stored in indigenously developed fish vending and display unit had a capacity of 224 L. The influence of ice storage on the fish was evaluated for 16 days. Physicochemical and microbial parameters like pH, TVB-N, TMA-N, PV, TBARS, FFA and TPC increased significantly during storage period. The results showed a gradual and proportional increment of pH values between 6.7 and 7.5 during the storage time. The sensory scores for overall acceptability were found to be decreased significantly ($p$<0.05). The sensory scores for overall acceptability reached 9.6 to 5.59 on 16 day but remained well above the acceptable range. Based on physicochemical, microbiological and sensory quality it is concluded that the iced threadfin bream could be acceptable condition for 16 days in fish vending and display unit without any deterioration in quality.

**Keywords:** threadfin bream, ice storage, physicochemical changes, microbial changes, sensory changes, Fish vending and display unit

## Introduction
Fish is an important part of a healthy diet since it contains high quality protein, vitamins, minerals, and are the primary food source for long-chain omega-3 fatty acids when compared to other meats. Studies suggest that omega-3 fatty acids are important during fetal brain and eye development, and may help to prevent heart disease in adults (Udochukwu1 *et al*., 2016) [1]. Health experts recommend that regular consumption of fish be included as part of a healthy diet. According to the American Heart Association (Kraus *et al*., 2000) [2] fish consumption frequency should be at least twice a week to enhance cardio protective effects. The threadfin bream, (*Nemipterus japonicus)* is a lean variety of a commercially important demersal fish species in India. Demersal finfish resources contributed 29% of the total landings of the country with major share of threadfin breams about 1.70% (CMFRI) [3]. The fresh and iced fish has high demand in market. Threadfin breams enjoy good demand for its meat, especially to produce surimi based products (Hanumantha *et al*., 2017) [4]. Fish is a highly perishable product due to its high water activity, pH is close to neutral and it contains a high content of nutrients that are easily utilized by micro-organisms (Hosseini., 2010) [5]. Fish quality is subjective in nature and is very complex concept (Adebayo *et al*., 2012) [6] which includes nutritional, microbiological, biochemical and physiochemical attributes. Fish shelf life is influenced by a number of factors such as initial microbiological load, season, handling, the limited and variable shelf lives of fish are major problems for fish quality and assurance (Konstantinos, 2001) [7]. The growth of bacteria, changes of enzymatic activity and chemical reactions are factors affecting fish and fishery products quality during storage, which are greatly influenced by storage temperature (Song *et al*., 2015) [8]. The quality of fish and fishery products has become a major concern in fish industry all over the world (Huss *et al*., 2003) [9]. Preservation is necessary to prolong shelf life and reduce the spoilage of fish. Icing is widely accepted method of chilling the fish, which is the economical and readily available method. Ice is the most important and ideal medium used for preserving fresh fish in both tropical and temperate climates (Surti *et al*., 2001) [10]. The preservation of fish in ice is one of the most efficient ways of retarding spoilage.

Ideal icing involves packing crushed ice and fish in layers in insulated boxes, in the fish to ice ratio of 1:1 (Jose *et al.*, 2005) [11]. The shelf life of white-fleshed lean fish was reported as 11-14 d in ice (Huss, 1988) [12]. It is estimated that, fresh fish caught in tropical and sub-tropical areas may be wasted with the major loss in quality due to improper handling. There are considerable problems in maintaining quality during the period of post-catch handling and distribution of fish. Fisher folks due to lack of infrastructural facilities and technical know-how sell the fish mostly in exposed condition with improper shelter and vending or display units. It is generally agreed that the use of properly designed containers with insulation for marketing of fresh fish can reduce the wastage of fish by spoilage. Therefore, this study was performed to investigate the physicochemical, microbial and sensory changes of iced threadfin bream fish stored in fish vending and display unit for 16 days.

## Materials and Methods

### Sample preparation

Fresh threadfin bream (*Nemipterus japonicus*) fish was used for this study. The average total length of 23.00 ± 1.73 cm, average standard length of 18.33± 0.93 cm and the average total weight of 154.98 ± 4.49 g (Table1) of threadfin bream was procured in fresh condition from Mangaluru landing centre in iced condition. The fish was segregated, washed in fresh chilled water and transferred to fish vending and display unit. Crushed ice and fish was used in the ratio of 1:1 (ice: fish). The fish vending and display unit was kept in shade for storage studies. The ice was replenished once in 24 h after draining the melted water. The total period of ice storage was 16 days. The samples were drawn for analysis at 0, 2, 4, 6, 8, 10, 12, 14, and 16 days of iced storage. The meat samples were analyzed for physicochemical microbial and sensory studies. Samples were randomly dissected by hand to get their muscles and were subjected to chemical and microbiological analysis in triplicate.

**Table 1:** Physical characteristics of threadfin bream

| Characteristics | values |
|---|---|
| Average total length (cm) | 23.00 ± 1.73 |
| Average standard length (cm) | 18.33 ± 0.93 |
| Average total weight (gm) | 154.98 ± 4.98 |

Values indicate mean ± SD, n = 3

### Physicochemical analysis

The pH of the fish samples was measured using pH meter of 'Eu Tech (pH 510') in the temperature range of 27-29 °C as method described by Suzuki (1981) [13]. Total Volatile Base Nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) was determined by Conway micro diffusion method (Beatty and Gibbons, 1937) [14]. TVB-N and TMA-N was calculated and expressed in mg/100 g of the sample. The peroxide value (PV) of fish sample was determined according to Jacob (1958) [15] by iodometrically**.** The peroxide value of an oil or fat is the amount of peroxides expressed as meq $O_2$/ kg of fat. Thiobarbituric acid reactive substance (TBARS) value was determined according to the method of Raghavan and Hultin (2005) [16] to evaluate the oxidation stability during storage and the results expressed in mg malonaldehyde/kg of fish sample. The Free fatty acids (FFA) of fish samples was determined by the method as described by Dyer and Morton (1956) [17] and results expressed as % of oleic acid. All determinations were performed in triplicate.

### Microbiological analysis

Total plate count was analyzed(TPC) using the method described by APHA (1995) [18], 10 g of fish sample was taken and macerated for 2 min. in sterile with 90 ml of sterile diluents of 0.85 % sodium chloride (NaCl) using a pestle and mortar. The serial decimal dilution of were prepared using 9 ml saline for making serial dilutions and it was well mixed in cyclomixer. Exactly 0.1 ml of inoculum from each of the dilution and was spread plated onto TPC agar plates using a sterile plastic spreader. The inoculated plates were incubated at 37 °C for 24 h. The average counts were expressed as log cfu/g.

### Sensory analysis

Sensory characteristics and overall acceptability of fish were assessed by a panel of six members on the basis of ten point scale on each sampling as suggested by Sukumar *et al.* (2007) [19] with little modification. Sensory characteristics study included general appearance, odour and texture of fish. The scores were given in the decreasing order scale with 10-9 for excellent, 8-7 for good, 6-5 for fair and acceptable, 4-3 for poor and 2-1 for very poor. The mean of the scores given by the panel represented the overall sensory quality.

### Statistical analysis

The data obtained from microbiological, biochemical and sensory analysis were further analyzed by using Statistical Package for Social Sciences (SPSS). Analysis of variance (one way - ANOVA) was performed to determine the differences between storage days. Significance of differences was defined at $p < 0.05$.

## Results and Discussion

### Physicochemical and microbial characteristics of fresh threadfin bream

The freshness of fish meat was assessed by physicochemical, microbiological and sensory parameters and it was found that fish was prime condition (Table 2). The TVB-N and TMA-N content was in fresh condition according to the connell (1990) [20] limit for fresh fish. Lipid oxidation analysis also showed fishes in fresh condition. The PV also shows fresh condition of fish. Similar results have been reported by Smith *et al*. (1980) [21] for a number of species e.g. 0.8-1.2 meq $O_2$/ kg for herring. Low TPC load was found for fresh threadfin bream. These freshness parameters conclude that fish is very fresh condition. Lakshmisha *et al.* (2012) [22] have reported similar physicochemical results for fresh threadfin bream.

**Table 2:** Biochemical and microbiological characteristics of fresh threadfin bream

| Characteristics | Values |
|---|---|
| pH | 6.77 ± 0.01 |
| TVB-N mg (%) | 5.02 ±0.98 |
| TMA-N mg (%) | 1.14 ±0.16 |
| PV (meq$O_2$ / kg of fat) | 0.77 ±0.01 |
| TBARS (mg malonaldehyde / kg) | 0.86 ±0.02 |
| FFA (% Oleic acid) | 2.59 ± 0.11 |
| TPC (Log cfu/gm) | 3.98 ±0.07 |

Values indicate mean ± SD, n = 3

### Physicochemical changes

#### pH

The values of pH obtained from the present study showed statistically significant increase ($p < 0.05$) with increase in storage time for fish. The initial pH value was observed as

6.77 which reached up 7.50 (Table 3) at the end of storage. This increase in pH levels of fish causes increase in volatile bases and accumulation of ammonia due to decomposition of nitrogenous compounds by the microbial activities. The results of this study are in co-relation with Vilma *et al*. (2009) [23] during the ice storage of mackerel.

**Total volatile base nitrogen (TVB-N)**

In this study, the TVB-N content significantly increased from 5.02 to 32.51 mg % for threadfin bream at the end of the storage in ice (Table 3). This observed increase during storage may be related to several enzymatic processes, including the deamination of free amino acids, degradation of nucleotides and oxidation of amines or microbial activities (Alcicek, 2011) [24]. Considering the acceptability limits reported by Connel (1975) [25], the results of present work indicated that, threadfin bream was acceptable even at the end of 16 days of storage in ice. The increasing value obtained for TVB-N was comparable with the increasing TVBN as reported by Gulsun *et al*. (2009) [26] for iced storage of red mullet (*Mullus barbatus*). This increase could be attributed to the production of ammonia and other volatile amines in the muscle during storage (Mazorra *et al*., 2000) [27].

**Trimethylamine nitrogen (TMA-N)**

The quantitative level of TMA in fish is considered as an important indicator of the quality of marine fish (Zhang *et al*., 2003) [28]. The changes in the TMA-N content of the fishes kept in iced condition are shown in Table 3. The content of TMA-N was 1.14 at initial stage and reached to 10.24 mg % on 16 days of storage. The gradual increase in TMA-N values was observed throughout the time of storage. Trimethylamine Oxide (TMAO) decomposition in fish and seafood results in to formation of trimethylamine and dimethylamine which are responsible for off flavor (Hui, 2006) [29]. The quantity and presence of this compound depends on the species, size, sex, station of year, etc. (Tsigarida *et al*., 2003) [30]. The increase in TMA-N content is also supported by the study conducted by Chuma *et al*. (2010) [31] during the iced storage of croaker (*Pseudotolithus elongatus*).

**Peroxide value (PV)**

Primary oxidation was measured by means of the PV. Oxidative rancidity development in fish is determined by the estimation of peroxide value and is expressed as meq $O_2$/kg of fat. In the present study, the peroxide value significantly ($p<0.05$) increases with increase in storage time for threadfin bream and suddenly drops at the end of storage as represented in Table 3. At the beginning of storage, low PV was detected which occurred during the induction period of lipid oxidation so rancidity was not detected at initial day. The maximum PV was found to be 8.27 meq $O_2$/kg of fat on 12 day and decreased by the end of storage period. The initiation of lipid oxidation process starts the free radical chain reaction and enters in to the monomolecular stage. The decreasing trend in PV is mainly due to the generation of aldhyde, ketones and other non-radical compounds. Similar findings were reported by Yesim *et al*. (2011) [32] for common sole stored in ice.

**Thiobarbituric acid reactive substances (TBARS)**

Thiobarbituric acid reactive substances are the secondary oxidative products of lipid oxidation and are expressed as mg malonaldehyde/kg of meat. In the present investigation, the TBARS value showed a significant ($p < 0.05$) increase during the ice storage period (Table 3). The TBARS varied between 0.86 to 4.72 mg malonaldehyde/kg of meat. The present finding is also supported with the results of Aubourg, (2001) [33]. The increase in TBARS indicated formation of secondary lipid oxidation products (Kolakowska, 2002) [34].

**Free fatty acid (FFA)**

Free fatty acid is the resultant products of hydrolytic rancidity of lipids which is expressed as % of oleic acid. The FFA is a result of enzymatic decomposition of lipid during storage leading to lipid hydrolysis (Tokur *et al*., 2006) [35]. In the current study, the values of FFA significantly increased ($p < 0.05$) from the initial value of 2.59 to 8.22 % of oleic acid in case of threadfin bream during the storage period (Table 3). The fat with high contents of poly unsaturated acids (PUFA) is readily prone to lipid oxidation. Hydrolysis of glycerol-fatty acid esters is one important change that occurs in fish muscle lipids postmortem with the release of free fatty acids. This is catalyzed by lipases and phospholipases (Pacheco-Aguilar *et al.,* 2000) [36].Similar increasing trend of FFA was observed by Sunil *et al*. (2010) [37] during the iced storage fish mackerel fish.

**Microbiological changes**

**Total plate count (TPC)**

The composition of fish muscle makes the fish favorable for microbial growth. Thus, fish spoiling occurs during storage mainly as a result of microbial activity (Souza *et al*., 2010) [38]. Microbiological problems which are associated with bacteria cause spoilage of seafood leading to huge economical losses. The initial TPC count was observed as 3.98 log cfu/g which increased progressively ($p < 0.05$) with end of storage (Table 3). At the end of storage day TPC was counted as 6.98log cfu/g. In this manner, International Commission on Microbiological Specifications for Food (ICMSF, 1986) [39] stated that the upper acceptability limit of TPC was 7 log10 cfu/g maximum permissible limits in chilled fish. According to limit of acceptability the threadfin bream was acceptable up to 16 days of storage. Antoine *et al*. (2002) [40] noted that a threshold value of 30 mg N / 100 g TVB-N is often correlated with a bacterial load of 6 log cfu/g which is evident in the present study.

**Table: 3:** physicochemical and microbial changes in threadfin bream during iced storage

| Parameters | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Storage days** | **pH** | **TVB-N** | **TMA-N** | **PV** | **TBARS** | **FFA** | **TPC** |
| 0 | 6.77±0.01 | 5.02±0.98 | 1.14±0.16 | 0.77±0.01 | 0.86±0.02 | 2.59±0.11 | 3.98±0.07 |
| 2 | 6.79±0.03 | 6.76±0.05 | 3.62±0.19 | 2.77±0.02 | 1.07±0.10 | 3.82±0.07 | 5.10±0.02 |
| 4 | 6.87±0.02 | 7.36±0.01 | 4.68±0.20 | 3.38±0.31 | 1.85±0.03 | 4.27±0.05 | 4.18±0.03 |
| 6 | 6.90±0.01 | 11.41±0.29 | 4.88±0.71 | 4.36±0.01 | 2.23±0.04 | 5.83±0.06 | 4.96±0.06 |
| 8 | 7.01±0.02 | 12.48±0.11 | 6.67±0.63 | 6.79±0.87 | 1.73±0.01 | 6.77±0.02 | 5.10±0.04 |
| 10 | 7.05±0.06 | 18.32±0.09 | 6.99±0.19 | 7.50±0.25 | 1.82±0.05 | 7.07±0.15 | 5.52±0.59 |
| 12 | 7.07±0.04 | 21.44±0.06 | 7.32±0.18 | 8.27±0.52 | 3.68±0.17 | 7.92±0.06 | 5.94±0.01 |
| 14 | 7.33±0.24 | 30.52±0.53 | 8.55±0.35 | 4.66±0.28 | 4.50±0.24 | 7.63±0.33 | 6.32±0.02 |
| 16 | 7.50±0.10 | 32.51±0.14 | 10.24±0.11 | 4.39±0.08 | 4.72±0.02 | 8.22±0.74 | 6.98±0.01 |

Values are Mean with the ± SD in the rows, n=3

**Sensory characteristics**

Initially the whole fish showed fresh sea weed odour, shiny appearance, elastic in texture, bright red gills and perfectly fresh eyes with convex black pupil in both pink perch and mackerel (Table 4). The sensory scores were showed significantly different ($p < 0.05$) between the fishes. There was a progressive reduction found in threadfin bream depicted. At the end of the storage period, fish used for study was still in acceptable condition. The composition of fish can affect the sensory properties that greatly influence the acceptability of fish as food. It may also affect microbial growth in fishes (Sallam, 2007) [41]. Sankar and Viswanathan, (1988) [42] found a progressive quality loss in sensory scores during chilled storage of silver pomfret and mackerel.

**Table 4:** Changes in sensory characteristics of threadfin bream during iced storage

| | Sensory characteristics | | | |
|---|---|---|---|---|
| **Storage days** | **General Appearance** | **Odour** | **Texture** | **Overall acceptability** |
| 0 | 9.71±0.10 | 9.57±0.08 | 9.78±0.03 | 9.60±0.00 |
| 2 | 9.21±0.10 | 9.55±0.05 | 9.60±0.00 | 9.40±0.05 |
| 4 | 8.94±0.05 | 8.91±0.03 | 8.94±0.04 | 8.6±0.04 |
| 6 | 8.67±0.3 | 8.53±0.04 | 8.80±0.00 | 8.20±0.00 |
| 8 | 8.26±0.18 | 8.48±0.03 | 8.48±0.03 | 8.00± 0.00 |
| 10 | 7.86±0.12 | 7.85±0.05 | 8.45±0.05 | 7.50±0.04 |
| 12 | 7.36±0.18 | 6.59± 0.01 | 7.83±0.05 | 7.14±0.06 |
| 14 | 6.87±0.14 | 6.20±0.18 | 7.55±0.05 | 6.44±0.09 |
| 16 | 6.28±0.20 | 5.00±0.10 | 6.86±0.05 | 5.59±0.00 |

Values indicate mean ± SD, n = 3

**Conclusion**

The present investigation proved that the results of the biochemical microbial parameters changed over storage time and all attributes related to the sensory analysis. The parameters concludes that, the keeping quality of threadfin bream fish stored in the indigenously developed fish vending and display unit extended up to 16 days in iced condition although based on sensory atributes, the fish shelf-life may extend beyond this period.

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