Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [9] August 2023 : 170-176

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

A Study on Changes in Some Quality Indices in *Mugil cephalus* Collected from Visakhapatnam Harbour and Frozen at -20°C

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ABSTRACT
The goal of the current study is to comprehend the changes in TMAN, TVBN, Total plate count, Peroxide Value and Thiobarbituric Acid value that have occurred during the 180-day storage at a temperature of -20°C in the ungutted fish species, Mugil cephalus. Despite an initial trend of slow increase in the quality indices, subsequent storage periods have shown a significant rise in the values of all the quality indices chosen for the present study. Results thus, conclude that much lower temperature of storage, than -20°C for a longer duration may be suitable to maintain the limits of consumer acceptability in whole ungutted Mugil cephalus.
Keywords: *Mugil cephalus, TMAN, TVBN, PV, TBA.*

Received 04.05.2023 Revised 14.07.2023 Accepted 03.08.2023

INTRODUCTION

Numerous essential elements for human health can be found in fish and fish products. Frequent susceptibility to change or deterioration in quality after being captured and death challenge the assurance of their quality and safety and it has become a problem of concern [1]. A variety of biochemical reaction mechanisms and microbial activities lead to the quality degradation. The species, size, fat content, environmental conditions at the time of the capture, the initial microbial content, and the storage temperature all affect how quickly the quality of fish degrades [2]. Fish that reside in warm regions, such as those from the tropics, can be kept in the ice for a longer period of time than fish that do. This is because tropical fish species have a dominating mesophyll microflora, which cannot grow rapidly in cold temperature conditions [3, 4].

Grey mullet (*Mugil cephalus*) is one of the most widely distributed food fish in the world. It is found in the coastal waters and estuaries throughout the tropics and subtropics [5]. Due to the high quality protein, fish is consumed by a significant portion of the global population. It is a source of energy for people and contains the most crucial nutritional components [6, 7].

Freezing has been the ideal method for fish preservation for a longer duration. Moreover, storage above the initial freezing temperature, enables the preservation of flavour and nutritional qualities. Additionally, according to Martino et al. [8], it has the benefit of reducing microbial and enzymatic activity. According to Woyewada et al. [9] the degradation and spoiling of a product are caused by the accumulation of various chemicals from the enzymatic breakdown of protein by microbes and biochemical changes.

Following the death of a fish, the decrease of TMAO to TMAN increases as the activity of spoilage bacteria increases [10]. Only in the logarithmic phase of bacterial growth does TMAO breakdown, which leads to TMA buildup, take place to a major degree. Three different varieties of fish were frozen for nine months in the study and TMA readings increased over that time [11].

Fish, both frozen and raw, are generally evaluated for their total volatile basic base-nitrogen (TVB-N) content. The total volatile base nitrogen (TVB-N) concentration of fish is the sum of the amounts of ammonia (NH_3), dimethylamine (DMA), and trimethylamine (TMA) in the fish. It is frequently used as a measure of spoilage and has been widely utilised as an index for the freshness of fish [12]. Fish was rated as "very good" if the TVB-N value was less than 25 mg/100 g, "good" if it was between 25 and 30 mg/100 g, "marketable" if it was between 30 and 35 mg/100 g, and "spoiled" if it was 35 mg/100 g or more, according to [13]. Studies conducted in the past have produced findings that were similar [14, 15]. According to Pearson et al. [16], oxidation of fish muscle tissue-stored lipids is another most significant

limiting factor during frozen storage of fish, especially fatty species. Increase and decrease in the value of TBA, particularly during long-term storage, are observed as the secondary products of lipid oxidation [17, 18].

Early post mortem alterations in fish tissue are related to lipid oxidation. Unsaturated fatty acids' carbon atoms lose their protons, which results in the production of free radicals, which spurs the process. When compared to products that are stored in the refrigerator, frozen products are more likely to experience lipid oxidation, which can happen enzymatically (using lipoxygenase or peroxidase) or non-enzymatically (using iron ions) [19, 20]. Microorganisms and chemical reactions have high probability of causing great loss of fish meat nutrient quality. The primary reason for microorganism and fat oxidation attack is the reduction in quality of fish with a high fat content [21].

High-fat fish have a short shelf life because fat conversion is simple. The degradation of quality is closely associated with hydrolytic and oxidative rancidity in fish muscles. Free fatty acids are produced during hydrolysis under the influence of lipases and phospholipases. These free fatty acids then go through additional oxidation to form low molecular weight components that give fish and fishery products their rancid fragrance and off-taste [22, 23]. The second stage of the autoxidation process, during which the peroxide was oxidised to aldehydes and ketones, was what led to the identification of the presence of reactive TBA compounds. A high TBA content can have a rotten smell, which is undesirable [21].

The present study has been taken up to observe the changes in some quality parameters during frozen storage of *Mugil cephalus* at -20^{o}C as the literature on this aspects with respect to the fish chosen is very meagre.

MATERIAL AND METHODS

Determination of Total Volatile Base Nitrogen (TVBN) and Trimethylamine (TMAN)

TMAN and TVBN in the present study were determined according to Egan et al. [24] in Pearson's chemical analysis of foods which involves steam distillation followed by titration method. 100gms of sample of the three fish species taken for the study was homogenized with 300ml 0f 5%m/v Trichloroacetic acid. 5 ml of the extract was transferred into semi-micro-distillation apparatus and was subjected to steam distillation. The distillate so obtained was collected in 15ml 0.01N Standard HCL. Rosalic acid indicator was added and titrated to a pale pink end point with 0.01N NaOH. A blank determination was also performed. One ml of 16 % m/v neutralized formaldehyde was added for every 10 ml liquid in the titration flask. The liberated acid was titrated with 0.01N NaOH.

$$\text{TVBN (mg/100gms)} = \frac{14\,(300+W) \times V_1}{50} \text{ mg/100gms}$$

$$\text{TMAN (mg/100gms)} = \frac{14\,(300+W) \times V_2}{500} \text{ mg/100gms}$$

Where, V_1 = Volume of standard acid consumed in the first titration.

V_2 = Volume of standard acid consumed in the second titration.

W = Weight of the sample.

Enumeration of Total Plate Count

10 gms of the sample is macerated with 90 ml of Phosphate buffer in a sterile homogenizer of stomacher type. The homogenate obtained was used to prepare sterile dilutions upto 10^6. One ml of each of the serial dilutions was transferred asceptically into sterile Petri dishes. Nearly 10 ml of the melted and cooled Tryptone Glucose Agar (TGA) was introduced into the Petri dishes and mixed gently by rotation. After about 30 minutes they were incubated at 37^{o}C for 48 hours. After 48 hours of incubation, colonies were counted.

Determination of Peroxide (PV)

Peroxide value was determined by according to Egan et al. [24] in Pearsons's chemical analysis of foods. Minced muscle was blended with twice its weight of anhydrous sodium sulphate in mortar. The blend was shaken with distilled chloroform for 5 to 10 minutes and filtered. For PV estimation 5 gms of oil was taken into 250 ml boiling conical flask, 30 ml of HOAc–$CHCL_3$ was added and swirled to dissolve, 0.5 ml saturated KI solution was added, shaken thoroughly and was boiled in water bath for not more than 30 seconds. 30 ml of water was added slowly and then the liberated iodine was titrated with 0.1N $Na_2S_2O_3$ with vigorous shaking until yellow was almost gone. 0.5 ml of 1% Starch solution was added and titrated by shaking vigorously so as to release all the iodine from the chloroform layer until blue colour just disappeared. Blank determination was carried out simultaneously. The Peroxide Value is often reported as the number of ml of 0.002N Sodium thiosulphate per gram of sample. The value so obtained was multiplied by 2, which then equals milliequivalents of peroxide oxygen per kg of sample (meq/kg).

Determination of Thiobarbituric Acid Value (TBA Value)

Thiobarbituric acid value was estimated according to the method described by Vynke [25]. 10 gms of fish muscle was homogenized with 50 ml distilled water and washed into distillation flask with 47.5 ml distilled water. 2.5 ml 4N HCl was added and heated by adding glass beads. 50 ml of distillate was collected in 10 minutes. 5 ml distillate was pipette into a glass stoppered tube; 5ml TBA reagent was added, stoppered, and heated in boiling water bath for 35 minutes. A blank was similarly prepared using 5ml distilled water with 5ml reagent, then the tubes were cooled and OD was measured against the blank at 538 nm. TBA number as mg Malonaldehyde per kg sample is equal to O.D. x 7.8.

RESULTS

Initial TMAN level recorded in fresh *Mugil cephalus* was 2.59 mg/100gms. An increasing trend was observed upto the end of the storage period. After 56 days TMAN levels reached 6.54 mg/100gms. After 70 and 84 days TMAN levels recorded were 10.25 and 14.75 mg/100gms respectively and were found to be within the acceptable limits. After 120 days TMAN levels exceeded acceptable limits. After 180days, levels reached 25.11 mg/100gms.

TPC in fresh *M. cephalus* was $9.9x10^3$ cfu/g. The count decreased to $9.5x10^3$ cfu/g after one day of frozen storage. But again an increased upto $9.6x10^3$ cfu/g was observed after 3 days of storage. Thereafter, TPC decreased and reached $8x10^3$ cfu/g after 28 days of storage. Upon further frozen storage TPC increased and reached $28x10^3$ cfu/g after 180 days. A high correlation value of 0.75 was obtained between TMAN and TPC in the present study.

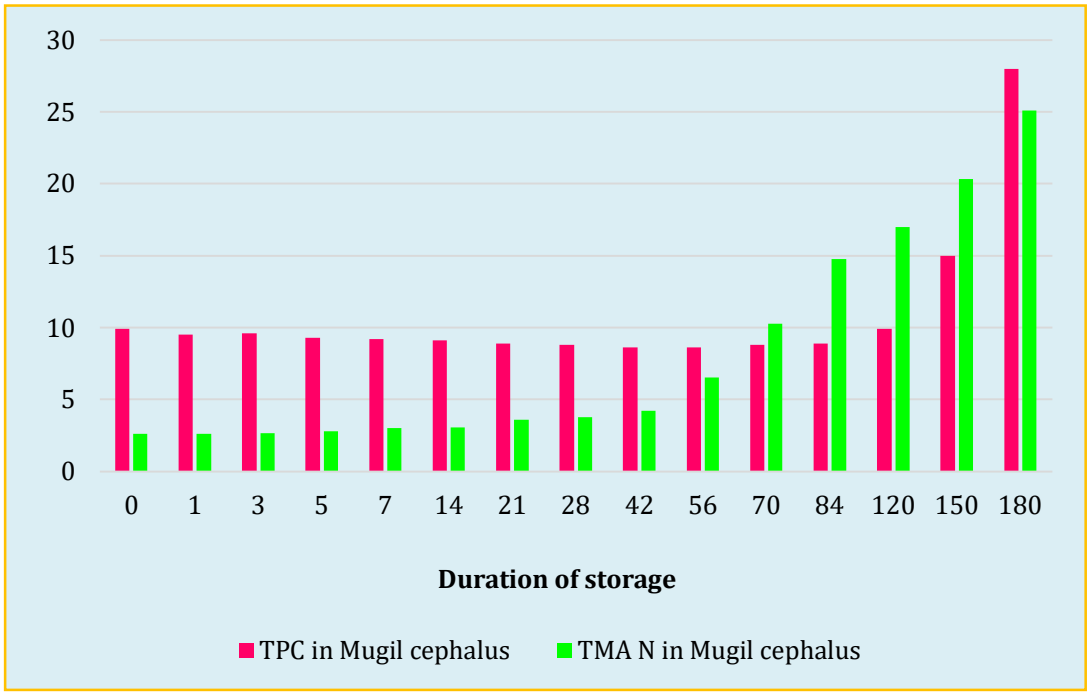

Figure 1. TPC and TMAN content in *Mugil cephalus* across 15 durations of storage.

Initial TVBN values in fresh *Mugil cephalus* recorded was 5.34 mg/100gms. Considerable increase was noticed in TVBN level during the entire storage period. The increase was slow during the initial period and reached 10.01 mg/100gms after 28 days. After 42, 56, 70, 84, and 120 days TVBN reached 11.45, 16.00, 20.75, 25.25, and 29.33 mg/100 gms respectively which were found to be below the acceptable limits. On further storage TVBN exceeded the acceptable limit and reached 43.20mg/100gms after 180 days. A high correlation value of 0.73 was observed between TVBN and TPC in the present study.

Initial Peroxide Value in Fresh *Mugil cephalus* was recorded as 0.10 meq/kg. After one day of frozen storage PV reached 0.15 meq/kg. Increasing trend of PV continued up and reached 15.20 meq/kg after 70 days of storage. Further storage resulted in a decrease of PV and after 180 days PV reached 8.25 meq/kg.

Fresh *M. cephalus* also showed no TBA content. After one day of frozen storage TBA value 0.20mg MA/Kg was recorded. TBA value increased further and reached 5.70 mg MA/Kg after 42 days of storage. Further Storage resulted in increased TBA values that exceeded the limit of acceptability 7 to 8mg MA/Kg as proposed by Huss [26]. Thus, by the end of the storage period TBA value reached to 17.25mg MA/Kg.

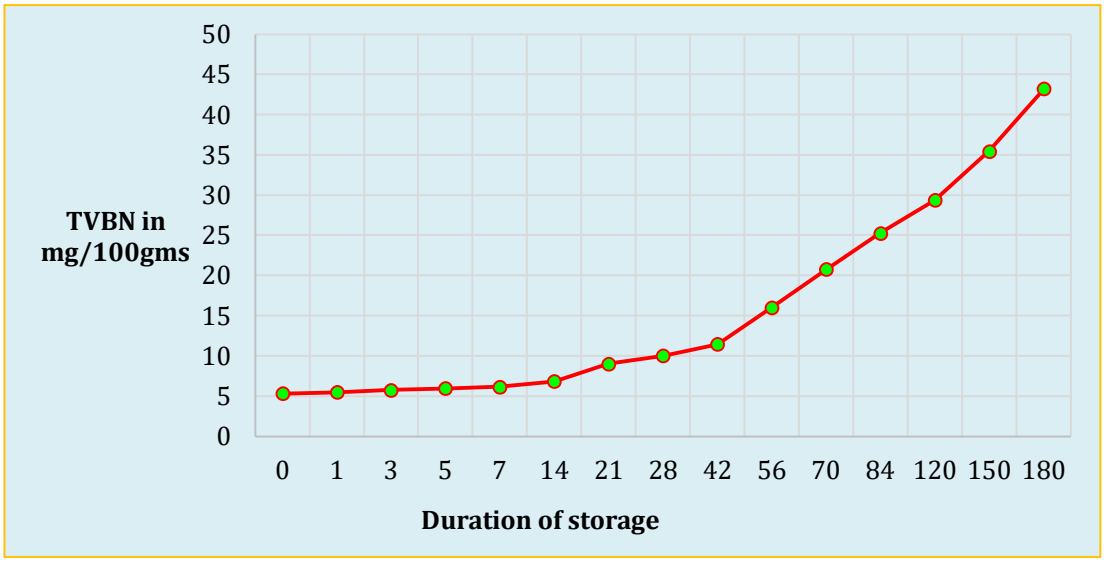

Figure 2. TVBN content in *Mugil cephalus* across 15 durations of storage.

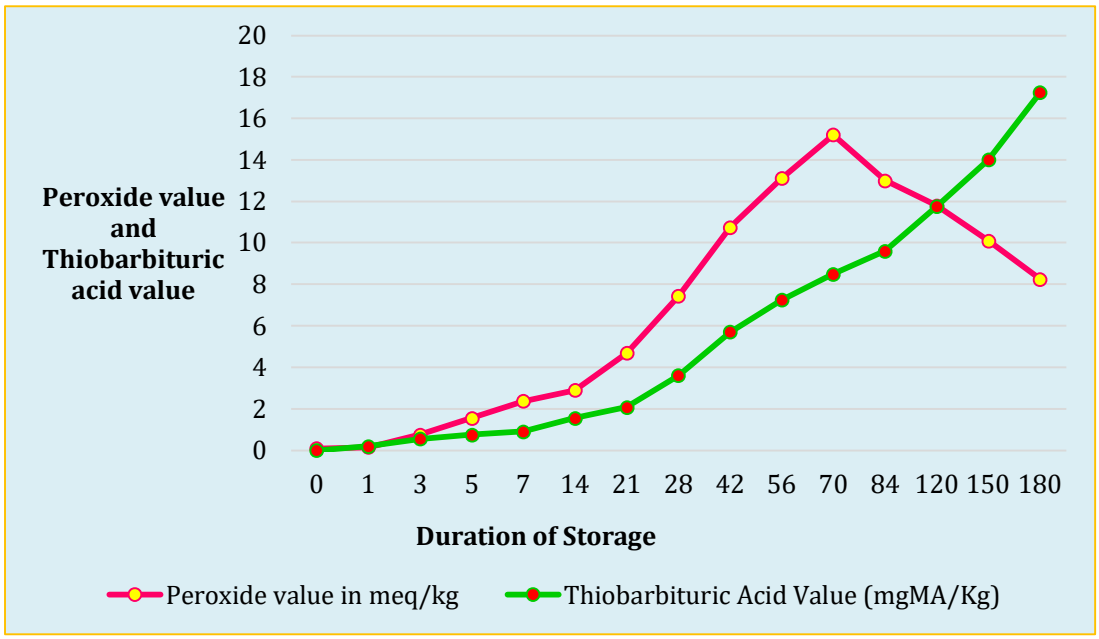

Figure 3. PV and TBA content in *Mugil cephalus* across 15 durations of storage.

DISCUSSION

Volatile substances like ammonia (NH_3) and trimethylamine (TMAN), which are formed during the fish storage by bacterial and autolytic processes, give off an ammoniacal and pungent fish odour that is indicative of fish spoilage. Trimethylamine oxide (TMAO), which is naturally produced by marine organisms to enable osmotic balance, is reduced by bacteria to form TMAN. TMAN is one of the principal substances evaluated by total volatile nitrogen (TVBN), along with ammonia and other volatile amines [27, 28]. In the absence of oxygen and ice preservation, bacterial action causes the conversion of TMAO to TMAN. The increase in TMAN levels observed in the present study may result from domination of free amino acid, oxidation of amines and degradation of nucleotides by autolytic enzymes and microbial activity as reported earlier by Chudasama et al. [29] in their studies.

The amounts of NH_3, TMA, and dimethylamine acid (DMA), which rise during the degradation process, are taken into account while determining TVBN to determine the freshness of the fish [30]. The levels of 30 to 35 mg N_2 per 100 g muscle are considered the limit of acceptability [31]. Upto the end of 120 days, the fish in the present study retained the acceptable limits. Chudasama et al. [29] in their studies on iced and refrigerated *Rastrelliger kanagurtha* also reported increase in TMAN values and TVBN values similar to the

present study results. The amount of TVB-N continuously increased during cold storage of mullet fish [32]. During the frozen storage of mullet fish, the amount of TMA steadily rose in the present study and these findings were consistent with those of [32-35]. Total volatile nitrogen is always taken into account when evaluating the quality of fish since it is closely associated to microbial development and the generation of essential substances including methylamine ammonia, diethylamine, and trimethylamine as a result of bacterial metabolism [36].

During the frozen storage of mullet fish in the present study, it was noticed that the total bacterial count increased steadily and these findings are consistent with those reported by [32, 37]. According to Zaitsev et al. [38] who noted that common salt has a bacteriostatic and bactericidal activity that delays the growth with eventual destruction of the bacterial cells, the total bacterial counts were rather high in the freshwater crushed ice samples. The increase in APC in present study may be attributed to an increase in simple nitrogenous molecules (amino acids and nucleotides) and fatty acids produced by the natural enzymatic breakdown of fat and protein, which leads to the creation of favourable circumstances for bacterial growth as reported by Aref et al. [39]. The maximum permissible limits (7 log CFU/g) has been specified by the International Commission on Microbiological Specifications for Foods for fresh fish [40].

During frozen storage in the current study, PV and TBA increased significantly, and oxidation of these unsaturated fatty acids results in lipid degradation as also reported by Karami et al., 2013. The amount of PV increased as a result of the attack by unsaturated fatty acids on red carp muscle during the freezing phase and this increase might be connected to a rise in hydroperoxide production as reported by Tenyang et al. [41]. The pattern of change in peroxide values in the present study strongly correlate with the reports of Ninan et al. [42], who noticed the increasing trend of peroxide value gradually for the species *Oreocromis mossambicus* until the 12th and 15th week of freezing, then reduction after that. The progressive increase in PV during the initial phases of storage and its gradual decrease during the later duration can be attributed to the fatty fish's limited shelf life caused by fat oxidation. Fatty acid hydroperoxide, which can be calculated as a peroxide number, is the primary byproduct of fat oxidation. Peroxides are unstable substances that break down into aldehydes, ketones, alcohols, and volatile substances, imparting an undesirable flavour [43, 44]. Acceptability limits for peroxide value of fish oils are (7-8 meq oxygen/ kg fat) [26].

TBA is frequently employed as a measure of the degree of fat oxidation. A good technique for the monitoring of oxidation processes in meat is using the Thiobarbituric acid assay (TBA) after conversion to malonaldhyde equivalents [45]. It is thought to be the end result of secondary oxidation of fat. The presence of TBA in fish oils indicates that oxidation has occurred in the samples [46, 47]. The TBA value increases as oxidative degradation in oily fish progresses, the peroxide value and the TBA value may decline again at a later stage of spoilage [48].

Yehia et al. [32] observed that fresh fish had low TBA concentrations, and that storage caused the TBA values to rise significantly as observed in the present study. Preservation studies on sardines (*Sardinella gibbosa*) in ice reported that the TBA value was 17.2 mg malonaldehyde/kg sample at the start of storage and grew by 97% by the conclusion of the storage period (15 days). The increased TBA level is a sign that secondary fat oxidation products are forming. Aldehydes in particular, which are relatively polar secondary products, have been measured using TBA. The value of the peroxide value showed a reduction in the present study and this may be due to the breakdown of hydroperoxides into secondary oxidation products, mainly aldehydes at an advanced stage of fat oxidation, while TBA increased dramatically on the day of storage after the sixth day of storage as reported by Chaijan et al. [22]. A gradual increase in TBA value with increasing frozen period for the species *L. aurata* similar to the present study observation was also observed by Chaijan et al. [22], where TBA recorded at -18°C in the days (15 and 180 days). Dragoev [49] reported a gradual increase in TBA value (0.15 and 7.22 mg malonaldehyde/kg fat for the species *Scomber scombrus* at -18°C in the days (0 and 360), respectively. That may be due to peroxides decomposition to secondary products of oxidation, especially aldehyde in the later stages of fat oxidation. The current study's findings are consistent with those of Lakshmisha et al. [50], whose work was done at -20°C on the Indian species *Rastrelliger kanagurta*, where TBA value increased consistently. The highest TBA value that determines the quality of frozen and chilled fish is 5 mg malonaldehyde/kg, while fish can be ingested up to a limit of 8 mg malonaldehyde/kg [51]. According to Nikoo et al. [52], acceptability limit of TBA for human consumption is 7-8 mg malonaldehyde /kg.

CONCLUSION

The results of this study showed that the frozen fish retained edible quality upto 90 days of frozen storage and the quality of fish is best in fresh condition. The rate of deterioration accelerated with an increase in the frozen storage time. The proliferation of bacteria, TVBN, TMAN, lipid oxidation increased as the storage period increased. Thus, the results clearly indicate that, the ungutted fish may retain longer acceptability

characteristics at temperatures lower than -20°C. There is also scope for application of suitable pretreatments prior to frozen storage for better fish meat quality retention.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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CITATION OF THIS ARTICLE

Shanti Prabha Y, Manjulatha C. A Study on Changes in Some Quality Indices in *Mugil cephalus* Collected from Visakhapatnam Harbour and Frozen at -20°C. Bull. Env. Pharmacol. Life Sci., Vol 12[9] August 2023 : 170-176.