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A Study on Some Chemical Quality Indices of Whole *Catla catla* from Visakhapatnam Harbour Frozen at -20°C

Shanti Prabha Y.[1]* and Manjulatha C.[2]

[1]Department of Zoology, Dr. V.S. Krishna Government Degree and PG College (Autonomous), Visakhapatnam 530 013, India
[2]Department of Zoology, College of Science and Technology, Andhra University, Visakhapatnam 530 003, India

**Corresponding Author*

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Abstract: Fresh and frozen samples of *Catla catla* were collected from Visakhapatnam fishing harbour, frozen at -20°C and stored across different durations upto the end of 180days. The samples were analyzed for Chemical quality indices namely TMAN (Trimethyl amine nitrogen), TVBN (Total volatile basic nitrogen), PV (Peroxide value) and TBA (Thiobarbitutric acid) during the storage period. Results are in consonance with the findings of earlier studies in terms of all the indices chosen for the present study.

Keywords: *Catla*, Trimethyl amine nitrogen, Total volatile Basic nitrogen, Peroxide value, Thiobarbitutric acid

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Introduction

According to the Food and Agriculture Organisation (FAO) of the United Nations (1997), fish is the most significant single source of high-quality protein, accounting for roughly 16% of the animal protein ingested by the world's human population. Aspects of nutrition science such as improved early development and growth, health maintenance, a decreased chance of obesity, and a decreased risk of chronic diseases are linked to diet. Protein, carbohydrates, minerals, vitamins, peptides, lipids, and essential fatty acids are just a few of the valuable nutrients found in fish. Fish is more significant as a food product in underdeveloped nations because it provides 75% of the daily animal protein and is referred to as "rich and poor food" as a crucial ally (Willette *et al.*, 2019; Masour *et al.*, 2021).

Fish provides eight necessary amino acids, including sulfur-containing lysine, methionine, and cysteine and 18–20% protein (Khalil *et al.*, 2019). It contains less fat than red meat and provides easily digestible protein of high biological value, which is essential for the body's growth and development, maintenance and repair

of worn-out tissues, and production of hormones and enzymes required for many bodily processes (Tacon *et al*., 2020). The highly prevalent polyunsaturated fatty acids (PUFAs) in fish have a significant physiological impact on the brain development, growth of foetus, neonates, and children. Fish should therefore be given the optimal place in children's diets for healthy development (Maulu *et al*., 2021).

Even in economically poor nations, fish is easily accessible and considerably less expensive when compared to other animal protein sources. According to Mohan *et al.* (2016), fish is a significant source of protein, fat, minerals, vitamins, and important omega-3 fatty acids that help ensure the security of food and nutrition. Despite this dietary significance, fish is a highly perishable food that, under tropical temperature conditions, could become unfit for human consumption within twelve hours of capture (Jim *et al*., 2017). The techniques used for catch, handling, processing, distribution, and storage have an impact on the quality of fish meat and its utility (Tesfay and Teferi, 2017).

In the meat, fish, and other animal protein-based industry, freezing is a common practice because it preserves quality for a long time and has several benefits, including minimal changes in product dimensions, colour, flavour, and texture deterioration (Obuz and Dikeman, 2003). Fish processing often involves the use of frozen storage. However, seafood that is frozen and kept in a frozen state always loses quality (Mackie, 1993). Changes in muscle integrity, proteins, and lipids are the main causes of quality loss in fish that has been frozen and preserved (Shenouda, 1980). Acid hydrolysis of lipids to produce free fatty acids can occur as a result of cellular disintegration during frozen storage. Due to the economic importance of these changes in fish muscle fibres, proteins, lipids, and textural qualities during frozen storage, studies on these alterations have been conducted for many years (Haard, 1992; Cappeln *et al*., 1999; Solanki *et al*., 2011; Gandotra *et al*., 2012).

Fish muscle proteins and lipids undergo various chemical and physical changes while being frozen, which, under certain circumstances (such as prolonged storage, inadequate freezing techniques, temperature fluctuations, etc.), may lead to a loss of quality, primarily manifested by an unacceptable texture as well as an undesirable flavour, odour, and colour (Sotelo *et al*., 1995). During frozen storage, fish and fisheries products may experience unfavourable changes, and deterioration may shorten the storage time. Lipid oxidation and enzymatic breakdown of lipid and proteins cause these changes (Sahari *et al*., 2013). Fish quality is significantly impacted by biological and chemical processes such as lipid oxidation and enzymatic activity during long-term frozen storage (Bahçeci *et al*., 2005; Strasburg *et al*., 2008).

The present study has been conducted to study the changes in some of the important chemical indices in Freshwater fish *Catla catla,* the most relished fish collected from Visakhapatnam Fishing harbor and stored at -20^{o}C across different durations.

Materials and Methods

The present study includes studies on TMAN (Trimethyl amine nitrogen), TVBN (Total volatile Basic nitrogen), Peroxide value (PV) and Thiobarbitutric acid (TBA) measurements of freshwater fish *Catla catla.* All the fresh samples were collected from Visakhapatnam Fishing harbor. Without any time lapse the tests were undertaken after thorough washing of the fish with water in the lab. The samples of fish were not eviscerated as the present study was on whole fishes. The sample was separated in 2 lots. The first lot was analysed in fresh condition and the second lot was packed in sterile polythene bags in the whole form and was stored at -20^{o}C. The frozen samples were analysed across fifteen durations of storage i.e., after 1, 3, 5, 7, 14, 21, 28, 42, 56, 70, 84, 120, 150, 180 days of storage.

Determination of Total Volatile Base Nitrogen (TVBN) and Trimethyamine (TMAN):

TVBN and TMAN in the present study were determined according to Egan *et al.* (1981) which involves steam distillation followed by titration method. 100 g of sample of the three fish species taken for the study was homogenized with 300 ml of 5% m/v Trichloacetic acid. 5 ml of the extract was transferred into semi-microdistillation apparatus and was subjected to steam distillation. The distillate so obtained was collected in 15 ml 0.01 N Standard HCl. Rosalic acid indicator was added and titrated to a pale pink end point with 0.01 N 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.

$$\text{TVBN (mg/100 g)} = \frac{14\,(300+W) \times V_1}{500} \text{ mg/100 g}$$

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

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.

Determination of Peroxide (*PV*):

Peroxide value was determined according to Egan *et al.* (1981). 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 min and filtered. For PV estimation 5 g 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 waterbath for not more than 30 sec. 30 ml of water was slowly added and then the liberated iodine was titrated with 0.1 N $Na_2S_2O_3$ with vigorous shaking until yellow colour 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 0.002 N 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, (1970). 10 g 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 min. 5 ml distillate was pipetted into a glass stoppered tube; 5 ml TBA reagent was added, stoppered, and heated in boiling waterbath for 35 min. A blank was similarly prepared using 5 ml distilled water with 5 ml 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

In *Catla catla*, TMAN content initially noted in fresh samples was 0.91mg/100 g. After one day of frozen storage, a slight increase 0.92/100 g was noted. After 3 days value dropped to 0.89 mg/100 g. After 5 days of frozen storage an increase to 0.95 mg/100 g was observed. Later TMAN content showed an increasing trend for the remaining duration of storage. The increase was slow until the completion of 90 days. Though the initial concentrations were low, TMAN levels recorded was 7.76 mg/100 g by the end of 90 days of storage. After 120 days of storage, the levels further increased to 10.96 mg/100,g which crossed the acceptable limit of 10–15 mg/100 g. After 150 days TMAN levels were 13.22 mg/100 g. At the end of storage period of 180 days the value obtained was 15.41mg/100 g (Fig. 1).

Initial TVBN levels in fresh samples of *Catla catla* was 3.11 mg/100 g. TVBN levels followed an increasing trend during the entire storage period. After completion of one day of frozen storage TVBN levels were 3.16 mg/100 g. Increase in

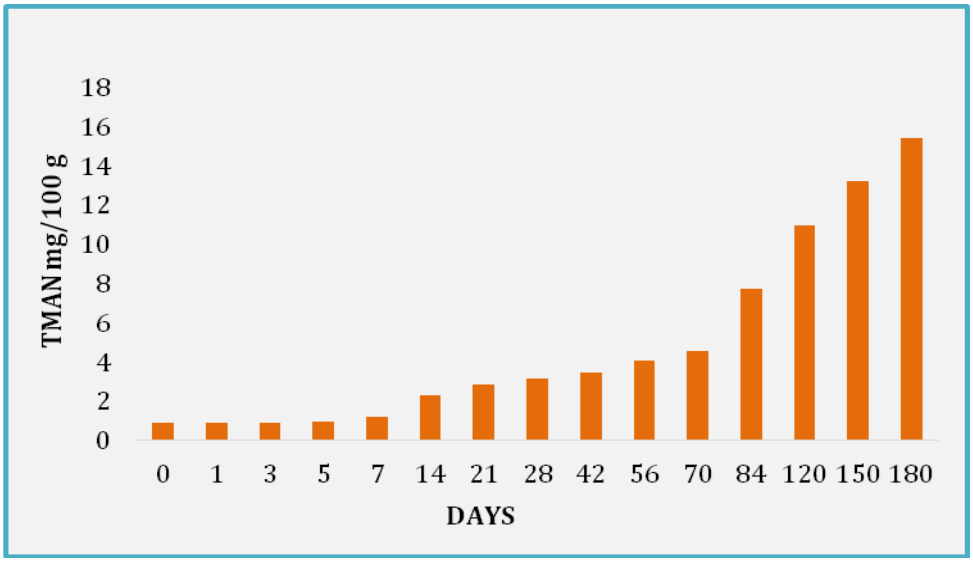

Fig. 1: Changes in TMAN mg/100 g in *Catla catla* frozen at -20°C.

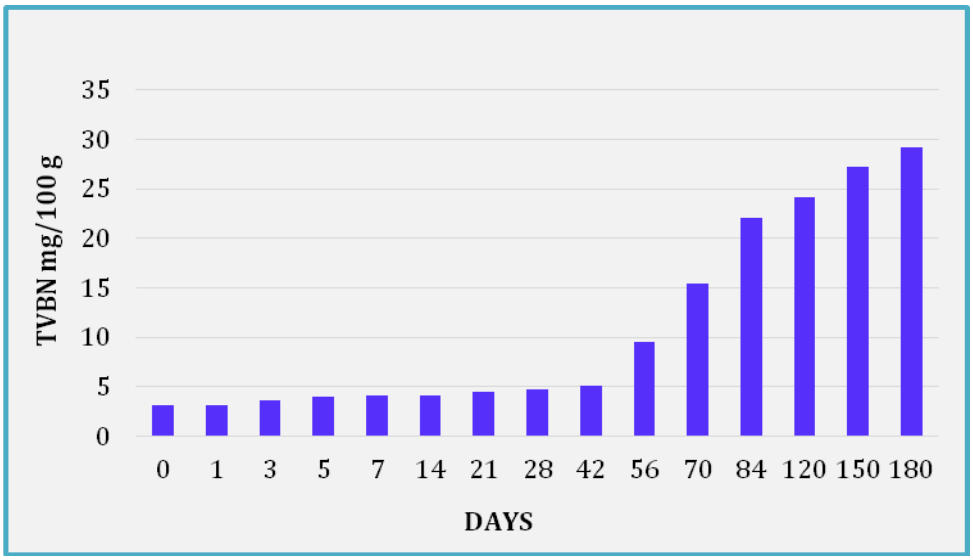

Fig. 2: Changes in TVBN mg/100 g in *Catla catla* frozen at -20°C.

TVBN level was very slow until the completion of 45 days of frozen storage. Thereafter, a value of 5.12 mg/100 g was recorded. A sharp increase to a level of 9.55 mg/100 g was recorded by the end of 60 days of storage. A rapid increase was noticed later until the end of the storage period. By the end of 180 days, the value recorded was 29.22 mg/100 g was recorded (Fig. 2).

Initial level of PV in fresh *C. catla* was 0.29 meq/kg. The value reduced to 0.28 and 0.21 meq/kg after 1 and 3 days of frozen storage, respectively. After 5 days PV reached 0.88 meq/kg. Gradual increase in PV was recorded until the completion of 120 days. PV reached 16.25 meq/kg after 120 days of storage. On further storage, decrease in PV was observed. By the end of 180 days, it was 10.36 meq/kg (Fig. 3).

TBA (Thiobarbituric acid value) in fresh *C. catla* was 0.05 mg Malonaldehyde/kg. after one day of frozen storage an increase in TBA value of 0.12 mg Malonaldehyde/kg was observed. TBA continued to increase throughout the storage

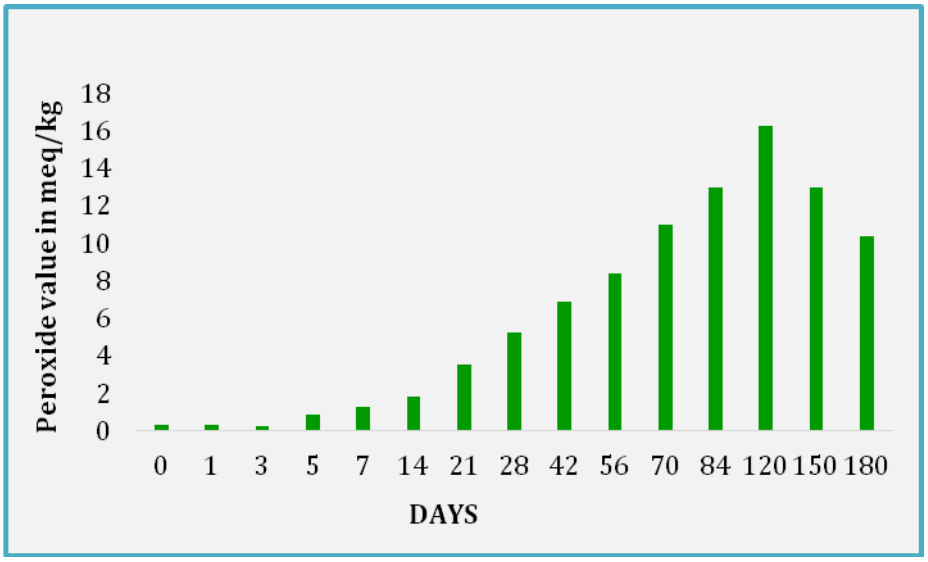

Fig. 3: Changes in Peroxide value (meq/kg) in *Catla catla* frozen at -20°C.

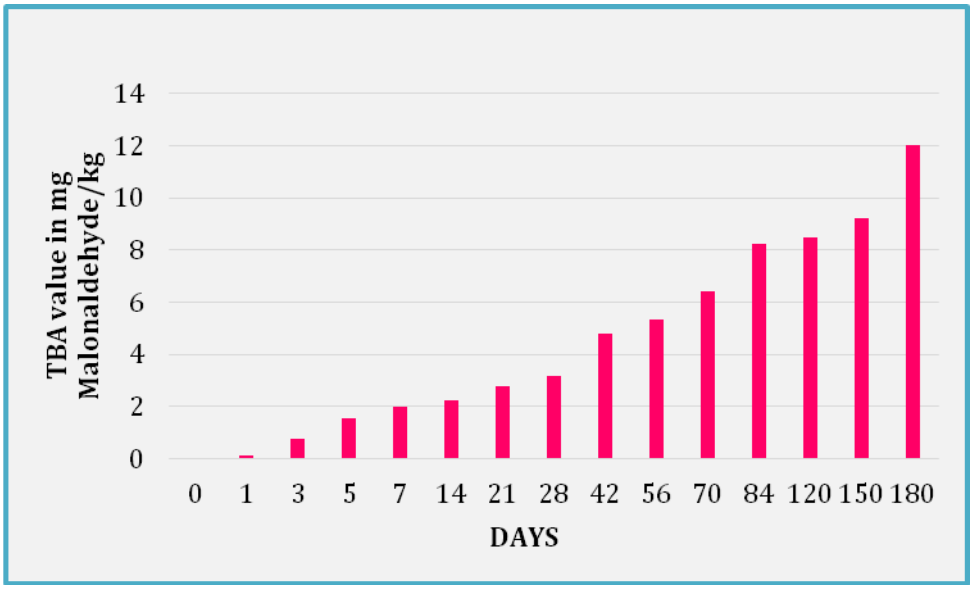

Fig. 4: Changes in Thibarbituric Acid Value (mgMA/kg) in *Catla catla* frozen at -20°C.

period. By the end of 90 days of frozen storage TBA values crossed the acceptable limit 7-8 mg Malonaldehyde/kg. By the end of storage period TBA value recorded was 12.00 mg Malonaldehyde/kg (Fig. 4).

Discussion

TMA is due to the activity of bacterial enzymatic decomposition of TMAO (Castel *et al.*, 1970; Pedrosa-Menabrito and Regenstein, 1988). According to Bligh (1971), freshwater fish cannot be classified by their TMA levels because they do not possess enough of the precursor trimethyl amine oxide (TMAO). Additionally, freshwater fish may degrade differently than marine fish. According to Balakrishnan Nair *et al*. (1971), autolytic processes rather than bacterial deterioration appeared to be the main determinant of quality in ice-caught freshwater fish. Regression analysis in the study conducted by Ali *et al*. (2008) on *Catla*, however, revealed a strong association between storage duration and

TMA readings. The TMAN values in the present study showed a slight but progressive increase as the length of the storage period increased. The levels were below the levels of acceptability as proposed by Connell (1975) upto the end of 84 days. Review of available literature showed that the studies on quality changes during frozen storage of whole *Catla catla* is very meager and hence, the present study has been performed. Azam *et al*., (2004) suggested that TMAN could be used as good indices to determine the freshness in freshwater fish samples. Thus, in the present study on freshwater fish *Catla catla* we have chosen TMAN as one of the quality indices.

In the present study the amount of TVBN in the fish was increased during the entire storage period. Kamal *et al.* (1996) also reported increase in TVBN values in *Hilsa Ilisha* stored at -20^{o}C for a period of 75 days. Ahamed *et al.* (1981) also noted a similar pattern of spoilage changes. TVBN in the meat is chiefly contributed by ammonia produced as a result of deamination of muscular proteins (Kumar *et al.*, 2013) and this may be one of the reason for increases in the present study. Tsironi *et al.* (2020) reported that TVBN was initially 6.8 and 8.4 mg N/100 g for gilt-head sea bream and sea bass fillets, frozen at -30^{o}C and increased with storage time up to approximately 20 and 23 mg N/100 g, respectively. For yellowfin tuna slices, initial TVBN was 7.6 mg N/100 g and increased with storage time up to approximately 18 mg N/100 g. Park *et al.* (2021) reported that the mackerel and croaker samples frozen at −18°C had the highest values of TBA and TVBN. A similar increase has also been observed in the present study.

Due to increasing oxidation and enzymatic hydrolysis of unsaturated fatty acids, lipid degradation is the primary factor in decreasing fatty acid shelf life (Sarma *et al*., 2000). The PV results in the present study on frozen *Catla* are in line with the earlier reports of Nuray and Ozkan (2007) that initially the PV and TBA values remained low for whole ungutted sardine. Tokur *et al.* (2006) reported that the scores did not exceed acceptable levels of filleted Trout (*Oncorhynchus mykiss*) during frozen storage (–18°C) for 12 months. In contrast, the present study showed an increase with storage time. Fish species differences could be the cause of disparities. Anchovy of adequate quality could only be kept frozen at -18°C for three months (Köse *et al*., 2001). Mazrouh (2015) reported from his studies on *Saurida* species, that during the period of frozen storage, the rate of deterioration intensified. As the storage duration lengthens, bacterial growth, protein denaturation, lipid hydrolysis, and oxidation parameters have increased. The increase in PV during low temperature storage is explained by Erickson (1997) and Sikorski and Kolakowska (2000) as a result of the presence of pro-oxidant enzymes (lipoxygenases, peroxidases) and chemical pro-oxidant molecules (hemoproteins and metal ions). According to Aubourg (1999), the PV at -30°C showed no variation over the first nine months of storage before suddenly increasing at month 12 for blue whiting. After 75 days at -20°C, the PV value of frozen oil sardine mince increased from 4.12 to 18.63 meq/kg (Verma *et al*., 1995) and from 5.53 to 16.2 meq/kg (Kamal *et al*., 1996). After four months of storage at -20°C, Losada *et al.* (2007) found that the PV and TBA values of slurry-iced sardines ranged from 1.50 to 7.57 and from 0.26 to 1.04, respectively.

Makri *et al.* (2009) noted an increase in TBA values in gilt-head sea bream up to 30 days of low temperature storage before they began to decline because the TBA reactive compounds were more likely to interact with biological components found in muscle, which caused the decline. No such decline was observed in the present study. The findings of Asgharzadeh *et al.* (2010) in silver carp were comparable. A higher anchovy oil concentration may hasten fish deterioration and keep anchovy oil from oxidising even at low temperatures, which can have detrimental effects on chemical and sensory quality as reported by Orak and Kayisoglu (2008). Jakhar *et al.* (2012) reported that total lipid content in *Catla catla* was very high and this high lipid content might have

contributed to the increase in lipid oxidation quality indices in the present study. In a study by Aubourg *et al.* (2004), Spanish horse mackerel was maintained in freezers at -80 and -20 ℃ for 12 and 5 months, respectively, after being caught. After five months of storage at each of the studied temperatures, the TBA content decreased. According to Aubourg *et al.* (2002), horse mackerel frozen at -80 °C for 2 h, then defrosted at -20 °C to reach a shelf life of 5 months and reported that the TBA values were higher in the final months of the study. Though the temperature of storage was low in the present study, a similar pattern of increase was noted. Calanche *et al.* (2019), examined whole, gutted, and filleted seabream, and also reported similar rise in TBA values as observed in the present study. According to research on herring fillets by Dang *et al.,* (2017) for 14 months at a steady temperature (between -12 °C and -10 °C) and stress settings, they found that the dark muscle is more vulnerable to lipid oxidation than the light muscle.

Karacam and Boran (1996) reported that the TBA value in anchovies stored at -18℃ increased significantly by the end of the storage period, indicating hydrolytic and oxidative changes. A similar pattern of increase in TBA value was also observed in the present study on frozen *Catla catla*. According to Bidlack *et al.* (1972), the reaction of malonaldehyde with several other muscle elements was likely what caused the fall in TBA which was observed initially in the present study also. According to Lakshmisha *et al.* (2008), fish may be consumed up to a level of 8 mg malonaldehyde/kg, with a maximum level of 5 mg malonaldehyde/kg indicating good quality for fish that has been frozen, chilled, or stored with ice. Thus, the present study shows that fish has retained the acceptable limits upto 120 days of storage in terms of lipid oxidation quality indices chosen.

Conclusion

Low temperature played a significant role in controlling TMAN production in the present study. TVBN values were found to be above the acceptable limits by the end of the storage period. PV and TBA values obtained in the present study indicate slow oxidation of lipids when compared with marine fishes.

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