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CHANGES IN SOME QUALITY INDICES IN *THUNNUS ALBACARES, SARDINELLA LONGICEPS* AND *RASTRELLIGER KANAGURTA* FROM VISAKHAPATNAM HARBOUR STORED AT -20^{O}C

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ABSTRACT
The present study has been undertaken to understand the changes that took place in the TMAN, TVBN, and total plate count during the storage of three whole (ungutted) fish species namely Thunnus albacares, Sardinella longiceps and Rastrelliger kanagurta for a period of 180 days at –20ºC. The study revealed the relatedness and distinctiveness of the quality indices chosen for all the three species chosen. Inspite of initial decreasing trend, an increase in the values of quality indices chosen is observed at different durations for different species during further periods of storage.

Keywords: *TMAN, TVBN, TPC*

INTRODUCTION
A food that is especially sensitive to oxidation and microbial degradation is fish. In general, fish has been a significant source of food for humans since it is one of the least expensive sources of protein, is very easily digested, and is rich in unsaturated fatty acids. Additionally, a significant number of other nutrients, including minerals and necessary amino acids for the synthesis of functional and structural proteins, are present in it (Mahboob *et al.,* 2015). Therefore, effective storage techniques must be used to extend the fish's shelf life and ensure its safety and quality from catch to eating. Preservation of food is essential to increase its shelf life and conserve its nutritional value, flavor and texture. Therefore, optimized methods for food preservation must prevent damage to food by microorganisms while maintaining its quality and nutritional value, (Ghaly *et al.,* 2010). The duration at which the fish may be stored depends on a number of variables, including the amount of ice used, the species of fish, the type of fish stored, and the temperature. High pressure processing, irradiation, pulsed light technology, pulsed electric field, microwave processing, radio frequency, and ultrasound are recent developments in modern fish storage technologies. The most common means of fish preservation on board continue to be chilling and freezing, notwithstanding these new technologies that have applications in fish processing. These techniques for preserving fish quality at low temperatures include refrigerated storage between 0 and 4 degrees Celsius or frozen storage between -18 and -40 degrees Celsius.

Fish chilling may involve the use of superchilling technology, which makes it less susceptible to deteriorative processes by allowing the product's modest water content (5–30%) to freeze. This technique enables the product to be transported without the need for external ice, which reduces the weight and expense of the shipment and extends the shelf life of the product compared to chilled foods. However, this technique can speed up autolytic, enzymatic, or other chemical reactions. The biochemical changes in the fish meat are also related to stress, weariness, freshness, freezing speed, storage temperature, temperature fluctuations, drying and thawing conditions have an impact on the meat components of fish and shellfish. During processing frozen fish meat, these elements should be taken into account since they affect the quality changes (Nakazawa and Okazaki, 2020).

Freshness loss from fish deterioration starts right away after catch (postmortem modifications), which justifies the significance of handling fish carefully from capture through processing/commercialization in preserving the quality of this product. Such care will determine the enzymatic, bacterial, and oxidative activity, whose pace and, consequently, the degradation process, depends on preservation techniques used as well as the fish species, size, capture method, temperature, storage type, and physical state prior to death (Sores and Goncalves, 2012).

The microorganisms, present are in the latency phase (also known as lag or delay) as they adapt to the new environment (dead fish) by adjusting their development and survival mechanisms in response to the lack of oxygen after the fish dies and is kept in ice for 5 to 6 days. After adjusting to the new environment, bacteria begin to multiply exponentially, and the start of the logarithmic phase (also known as log) is the primary factor in fish degrading after the sixth day under ice. In actuality, bacterial action, as opposed to autolytic action, which is also in charge of quality loss, is the principal cause of fish degeneration. This fact is supported by the fact that the digestive tract, which can be eviscerated, is where autolysis starts to occur. Actually, when evisceration is carried out under sanitary conditions, the action of digestive enzymes and the migration of bacteria from the intestinal flora to fish meat are prohibited (Jay *et al.,* 2005; Gill and Barbosa, 2011).

Volatile substances like ammonia (NH_3) and trimethylamine (TMA), 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 that has deteriorated. Trimethylamine oxide (TMAO), which is naturally produced by marine organisms to enable osmotic management, is reduced by bacteria to form TMA. In the absence of oxygen and ice preservation, bacterial action causes the conversion of TMAO to TMA. Additionally, TMA makes from 1 to 5% (in dry weight) of the muscle tissue of fresh fish, mostly from maritime settings (varies by species, catch area, size, and physical condition). TMA, along with ammonia and other volatile amines, is one of the primary substances evaluated by total volatile nitrogen (TVBN) (Huss, 1995; Huang *et al.,* 2020). When determining TVBN to evaluate fish freshness, all volatile nitrogen compounds in the sample are taken into account, particularly the amounts of NH_3, TMA, and dimethylamine acid (DMA), which rise during the degradation process (Senapati and Sahu, 2020). This time-consuming technique is utilised in research, but it is not frequently used in the fish industry due to the requirement for expensive laboratory equipment and skilled operators (Ólafsdóttir *et al.*, 1997).

One of the key factors contributing to food spoilage or seafood contamination is bacterial development in frozen fish. Therefore, the microbiological examination of frozen fish samples and the ingredients used in fish processing (water and ice) serves as the indication for determining the quality of the fish. In microbiological analysis, TPC is used to estimate the populations of bacteria in order to define the freshness of fish, cleanliness, and/or assess the likelihood of the existence of organisms that are crucial to public health (Huss, 1994).

The TPC is of significant consideration in assessing the microbial quality of food products, according to the International Commission on Microbiological Specifications for Foods (ICMSF) (International Commission on Microbiological Specifications for Foods, 1996). It also serves as a general indicator of the level of microbial contamination of foods. Food quality, shelf life, and safety are all determined by estimating the quantity of microorganisms present (Dalgaard, 2000).

The present study aims at studying the chemical quality changes in three commercially important fishes landed abundantly at Visakhapatnam harbor across different durations of storage at -20^{o}C without evisceration.

MATERIALS AND METHODS

The present study includes studies on TPC, TMAN and TVBN measurements of three marine species namely *Thunnus albacares, Rastrelliger kanagurta* and *Sardinella longiceps.* 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 samplesof 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 -20ºC. 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 Basic Nitrogen (TVBN) and Trimethyamine Nitrogen (TMAN)

TVBN and TMAN in the present study were determined according to Egan *et al.,* (1981) 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 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 15ml 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.

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

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

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 up to 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 37ºC for 48 hours. After 48 hours of incubation, colonies were counted.

RESULTS

Initial levels of TMAN in fresh *T. albacares* recorded was 2.81mg/100gms. After 1 and 3 days of frozen storage TMAN decreased to 2.55 and 2.20 mg/100gms respectively. On further storage increase in TMAN levels was recorded. After 7 days of frozen storage it reached 3.90 mg/100gms. After 28 days of storage the value reached 8.00 mg/100gms. TMAN levels recorded after 45 and 60 days of storage with 10.75 and 12.66 mg/100gms respectively were found to be within the acceptable limits (10-15 mg/100gms). Much higher values were recorded during the remaining storage period. After 75 days of storage, TMAN levels reached 15.75 mg/100gms which were above the acceptable limit mentioned. After 90, 120 and 150days of storage, TMAN values recorded were 18.00, 21.11 and 25.25 mg/100gms respectively. At the end of 180 days of storage, TMAN reached 27.00 mg/100gms.

TMAN levels recorded in fresh *S. longiceps* was 2.62 mg/100gms. An increase was observed throughout the storage period. A slight increase was observed after one day of frozen storage. Increasing trend was gradual until 7 days of storage. Later, the levels rose to 3.30 mg/100gms. After 14, 21, 28 and 42 days of storage TMAN levels were 3.75, 4.06, 4.85 and 5.50 mg/100gms respectively. After 70 day the value reached 11.10mg per 100gms. By the end of storage period the value reached 17.48 mg/100gms which was found to be above the acceptable limit of 10-15 mg/100gms. After 150 days of storage, high levels of 26.24 mg/100gms were recorded. At the end of 180 days levels reached to 31.36mg/100gms.

Initial TMAN levels in *R. kanagurta* in fresh condition were recorded as 2.92 mg/100gms. After 1 day of frozen storage TMA N levels decreased to 2.90 mg/100gms. But after 5 days the value increased to 3.33 mg/100gm. After 28 and 56 days TMAN levels recorded were 4.25 and 8.90 mg/100gms respectively and were found to be below the acceptable limit. On further storage TMAN levels gradually increased and reached 23.42 mg/100gms by the end of the storage period.

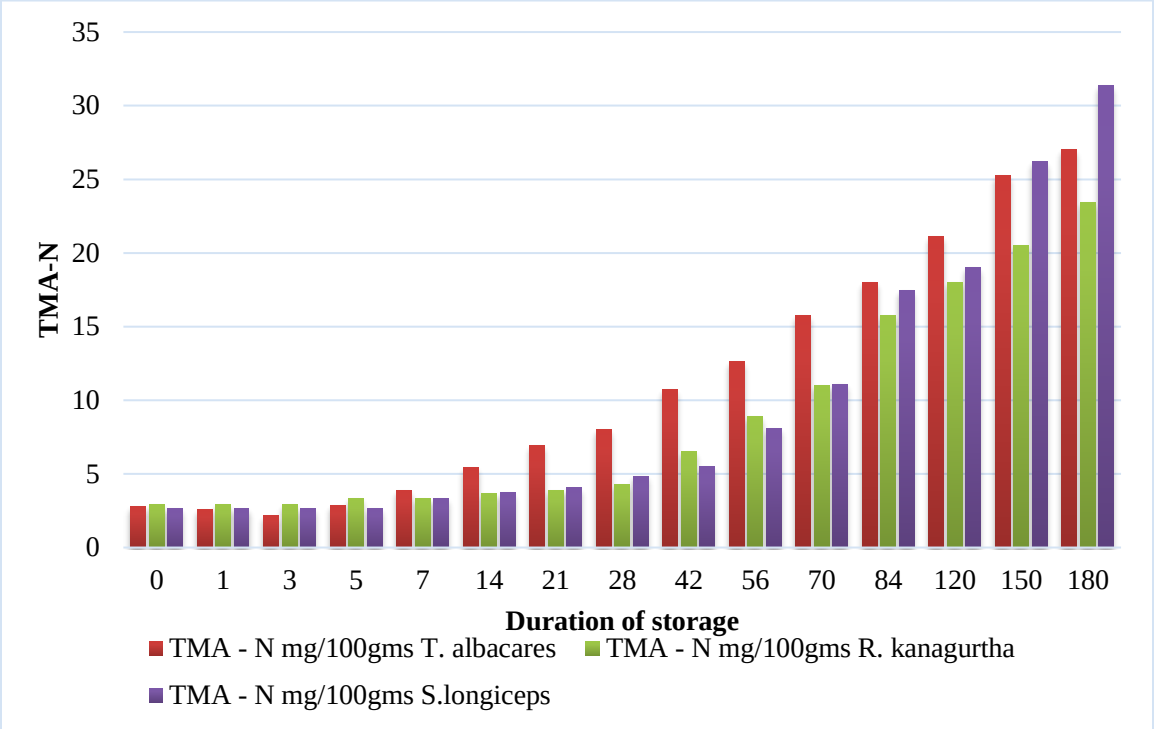

Figure 1. Variations in TMAN content during the storage period

Initial TVBN content was found to be 11.36 mg/100gms in *T. albacares*. After 1 day of frozen storage TVBN content increased to 11.40 mg/100gms. A gradual increase was noticed upto the end of the storage period. After 84 days of storage TVBN reached 27.75 mg/100gms. After 120, 150 and 180 days TVBN content reached 31.00, 36.75 and 39.23 mg/100gms respectively.

TVBN content recorded in fresh *S. longiceps* was 13.07 mg/100gms. TVBN content gradually increased upto the end of the storage period. TVBN content was found to be below the acceptable limits after 70 days of storage with a recorded value of 24.00 mg/100gms. On further storage, TVBN content exceeded the acceptable limits. By the end of 180 days TVBN reached 42.44 mg/100gms.

Initial TVBN content recorded in fresh *R. kanagurta* recorded was 9.38 mg/100gms which gradually increased during frozen storage. After 21, 42, 70 and 84 days of storage the values of 11.75, 16.00, 21.25 and 24.00 mg/100gms were recorded respectively. After 150 days the value increased to 28.20 mg/100gms which was below the acceptable limit but at the end of the storage period a value of 32.90 mg/100gms was recorded which exceeded the acceptable limits.

Total Place count (TPC) in fresh *T. albacares* was $25x10^3$cfu/gms. During frozen storage it gradually decreased and reached $19x10^3$cfu/gms after 21 days. Further storage resulted in an increase to $77x10^3$cfu/gms after 180 days of storage. High Correlation value of 0.85 was recorded between TPC and TMAN in *T. albacares.*

TPC in fresh *S. longiceps* was 30 $x10^3$cfu/gms. Frozen storage resulted in a decrease to 22 $x10^3$cfu/gms that was recorded after 28 days of storage. Upon further storage an increase in the count was observed and that continued until the end of storage. TPC reached $50x10^3$cfu/gms after 180 days of frozen storage. High Correlation value of 0.88 was recorded between TPC and TMAN in *S. longiceps.*

TPC in fresh *R. kanagurta* was 87 $x10^3$cfu/gms and that was very high when comparedto counts in other marine fishes taken for the present study. After one day of frozen storage, TPC increased to 88 $x10^3$cfu/gms. The value decreased to 83 $x10^3$cfu/gms after 14 days of storage. Further frozen storage led to an increase in the count. Finally at the end of 180 days TPC reached 95 $x10^3$cfu/gms. High Correlation value of 0.892 was recorded between TPC and TMAN in *R. kanagurta.*

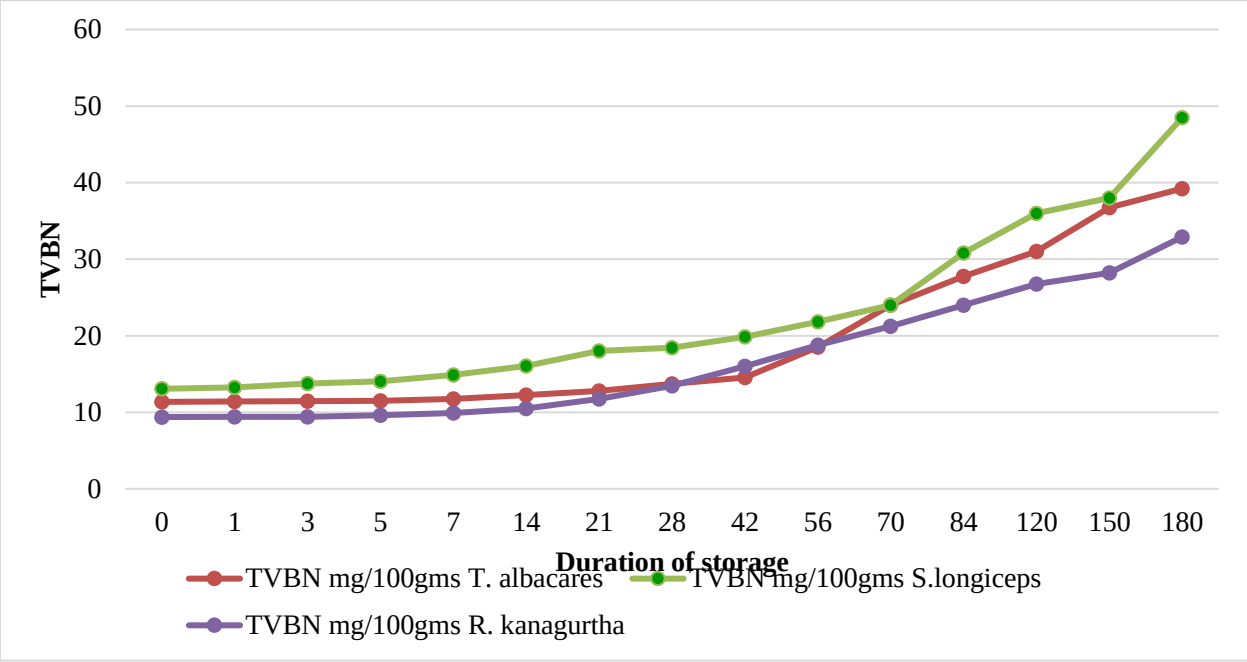

Figure 2. Variations in TVBN content during the storage period

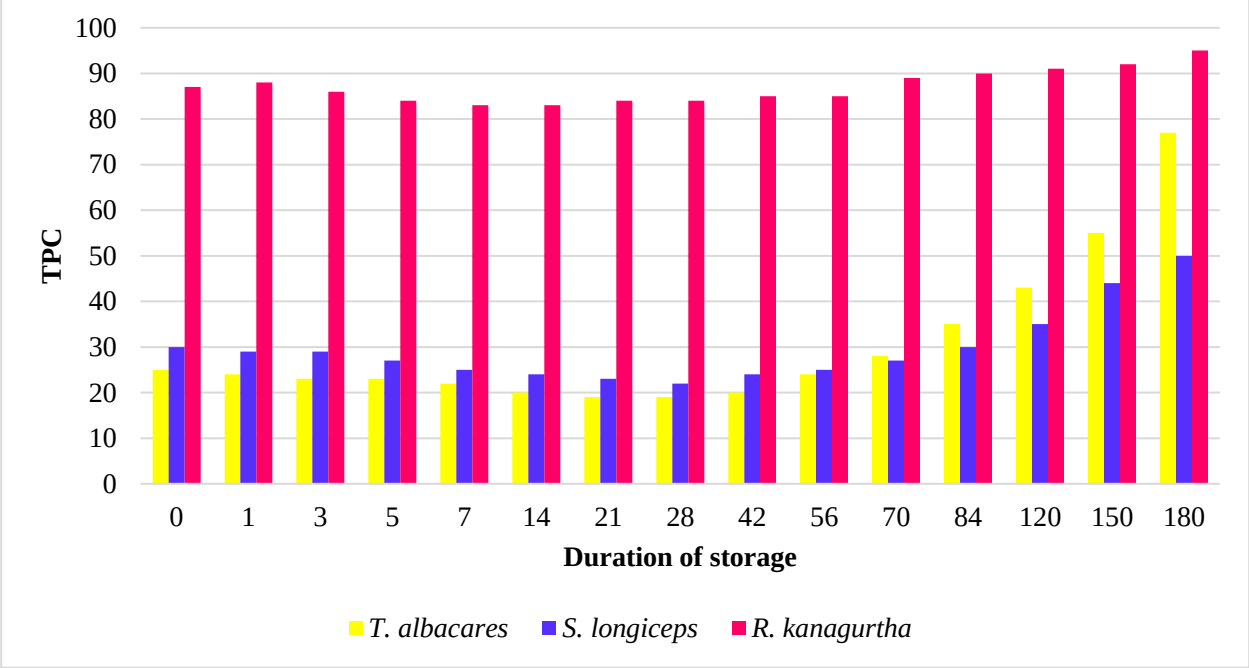

Figure 3. Variations in TPC during the storage period

DISCUSSION

Shellfish and marine fish contain trimethylamine oxide (TMAO). According to Hebard *et al.*, (1982) and Sotelo and Rehbein (2000), tissue concentration varies greatly depending on the species, their habitats, and

the seasons. According to Sotelo and Rehbein (2000) and Seibel and Walsh (2002), it also has a significant impact on the physiological function of living fish and the postharvest deterioration of seafood products.
Trimethylamine (TMA), is one of the end products of the post-mortem TMAO degradation, which occurs via both non-enzymatic and enzymatic pathways (Sotelo and Rehbein 2000). These compounds contribute to quality losses in both chilled and frozen fish products during storage. At cold temperatures, the breakdown of TMAO results in the production of volatile amines, mostly TMA (which is formed by bacterial TMAO-reductase enzymes) but also other substances, including trace amounts of DMA (Mizuguchi *et al.,* 2011). The characteristic "fishy" odour associated with fish rotting is brought on by the presence of TMA and DMA. Moreover, TMA along with ammonia and other volatile amines is one of the primary components measured by total volatile basicnitrogen (TVBN) (Huang *et al.,* 2020).
Fish spoilage is frequently determined by measuring the concentrations of volatile amines in fish tissue, which are typically quantified as TMA-N, DMA-N, or total volatile basic nitrogen (TVBN), which is a combination of TMA, DMA, ammonia, and other amines (Etienne and Nantes, 2005). TMAN concentrations in really fresh fish are typically less than 2 mg/100 g of tissue. In cold water Atlantic cod (*Gadus morhua*), TMAN has been used to detect spoilage. Good-quality chilled fish often contain less than 1.5 mg TMAN/100 g, and 10-15 mg TMAN/100 g is typically recognised as the limit of tolerance for human eating (Huss, 1988).
Jack mackerel (*Trachurus novaezelandiae*) had a shelf life of seven days when stored on ice, according to Ryder *et al.,* (1984), and the sensory panel disapproved of the fish on day nine. Although the bacterial counts in the study had exceeded the upper limit of the International Commission on Microbiological Specifications for Food (ICMSF, 1978) guideline of 7 log10 cfu/g by the Day 9, the TMAN concentration was approximately 1.4 mg/100 g at Day 9 and did not exceed the guideline of 2 mg/100 g until Day 12. It is confirmed that TMA-reducing bacteria exist in the fish spoilage flora of New Zealand fish since the TMA concentration rose over time (Summers *et al.,* 2017).
Indian Mackerel fish held for a duration of 0–3 days in both storage systems had the highest level of freshness and the lowest TVBN and TMA levels. However, it was shown that the quality of fish was inversely related to temperature and time. The findings imply that regulating time and temperature during processing and storage is helpful to preserve consistent freshness and quality of Indian Mackerel for longer periods of time under refrigerated conditions (Chudasama *et al.,* 2018). During the storage period, the TMA values for chilled mackerel considerably increased, whereas the values for frozen mackerel steadily decreased (Emilia *et al.,* 2016). Production of TMAN is slow at the start of chemical change in fish and gradually increases exponentially in pace after few days in chilled storage (Onyeanula *et al.,* 2022). In the present study though there was a slight drop in the TMAN content in *R. kanagurta* and *T. albacares*, a gradual increase was observed during later stages of frozen storage and this may be due to the presence of gut which serves as reservoir of microorganisms. Hoyles *et al.,* (2018), reported reduction of TMAO by the gut microbiota (predominantly *Enterobacteriaceae*) to TMA and also stated that Clostridia (*Sensu stricto*), bifidobacteria, and coriobacteria were significantly correlated with TMA production in the mixed fermentation system.TMAO does not serve as a substrate for bacterial catabolism but is instead important as an alternative electron acceptor enabling some bacteria to exhibit rapid growth under anaerobic conditions. The product of this reaction is trimethylamine (TMA), which is an important component of the odor of stored fish, giving the typical fishy smell (Leisner and Gram, 2014). The trend for *S. longiceps* in the present study was different and coincided with the study of Leelapongwattana, (2005), the speed of TMAO degradation depends on many factors, including storage temperature, species, muscle integrity and reducing conditions (Parkin and Hultin, 1982).
Fish quality is frequently assessed using Total Volatile Base Nitrogen (TVBN) and Trimethylamine (TMA), which are excellent indicators of the trimethylamine, dimethylamine, ammonia, and other basic nitrogenous compounds produced by spoilage bacteria, autolytic enzyme at frozen temperatures, deamination of amino-acids, and nucleotide catabolites that are linked to fish spoilage (Emelia *et al.,* 2016). TVBN includes measurement of trimethylamine, dimethylamine, ammonia and other volatile compounds associated with fish spoilage, which increases as spoilage progresses. In the present study, TVBN values for all the three

species showed an increase which is coinciding with similar reports of Park *et al.*,(2021) which states that a steady increase in TVBN proportional to the storage period and temperature was observed in mackerel and croaker preserved under frozen storage. The TVBN of chilled mackerel increased significantly during storage time, but the values for frozen mackerel declined slowly (Emelia *et al.*,2016). According to Raouf *et al.,* (2009), this trend was due to the denaturation of muscle protein by surviving microorganisms. On the other hand, the decrement of TVBN values in frozen mackerel may due to the fact that low temperature caused the bacteria become inactive and hence slower the rate of deterioration. The acceptable limit for TVBN that had been stipulated by literatures (Orak, and Kayisoglu, 2008); Ojagh *et al.,* (2010) was 35 mgN/100g sample. After six months of storage at -18 ^{o}C, Spanish mackerel (*Scomberomorus commersoni*) samples examined by Nazemroaya *et al.,* (2011) revealed a cumulative rise of 15 mg TVB-N/100g. While the TVBN total increment in samples of a lean fish, the sea bass (*Dicentrarchus labrax*), measured by Zyurt, *et al.,* (2007), was 2.87 mg/100 g after nine months of frozen storage at -18 ^{o}C.

Ibrahim *et al.,* (2008) reported that the TVB-N of raw tilapia fish was 11.02 mg/mg. The values of TVB-N increased after 60 days from frozen storage, reached to 19.32 mg/100 g. According to Orak *et al.,* (2008) and El-Sherif *et al.,* (2018), tilapia fish contained 14.31 mg of total volatile basic nitrogen (TVB-N) per 100 g of meat at the point of freezing. These values increased as the frozen storage duration progressed, reaching 18.08, 21.2, and 29.31 mg/100 gm after 60, 120, and 180 days of frozen storage at -18^{o}C, respectively. The activity of proteolytic enzymes of microbial origin, which break down nitrogenous compounds, may be responsible for this increase.

According to Shimelis and Mekonnen, (2009) 2.57 x 106 cfu/g of bacteria were found to be the initial overall bacterial load in tilapia. The initial sample's elevated microbial load may have resulted from improper handling techniques or unsanitary conditions at the landing centre. The overall bacterial load in tilapia fish fell by about two logs (from around 106 to 104 cfu/g) on day 60 of storage, which may have been caused by the cold shock during storage under freezing conditions. For cuttlefish and crab, the same findings were reported by Thailambal (2007). The microbial load began to rise, though, starting on the 75th day of storage and beyond. The sudden rise in TVB-N could be a sign that bacterial action has spoiled the fish. Although it rose linearly with storage time, the TVB-N content, after being preserved for 90 days, it has not attained the maximum concentration of 35 mg N/100 g, as reported by Shimelis and Mekonnen, (2009).

The removal of the stomach and gut enzymes and bacteria may have decreased autolytic and bacterial activity, resulting in a lower TVB-N value initially in dressed fish. This may have extended the shelf life of dressed pabda over whole pabda according to Pal *et al.,* (2019). For refrigerated storage of red mullet (*Mullus barbatus*), Ozyurt *et al.,* (2009) reported an increase in TVB-N value that was comparable to an increase in TVBN value. According to Mazorra *et al.,* (2000), this rise may be explained by the muscle's storage-related synthesis of ammonia and other volatile amines.

According to Alkuraieef *et al.,* (2022), a study was conducted on the chemical and microbiological quality of imported chilled, frozen, and locally cultured fish in Saudi Arabian markets. They have reported that all of the locally cultured fish samples were under the limit of total volatile basic nitrogen TVB-N (30 mg/100 g) for fish, while 22.73% and 7.16% of frozen imported and chilled imported fish respectively were over TVB-N limit. With increasing frozen storage duration (up to 60 days), the total volatile base-nitrogen (TVB-N) value grew significantly ($p>0.05$), but it did not exceed the undesirable level (35 mg N/100g), which is comparable to the EC recommendation (2003) as reported by Chakma *et al.,* (2022) in frozen Skip Jack Tuna. Similar to the findings of Seong *et al.,* (2017), this growing trend may have resulted from increased protein breakdown by muscle enzymatic activity.

TPC for the three species chosen for the present study gradually increased after 56 days of storage at –20^{o}C. This in consonance with the reports of Bao *et al.,* (2007) who reported a faster microbial growth in chilled than in superchilled samples of Arctic Charr fillet under the effect of dry ice and superchilling. Obemeata *et al.,* (2011) showed an increase in bacterial count from 7.9×10^{3} to 7.6×10^{7}cfu/g when Tilapia fish was stored at 4^{0}C and from $5.4\times10^{1}$to 7.9×10^{3}cfu/g when Tilapia was stored at -18^{0}C. Furthermore, chemical and microbial contamination in some fish samples exceeded the recommended permissible levels. Microbial analysis results of the study of Alkuraieef *et al.,* (2022) clearly showed that the mean TPC values in the

examined fish samples varied, with 2.67×10^4 CFU/g, 1.45×10^5 CFU/g, and 4.42×10^7 CFU/g for locally cultured, frozen imported, and chilled imported fish, respectively. A significant difference ($P < 0.05$) was shown between frozen imported fish and chilled imported fish and locally cultured fish, as well as between chilled imported fish and locally cultured fish.

The International Commission on Microbiological Specifications for Foods (ICMSF) stated that the APC is an important factor for evaluation of microbial quality assessment in food products and is an indicator of the overall degree of microbial contamination of foods (International Commission on Microbiological Specifications for Foods, 1996**).** Estimating the numbers of microorganisms is used to determination food safety, shelf life, and quality as stated by Dalgaard, (2000). Fresh mrigal fish had an overall plate count of 8.36 x 10^2cfu/gram of flesh. The overall bacterial count during the freezing and frozen storage study increased up to the 30^{th} day at 15.00 x 10^2cfu/gram and subsequently dropped until the end of the frozen storage period 2 at 24 x 10^2cfu/gram (Kumar *et al.,* 2021).

CONCLUSION

The rate of deterioration was accelerated during frozen storage time in the present study. The TPC, TMAN, TVBN values increased as the storage period increase. Quality of the ungutted, whole fish may be enhanced by preserving at temperatures lower than -20^{o}C. The degradation of the fish begins at the time of capture, making this a vital phase in determining the product's quality and safety as food. So, the sort of product that will be advertised and afterwards consumed by the client is greatly influenced by the refrigerated and/or frozen storage conditions.

CONFLICT OF INTEREST

None.

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