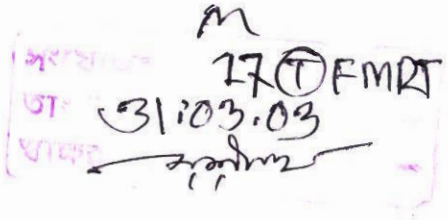
Shelf Life of Tilapia (*Tilapia nilotica*) and Bhetki (*Lates calcarifer*) Stored in Ice and At Ambient Temperature



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Shelf Life of Tilapia (*Tilapia nilotica*) and Bhetki (*Lates calcarifer*) Stored in Ice and At Ambient Temperature

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DECLARATION

The results submitted in this thesis are entirely of the candidate's own investigations and no part of the results has been accepted for any degree, nor it is being concurrently submitted for any degree

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TO

My Younger Sister

RASHNA SHARMIN RAZZAQUE (RIPA)

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ABSTRACT

Investigations on quality changes of *Tilapia nilotica* and *Lates calcarifer* were carried out. Parameters assessed were organoleptic, biochemical (TVB-N, TMA-N, TMAO-N and pH) and microbiological methods (TVC). The nature of spoilage were studied during iced storage for 25 days and at ambient temperature for 24 hours. Insulated ice boxes and bamboo baskets covered with banana leaves were used . The rate of spoilage was found to be slower in *Lates calcarifer* than *Tilapia nilotica* during storage in ice and at ambient temperature. In iced storage study, the rejection time of *Tilapia nilotica* was between 10 and 13 days as judged by taste panellists. During this period, the value of TVB-N, TMA-N, TMAO-N, pH and TVC ranged between 20.64±0.10 to 28.03±1.42, 2.52±0.22 to 3.01±0.13, 1.98±0.06 to 2.23±0.10 mg-N/100g , 6.97±0.10 to 7.05±0.11 and $\log_{10}$6.90±0.05 to $\log_{10}$7.72±0.05 cfu/gm. While the shelf life of *Lates calcarifer* was found to lie between 18 to 19 days as judged organoleptically. Within time, the value of TVB-N, TMA-N, TMAO-N, pH and TVC varied between 32.90±1.88 to 76.51±3.07, 9.56±1.42 to 26.05±2.6, 24.45±2.42 to 26.45±0.53 mg-N/100g, 7.03±0.14 to 7.21±0.16 and $\log_{10}$6.77±0.04 to $\log_{10}$7.91±0.08 cfu/gm. At ambient temperature, the keeping time of *Tilapia nilotica* and *Lates calcarifer* were 10 – 17 and 17 – 20 hours respectively. On the 17th hour in tilapia, the muscle pH, TVB-N, TMA-N and TVC values were 6.86±0.08, 48.80±0.24 mg-N/100g, 2.27±0.07 mg-N/100g and $\log_{10}$6.94±0.05 cfu/gm respectively. TAMO-N at this time could not be detected. However, TVB-N, TMA-N, TMAO-N, pH and TVC were 109.54±2.96, 40.75±2.50, 17.98±0.62 (mg-N/100g), 7.23±0.05 and $\log_{10}$7.87±0.56 cfu/gm on the 20th hour in bhetki. A comparative study made on the quality changes of *Tilapia nilotica* and *Lates calcarifer* in iced storage and storage at ambient temperature, showed that the pattern of spoilage rates or quality changes were different between the two species. *Lates calcarifer* had better keeping quality than *Tilapia nilotica* in both the storage conditions. Proximate composition of the two species were found to vary between different anatomical portions, sizes and also in different sexes. The protein content was higher in all the anatomical portions in *Lates calcarifer* compared to *Tilapia nilotica*. Lipid and Ash content was found to have almost similar values in both the species. While moisture was higher in *Tilapia nilotica* than *Lates calcarifer*. Large size fish had higher protein and lipid content in *Lates calcarifer* than *Tilapia nilotica*. Ash percentage did not vary much between the sizes and species. Moisture content for all sizes in *Tilapia nilotica* were higher. As with the other result, protein was higher in male of *Lates calcarifer*. Similar result was also observed for lipid. Ash content did not vary between the species and sexes. While moisture content was higher in both sexes.

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1 INTRODUCTION

Fish inhabiting in rivers, lakes, ponds, estuaries and seas are a treasured resource both in terms of their utility as food and as materials for scientific study. It constitute a cheap, self sustaining resource, which through appropriate management, can yield high returns in terms of proteins. Fish occupy a significant position in the socioeconomic fabric of the country by providing not only nutritious food but also income and employment opportunities.

From ancient times, fishing has been a major source of food for humanity and a provider of employment and economic benefits to those engaged in this activity. The wealth of aquatic resources was assumed to be an unlimited gift of nature. However, with increasing knowledge and the dynamic development of fisheries after the second world war, this myth has faded. In face of the realisation that aquatic resources, although renewable are not infinite and need to be properly managed, if their contribution to the nutritional, economic and social well- being of the growing world's population is to be sustained' (FAO, 1996).

Over the past two or three decades, fisheries issues have emerged from being an obscure sectoral concern or primarily welfare consideration for a few coastal people to an important growth sector having a significant role in economic development and food security in many developing countries.

World fisheries has undergone rapid changes during the last decades. New technologies, creation of Exclusive Economic Zone (EEZ), the 1982 UN Convention of the Law of the Sea (UNCLOS) and other development have brought about drastic changes in the fisheries management and enhanced access and significant expansion of effort and production (Ahmed *et. al.* 1999).

The world supply of fish rose steadily to 112.3 million ton in 1995 from only 20 million ton in the early 1950's (FAO, 1996). Of the total world production of 112.3 million ton in 1995, 90.70 million ton (81%) was from marine environment and the remaining 21.60

million ton (19%) from inland water (FAO, 1997).

Fish as food recently been estimated to account for 19% of animal protein and 4% of total protein supply (Alexandratos, 1995). Globally, fish consumption per capita has grown only modestly over the past quarter century from about 10.5 kg in 1970 to 13.4 kg in 1994 (Westlund, 1995).

About 100 million people are partly or wholly economically dependent upon fish. Most of them are relatively poor people in the developing countries. Fish is also an important source of foreign exchange earning. Net export of fishery products by developing countries were more than 16 billion in 1994. The share of developing countries in the fish trade was 51% (Alexandratos, 1995).

The role of fisheries in the national income, employment, health, nutrition and foreign exchange earning is being increasingly realised in the present days (Shah, 1998). The same would continue to be reckoned at great prudence in the future. This is due to present scenario of increased demands and intersectoral conflicts in resource utilisation and exploitation.

Fish accounts 63% of the country's animal protein supply and 3.4% of its gross domestic product (GDP). The role of fishery in earning foreign exchange is also commendable. They account for 6.28% of the country's total foreign exchange earning (1999-2000). It has been reported that 12. 8 percent of the population directly or indirectly depends upon fishing and its ancillary industries for their livelihood (DOF, 2001).

It has been well established through investigations that, fish and fishery products are very rich in nutritional properties. Muscle of fish is largely composed of protein of the globulin and albumin classes. About 80% to 90% of fish protein is digestible and contain all necessary dietary amino acids (Nilson, 1946; Dea and Tarr, 1949). The internal organs and the muscle contain all of the vitamins of the B-complex (Tarr, 1954; Guha, 1962). Fish oil is the richest known sources of vitamin A and D. It has the known distinction of possessing the highest calorie density of any foodstuff. Fish oil generally

contains high proportion of polyunsaturated fatty acids, which play an important role in reducing the cholesterol level in the human blood (Roa, *et. al.*, 1977).

Fish is therefore recognised as an important food item for human consumption throughout the world. Fish is the main source of animal protein in the diet of the people of Bangladesh. More than 63% of the animal protein in Bangladesh comes from fish (DOF, 2001).

Bangladesh is an agricultural dependent country with enormous fisheries potential. It is a land of water that is a storehouse of varieties of fish resources. The country has vast area of water bodies which includes river, pond, beel, baor, lake etc. with large bodies of brackish water. The country has about 49,20, 316 ha. of inland water of which about 28, 32, 792 ha. remains under floodwater for a period of 2 to 6 months every year (DOF, 2001). This flooded area is breeding and feeding paradise for various species of fish and considerable quantity of fish are caught from these area. The country has a 480 km. long coastline and approximately one million ha. of territorial water (Karim, 1978). The Exclusive Economic Zone of Bangladesh extends 320 km out of the sea from the coastline.

At present the total requirement of fish in Bangladesh is about 23,00,000 metric ton, but the present production is 16,61,151 metric ton of which about 54,00,000 metric tons are marine. Fish consumption per capita in Bangladesh is about 12.04 kg (DOF, 2001). The catch is almost entirely consumed at home; only very little portion is made available for export. Even then, it is one of the major exportable item of the country.

During the last fiscal year (1999-2000), Bangladesh has exported 39,391metric ton fish and fisheries products and earned 1811.56 crore taka (DOF, 2001). Thus, it is clearly evident that fish and fishery products play an important role in the national economy and also in the countries occupational life.

In Bangladesh, fishermen do not get proper return of their catch because of qualitative and quantitative loss. This is because fish is highly perishable commodity and spoils

rapidly. Losses can be classified as a quantitative or qualitative. Quantitative losses include fish which are disposed of because of low commercial value while qualitative losses occur through spoilage or insect attack. The two largest sources of quantitative losses accounted to 6% of the total marine catch and 2% of the inland catch (Coulter and Disney, 1988).

Qualitative losses consist of losses in commercial value, but not in physical biomass, through loss of quality. An estimate provided overall qualitative losses of Tk. 3,550 million (US$ 117 million) in 1986. Losses due to downgrading of fresh to dried fish account to 52% (Coulter and Disney, 1988).

Fresh fish trade in Bangladesh faced many serious problems because of the perishable nature of fish. There are wide variations in the supply of fresh fish in the country causing fluctuation in the prices. During season of abundance, prices of fish drops sharply. On the other hand, where fish are scarce, the prices are high. Appropriate preservation of fresh fish by ice would extend their keeping quality; help level out shortage and excesses thereby stabilising prices.

Preservation is particularly necessary during the period of abundance when all the fishes are not possible to be consumed. There is no efficient system for handling and transportation. By the application of appropriate preservation techniques, fish can be made usable during the period of scarcity. This also helps in maintaining an even nutritional status throughout the year. In some areas fresh fish are abundance during the monsoon. Thus, for solving regional and seasonal fluctuation of supply, preservation of fish is essential. Fluctuation of price may also be controlled in this way. A good preservation technique is very essential for the purpose of exporting fish and fisheries product to earn foreign currency. This is also to eliminate or to minimise the wastage of this perishable commodity. Considering the above facts it may be concluded that proper preservation method is very helpful for fish culturists, producers, fishermen, processors, traders and consumers.

With the increasing population pressure in Bangladesh, the demand of fish and fishery products is increasing day by day. Flesh of fish is one of the highly perishable and requires proper handling, transportation and preservation to prevent qualitative and quantitative loss. Aims of good preservation techniques are to (a) maintain quality of the product (b) minimise wastage and (c) maintain constant and uniform supply of fish to the consumers at a responsible price throughout the year.

In developing world, like Bangladesh, there is a need to increase food production and preservation because of demanding and ever increasing human population. But it is only possible by proper utilisation of natural resources. The utilisation or study of natural resources or habitat resources is by no means a complicated effort mainly in a country like ours where a large cross-section of people are engaging there livelihood on fisheries. Therefore, any research work on fish and fisheries should be encouraged.

Preservation of fish products for human consumption is not a simple task. Various factor contribute to the spoilage of fish and deterioration in the quality of the products.

From very early times, fish has been preserved by drying, salting, smoking or combination of these techniques. A major portion of the world catch is still being preserved by these traditional techniques. But with the raising living standard, the demand for such crude products has been falling steadily. As such, preference for fish that has retained the flavour, odour, appearance and texture of the freshly caught fish has been increasing to a great extent (Anon, 1988). Among various methods of preservation, iced storage is considered to be one of the most efficient short term storage method which retain the original characteristics of the fresh fish without much change (Curran and Disney, 1979; Lima dos Santos, 1981 and Howgate, 1985).

The rate of deteriorative changes are dependent on the storage temperature and length of storage time. There are no regular and definite patterns of degradation for iced stored fish. It varies from species to species. This is due to the variation in the composition of the muscle of different species, size of the species and maturity of the species (Clucas 1985).

In landing centers, fishermen usually use bamboo basket, wooden box insulated with hogla mat or similar materials for packing fish with ice during transportation and distribution while in other circumstances fishermen also use various kinds of aquatic weeds like water hyacinth, banana leaf etc where ice is not available.

In Bangladesh, investigation on the effect of ice storage, processing and preservation of some fish had been carried out by few workers (De and Mea, 1966; Rahman, 1974; Ahmed, *et. al.* 1981; Rubbi *et. al.* 1978).

Information on the changes of iced stored fish under various condition are therefore needed in order to avoid qualitative and quantitative looses and also to ascertain or predicts its quality standard. A similar pattern of study is also required for fish not stored in ice. There is also needed to carry out comparative study between different species related to their shelf life. Species vary from one to another in their proximate composition. Thus, study on the nature of spoilage in relation to different anatomical portion, different sexes and different sizes of fish are so important.

Thus, the present investigation has been undertaken to assess the quality of fish during iced storage and those stored at ambient temperature. Two important fresh and brackish / marine water species i.e. tilapia (*Tilapia nilotica*)and bhetki (*Lates calcarifer*) were considered for the study.

Lates calcarifer (Bloch) is a coastal and estuarine species, and much of the catch is landed in winter. It is a bottom feeder (on small fish and crustaceans). It enters estuaries and backwaters in pursuit of food and shelter but always returns to marine environments for spawning. This species is esteemed as a food fish and marketed mostly fresh. The utilisation of this fish is fresh or frozen, salted or dried is essential. The maximum size attained is 150 cm while the common size 35 – 60 cm. At present, it possesses a great potential interest to pisciculture.

Tilapia nilotica (Linnaeus) is an exotic fish that has been introduced in Bangladesh in 1974 (Karim, 1975). This species is native to Africa; its initial discovery was made in

1939 (Chimitis, 1954, Vass and Hofstede, 1952). The fish has become very important due to its rapid growth, early maturation (at the age of three months) and frequency of breeding (4 times a year). It can live in fresh, brackish as well as seawater. Although, Trewavas (1982) has renamed this species as *Oreochromis niloticus*, yet the former name (*T. nilotica*) is very popular in Bangladesh.

The present research work was undertaken with the following aims and objectives:

1. To study the organoleptic, biochemical (TVB-N, TMA-N, TMAO-N and pH) and microbiological (Total viable count) changes in *Tilapia nilotica* and *Lates calcarifer* during iced storage.

2. To study the organoleptic, biochemical (TVB-N, TMA-N, TMAO-N and pH) and microbiological (Total viable count) changes in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature.

3. To compare the spoilage rate (organoleptic, biochemical and microbiological) of *Tilapia nilotica* and *Lates calcarifer* during iced storage.

4. To compare the organoleptic, biochemical (TVB-N, TMA-N, TMAO-N and pH) and microbiological (total viable counts) changes in *Tilapia nilotica* and *Lates calcarifer* during storage at ambient temperature.

5. To compare the proximate composition of *Tilapia nilotica* and *Lates calcarifer* in relation to size (Large, medium and small), sex (male and female) and anatomical portion (Head, middle and tail portion).

REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

From the onset of civilisation, the relationship between man and fish are inseparable as fish are the main source of protein of mankind. With the ongoing of civilisation, man came to know that fish couldn't be kept raw for a long time as they get spoiled. Spoilage of fish has drawn the attention of intelligent people and had put effort to know the reason of spoilage. There is no documentary evidence as at what time people had started investigation about spoilage of fish. But steadily the concept gain momentum in the mind of the people and with lay effort, it is only at the years of 20th Century, scientific investigations about spoilage of fish got success (Rabby, 2000).

The literature are reviewed here include research work on proximate composition and quality changes (sensory, biochemical and microbiological) associated with fish during iced storage and at ambient temperature. Some literature of proximate composition in relation to sexes, sizes and different anatomical portion are also included.

The length of time that the fish will remain edible if stored in ice is known as the storage life or shelf life. Ice storage is relatively a short term method of preservation with storage lives varying between a few days and about four weeks. The length of storage times dependent on a large number of factors including species, size, method of capture, fat content, breeding condition, feeding condition and methods of killing (Clucas, 1985).

A considerable amount of experimental work has been carried out on spoilage of temperate and cold water marine species of commercial importance in different parts of the world. Published papers on iced storage experiments have been reviewed and attempts have been made to draw general conclusions about the storage lives of fish in different broad categories (Curran and Disney, 1979; Lima dos Santos, 1981; Howgate, 1985). These reviews confirmed that there is wide variation between species and the same species can have different storage lives under different conditions.

2.1 Biochemical Composition

The knowledge of chemical composition of fish can help to suggest appropriate diet of the patient. Some informational studies of the biochemical and nutritional studies of fresh water fish of this region had been carried out by Kamaluddin *et. al.* (1977) and Rubbi *et. al.* (1978). The chemical composition of fish is affected by numerous factors, either of intrinsic in nature or environmental. The physical properties and chemical composition of the fish may vary with species depending on the state of maturity, age, size and season.

Jacquot (1961) and Jafri (1969) recorded the seasonal effect on the proximate composition of the fish.

The nutritive value of the fish depends on the chemical composition of the fish, which varies widely from species to species and from one individual to another (Stansby, and Olcott, 1963).

Kordyl (1957) reported that the flesh of female contains less protein and more water than that of the males, regardless of state of maturity.

Thurston (1958) reported that Alaskan pink salmon had inverse relationship between fat and moisture and positive correlation between protein and moisture. He also obtained wide variation in chemical composition due to sizes of fish and noted lower oil content in female.

Thurston *et. al.* (1959) while studying the chemical composition of 21 species of river and lake fish observed noted small variation in protein content and maximum variation in oil content among the species.

Thurston (1961) studied the chemical composition of 9 species of rockfish of single genus *sebertodes* and observed small variation in their composition and smaller variation in chemical composition due to sizes.

Khuda *et. al.* (1962) reported in carp that with the increase of age and body weight, moisture content and protein decreased whereas non protein nitrogen (NPN) increased.

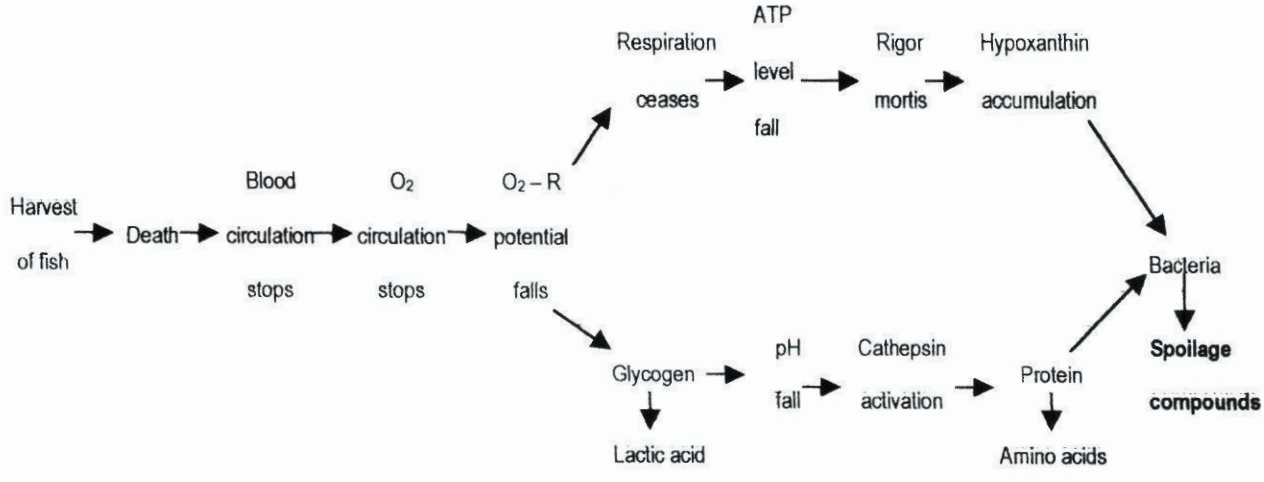
Adhikari and Noor (1967) recorded inverse relationship between fat and moisture contents and positive correlation between protein and moisture.

2.2 Spoilage of Fish

Fresh *fish is* one which is newly produced not stored or preserved and has its original qualities unimpaired in anyway (Dassow,1963).

As soon as the fish dies characteristic phase of postmortem changes occur and flesh stiffens. This phenomenon of stiffening of fish flesh post-mortem is known rigor mortis. After this period is over, the stiffness of muscle end, the body become soft, limp and finally flabby. This stage is referred to 'Post-rigor' (Srivastava 1985).

Spoilage refers to any change in the condition of food which become less palatable, or even toxic accompanied by alternation in taste, smell, appearance or texture (Singleton *et. al.* ,1978). Spoilage is the result of whole series of complicated deteriorative changes brought about in the dead fish tissue by its own enzyme, by bacteria and by chemical action (Shewan, 1976). The spoilage is reflected with the development of objectionable off flavour, discoloration, off odour, stiffness & watery textured in the flesh. Spoilage pattern of fish is shown in the following figure:



Spoilage pattern in Fish (Eskin *et. al.* 1971)

Temperature humidity & time of storage are the most important factors influencing the quality of fish (Stansby and Olcott,1963). Exposure to high temperature significantly increases the rate of spoilage.

It is difficult to give a consistent pattern on the fish spoilage as much depends on the species of fish, the catching methods, fishing seasons and bacterial load. However in fish and shell fish, it is generally accepted that autolytic changes begin soon after the fish dies (Pollar, 1998).

The bacterial spoilage is one of the very potent factors in fish spoilage, although the influence of denaturation of protein & the rancidity of fat are not negligible. The keeping quality of fish depends on the species of fish, fishing methods (Shewan, 1951 and Jones, 1954) and temperature of storage time, as well as various conditions influencing the action of macro organism (Ranke,1959).

2.3 Mechanism of Spoilage

2.3.1 Autolysis

When fresh fish spoils, it passes through several stages. On of the first change after death of fish is the onset of rigor mortis (Amlacher, 1961) due to the formation of lactic acid from glycogen by a series of enzymatic reaction in the tissue which decreases pH value (Tarr, 1954). Immediately there after, decomposition of the highly complex protein of fish flesh into simpler protein, polypeptides and amino acids starts to take place. This is known as autolysis.

Stansby & Lemon (1933) reported that fish detonation can be divided into two main stages based on the type of products (i) Primary change, which lead to formation of amino acids from protein or to certain types of intermediate products such as polypeptides and peptones (ii) Secondary change includes formation of such products as amines, indoles, hydrogen sulphide caused by microbial action. In general the decarboxylas of bacteria catalyses he formation of amine from amino acid on the acidic side and the amino radical of amino acid is spliced off by deaminase in slightly alkaline

condition (Tomiyasu & Zenitani, 1957). During bacterial spoilage, amino & ammonia nitrogen increases, presumably caused by the increase of protein hydrolysis (Beatty & Collins, 1939), which generally occur at advance stages of spoilage (Tarr, 1954).

After the death of fish, the oxygen supply in the tissue ceases due to disruption of the circulatory system. In a short time of postmortem, the mitochondrial system ceases to function. Adenosine triphosphate (ATP) is gradually depleted through the action of various ATPase (Foegeding *et. al.*, 1996). Some ATP is temporarily regenerated by conversion of creatine phosphate to creatine. After residual supplies of creatine phosphate have been depleted, , anaerobic glycolysis continue to regenerate some ATP with the end product, lactate accumulating (Foegeding *et. al.*, 1996)

Glycolytic activity cease because of exhaustion of substrate or because of reduced pH caused by ATP hydrolysis. The rate and extent of pH decline affect the metabolic rate and the buffering capacity of the muscle. Generally white muscle has relative amount of the amino acids carnoisine and serine and these probably function as buffering agents in the muscle cell (Foegeding *et. al.*, 1996).

When the pH reaches a critical value, the ultimate pH, certain critical enzymes, especially phosphofructokinase, are inhibited and glycolysis ceases. The ultimate pH has an important influence on the textural quality of the meat, its water holding capacity, its resistance to growth of microorganisms, and its colour (Foegeding *et. al.*, 1996).

In the absence of ATP, the myofibrilar proteins become intimately and irreversibly coupled and the muscle cannot relax. This state is known as rigor mortis and is characterised by a stiffening of the muscle.

The autolytic reaction proceeds in the same manner in all muscle foods but the kinetics of this reaction is species specific. Since autolysis is associated with enzymes, rates of reaction are temperature dependent. According to Jones *et. al.* (1964) the early reaction of this sequence are autolytic and bacterial enzymes become progressively the more active in the later stages.

2.3.2 Microbial Spoilage

Considerable attention has been focused on the role of microorganisms on fish spoilage and this has resulted in the development of several preservation strategies. These strategies aim to curtail proliferation of microorganisms in foods.

Newly caught fish is generally not harboring pathogens in its flesh & body fluids. But the external surface, the slime of skin, gill & the intestinal fluid of the fish carry heavy bacterial loads (Shewan,1961),which do not cause any harm in live fish. The bacterial load of fish depends upon both intrinsic & extrinsic factors. Fish from warm water frequently carry greater number of bacteria than cold water fish & yield higher count when incubated at temperatures of 35 -37^{0}C (Shewan, 1977). This suggests that the population of warm water is more mesophilic.

Within short period after death, the fish goes into rigor and after that humoral defences against bacterial invasion cease to operate and the mechanical barriers such as skin & membranes gradually loose their impermeability. The bacterial population then enters a phase of more or less exponential growth corresponding to the initial appearance of spoilage indicator substances as trimethyl amine from TMAO (Shewan,1938), ammonia from protein (Hilling *et. al.* 1958), histamine from histadin, certain acid from glucose & other related bases. Technologically these changes in the bacterial flora are of enormous importance, since together with associated endogenous biochemical & perhaps physical changes in the fish tissue, they lead to the process of spoilage and ultimate decay and desolution of the evader (Borgatrom 1965).

It is generally accepted that bacteria on the skin of fish, in the gills and those found in the gut invade the otherwise sterile fish and began to metabolise surrounding low-molecular weight compounds thus producing the volatile compounds associated with spoilage. Under chill storage conditions, this is a surface phenomenon. However, under conditions of temperature abuse or cuts in the fish skin as a result of improper handling, the ingress of bacteria rapidly increases.

Shewan (1977) quotes data indicating fish from warm water frequently carry greater number of bacteria than cold water fish and yield higher count when incubative temperatures are 35 – 37^{0}C. This suggested that the population of warm water is more mesophilic. Bacterial loads on newly caught marine and fresh water fish is shown below (Shewan, 1977).

Table – 1 Bacterial loads on newly caught marine fish (Shewan,1977)

Area	*Species*	*Temperature*	*Skin /^{2}cm*	*Gills /^{2}cm*	*Gut content /^{2}cm*
Temperate	North sea fish	20^{0}C	10^2 - 10^5	10^3 – 10^7	10^3 - 10^5
Tropical	Indian sardines	30^{0}C	10^5 – 10^7	10^6 – 10^8	10^7 – 10^8
Subtropical	Japanese flat fish	20^{0}C	10^4	10^3 – 10^3	10^3 – 10^7

Table – 2 Bacterial loads on newly caught fresh water fish (Shewan,1977)

Area	*Species*	*Temperature*	*Skin /^{2}cm*	*Gills /^{2}cm*	*Gut content /^{2}cm*
Temperate water	U. K. salmon	20^{0}C	10^2 – 10^3	-	10 – 10^2
Tropical	Bangladesh hisha	30^{0}C	10^3 – 10^4	10^6	10^4 – 10^6
Subtropical	African Kariba	20^{0}C	10^3 – 10^5	-	-

Vanspreekins (1977) showed that high quantities of ammonia and volatile acids were produced in the later stages of spoilage and this was due to the oxidation of amino acids and other non-protein nitrogen compounds by bacteria. Chung (1968) showed protein production by bacteria is repressed in the early stages of spoilage but is derepressed in the latter stages of spoilage, thus causing increase protein hydrolysis, which leads to a rapid replenishment of the amino acid pool. This presumable influences production of all end products derived from amino acids e.g. H_3SH from methiomine. These substances were noted by Shewan (1977) to be produced rapidly after eight to then days of storage.

The general bacteriology of spoilage can be summarised according to the following scheme (Horner, 1994):

Sequence of events during spoilage

1) Spoilage bacteria naturally present on fish,
2) Amino acids and other non protein nitrogen substrate pool present,
3) Selective growth of bacteria, (mostly rod-shaped, Gram negative Pseudomonas), which actively oxidatively deaminate amino acids,
4) Repression of proteinase production derepressed by selective use of amino acids by Pseudomonas bacteria,
5) Amino acid recruitment to substrate pool by bacterial hydrolysis of protein,
6) Ammonia and volatile fatty acid production sharply increases due to 5,
7) Specific 'spoiler' types of bacteria produce sulphur containing and other odorous compounds e.g. TMA and NH_3.

2.3.3 Production of off Flavour and Volatile

Autolytic degradation products are implicated in several organoleptic changes for example IMP and other nucleotides are associated with freshness. They function as strong flavour enhances and together with glutamic acid, they give rise to a meaty flavour (Huss, 1988). On the other hand, hypoxantine is a bitter tasting compound and its concentration in the fish muscle may be used as a quality index for certain species of fish.

Bremner *et. al.* (1987) reported that concentrations of 3.3 μmole or above correlated with a bitter taste with their studies on long-spined sea bream and snapper.

During anaerobic glycolysis, lactic acid accumulation causes a drop in the pH of the muscle thus triggering the release of proteolytic enzyme such as *cathepsin*. Proteins are broken down into amino acids and / or intermediated such as polypeptides or peptones.

Enzymes from spoilage microorganisms can metabolise the amino acids of the fish muscle producing a wide variety of volatile compounds resulting in off-flavours and odours. Lysine for example degrades under post-mortem microbial attack to malodorous biogenic amines whilst carbonyl compounds can be divided from lipids. Similarly

sulphur containing amino acid may produce hydrogen sulphide, dimethyl sulphide (Herbert and Shewan, 1976; Smith, *et. al.*, 1984; Watts and Brown, 1982).

Trimethylamine Oxide an osmoregulatory substances found in many marine species, is degraded under post-mortem microbial attack to the fishy smelling Trimethylamine. TMAO found in a large number of marine fish and shellfish, is broken down to TMA by either endogenous enzyme trimethyiene oxidase (Banwart, 1981). When the oxygen supply is depleted, many of the spoilage microorganisms use TMAO as a terminal hydrogen acceptor, thus allowing them to grow under anaerobic/ anoxic conditions. This may be a reason why seafood spoil more rapidly (Sikorski *et. al.*, 1976).

2.4 Quality of Fish

In simple terms, the quality of food can be defined as those characteristics which make it acceptable to the consumer (Connell, 1975). Generally the consumer will pay more for fish that he or she consider to be higher quality and will continue to buy it's long as the quality remains constant.

Quality control can be defined as the maintenance of quality at a level that satisfies the consumer and that is economical to the producer (Connell, 1972). The importance of quality control is to satisfy the customer and thus to get better return from the product.

The keeping quality of fish depends on the species of fish, fishing methods, size, season (Shewan,1951; Jones 1954) and fishing grounds, storage temperature, storage time and various conditions influencing the action of micro organisms (Ranke,1959). There are various methods for assessing the quality of fish, but the followings are most widely used.

2.5. Methods For Estimating the Freshness of Fish

2.5.1 Organoleptic Evaluation

Organoleptic methods are universally accepted for estimating the quality of fish (Reay & Shewan, 1949). The basic method of evaluating the quality and shelf-life is by periodic sensory testing which is based on the characterisation and differentiation of the various sensorily perceptible factors such as appearance and odour of the raw fish and odour, flavour texture etc. of the cooked fish, which effects the sensory judgement of the buyers or consumers.

The organoleptic method may be influenced by the physiological and psychological state of the judges. Considerable variations are often observed in the sensitivity and the test of the judges of the panel at different times and the different circumstances under which the examinations are performed (Reay & Shewan, 1949; Farber, 1965).

At the Torry Research station, Aberden, UK. a good technique. has been evolved for organoleptic evaluation of fish and fisheries products. This method is consisting of using a trained taste panel, to *assess* the main organoleptic characters. Peryam & Pilgrim (1957) has developed an useful method for assessing overall acceptability of food products. Quality in this method has been defined by Miwa and Ji (1992), which is based on Shewan scheme (1938). This method has been used in great deal for consumer's studies of foods all over the world.

2.5.2 Chemical Test:

Certain chemical changes in spoiling fish appear to run parallel with changes in odour, texture, appearance etc. Various attempts have been made to measure freshness by estimating the quantities of *some* of these end products as a result of enzymatic and bacterial activity (Yamamura, 1933; Luke and Geidel,1935; Tanikawa,1935, Filler et al,1957 Sato,1960). Chemical tests of assessing the quality of fish and fishery products are based on estimating the products of spoilage either as individuals or as groups.

Ammonia (NH3), trimethylamine (TMA) and dimethylamine (DMA) are nitrogenous, non-protein bases. These bases, other than ammonia, are known chemically as amines. The combined total amount of ammonia, dimethylamine and trimethylamine in fish is called the total volatile base nitrogen content of the fish and is commonly used as an estimate of spoilage. The level of the total volatile bases increases after spoilage begins both enzymically and bacterially and hence, can be used as a quality index for fish..

Measurement of Total Volatile Basic Nitrogen (TVB-N) was probably the first chemical method to be used. as a practical index of freshness (Tillmans and Otto,1924) and it is still the most popular. Total volatile nitrogen has been widely used as an index for freshness of fish (Stansby *et al.* 1944).

This Total Volatile Base (TVB) Nitrogen (TVB-N) test is quite generally applicable; it usually gives reasonable good correlation with organoleptic freshness and can be carried out rapidly without the use of special equipment (Stansby *et. al.*, 1957; Hilling *et. al.*, 1958).

TVB does not increase much in the early stages of spoilage but rises rapidly with bacterial activity. The variance of TVB a sample of fish of the same origin and subjected to the same treatment is high compared to the average change over the acceptability. So, it is not sensitive indicator of freshness, until the fish is spoiled rapidly (Connell and Howgate, 1986).

Favourable results for the increase of the Total Volatile Basic Nitrogen content with fish spoilage were found by Luke and Geidel (1933), Shewan (1938), Stansby *et. al.* (1944), and Ota (1958).

The Total Volatile Basic Nitrogen (TVB-N) content of most fish is mainly composed of ammonia, trimethylamine and dimethylamine. The level of total volatile nitrogenous bases increases after spoilage begins, both enzymically and bacterially, and thus can be used as an index of spoilage. Using TVB-N as such an index does not distinguish the origin nor component of these volatile compounds, hence its use is more general. The use of TVB-N as an index of spoilage was first proposed by Shewan (1938).

Jenson *et. al.* (1979) studied the pattern of increase in TVB-N during the storage of cod muscle at 2^0C and compared these results with sensory method. A good correlation was obtained. Using the total volatile base as a quality index does not distinguish the origin or component of these volatile compounds. Hence its use is more general.

The increase in the amount of TVB parallel with the increase in TMA during spoilage. Although, it might be thought that the TVB test would give a more accurate indication of the freshness of a sample than the TMA test. This is only true during the later stages of spoilage since a group rather than one substance is being tested.

The determination of total volatile nitrogen is also referred to as total volatile base nitrogen, since the results of the analysis are always given in terms of nitrogen content of the bases. It is probably the oldest chemical method to assess fish spoilage developed e.g. used as a index of spoilage by Shewan (1938). This index has widespread usefulness and correlates reasonably adequately with sensory changes during spoilage or deterioration. It has the advantage of simplicity, cheapness and relative rapidity. The main disadvantages are that the sample is destroyed, the conditions of volatilisation have to be standardised or specified exactly and it isn't sensitive for early stages of spoilage when TVB levels are low (The major constituent of the TVB-N content of fish during early stages of spoilage is ammonia).

Another source of variability with the TMAO method appears to be from the leaching of volatile amines from different parts of the fish body during iced storage (Horner, 1994). A further source of variability arises through biological variations in the concentrations of the precursors. It has been found that there are seasonal changes in the levels of TVB-N (Linztel *et al*, 1934; Lovern and Wood, 1937; Reay *et. al.*, 1950), e.g. during the spawning migration of Atlantic salmon (Duncan and Tarr, 1958). There is evidence to suggest that there is a correlation between the age of fish and TVB-N content i.e. TVB-N content of fish increases with age (Shewan, 1951; Hughes, 1959).

The magnitude of TVB content depends to some extent on the method of measurement e.g. steam distillation in the presence of strong alkalinise generally results in higher

values of TVB ammonia result from the breakdown of nitrogenous compounds during distillation (Hughes, 1959).

TVB-N increases only slowly during the chilled storage of most freshwater fish (Nair *et. al.*, 1971), principally because of their low negligible content of trimethylamine oxide. Therefore, it is of little use for freshwater species. The use of TVB-N is hence, mainly restricted to marine fish and crustaceans (Cobb *et. al.*, 1973; Cobb and Vanderzant, 1975).

A range of methods are used to measure TVB-N. In all of these the fish or fish extract is made alkaline. The bases are distilled off and collected, then measured by titration. Dyer (1952) first suggested the fuming of dilute hydrochloric acid solution in alcohol to measure TVB-N in fish. Beatty and Gibbons (1936) presented the simple Conway's micro-diffusion technique. In recent years the scope of the method for chilled fish has been further explored, (Pearson and Musslemuddin, 1969; Uchiyama *et. al.*, 1970; Amu and Disney, 1973; Ota *et. al.*, 1975). Much of this research has concentrated on the development of a steam distillation technique to measure TVB-N.

Beatty and Gibbon (1938) suggested that the TMA could be used as an index of freshness of fish. During fish spoilage Trimethyl Amine Oxide (TMAO) is reduced by bacteria to Trimethyl Amine (TMA) (Ota *et. al.*, 1975). Connell (1975) reported that TMA could serve as an index of spoilage for marine fish. TMA contents fluctuates with seasons as well as within a single species (Shewan & Jones,1957) and its production is restricted to the marine species only.

Different workers have suggested a number of method for determining TVB. Among the commonly used methods, the Conway micro diffusion technique *is most* suitable and applied in this work for the determination of TVB and TMA.

As the activity of spoilage bacteria increases after the death of a fish, a subsequent increase in the reduction of TMAO to TMA occurs (Wood, Sigurdisson and Dyer, 1942). Thus, the amount of TMA produced can be used as an index of spoilage.

Trimethylamine oxide (TMAO) is a non-protein nitrogenous compound present in marine organisms (Love, Lovern and Jones, 1959). It is known that in many fish, particularly in the elasmobranchs, TMAO functions as an osmoregulater (Cohen, 1958). In marine fish, TMAO is broken down during spoilage, either by bacterial enzymes, endogenous enzymes or both. The main product of the bacterial reduction of TMAO is trimethylamine (TMA), since the role of autolysis is negligible (Shewan, 1977).

However, in gadoid fish, TMAO may be broken down by autolytic processes where bacterial activity is minimal, reducing TMAO to the intermediate dimethylaminomethygol which yields dimethylamine (DA) and formaldehyde (FA). This process is particularly dominant in frozen gadoid fish where bacterial is reduced to zero, (Castell *et. al.*, 1970; Harada, 1975).

Most marine species, and pike amongst the freshwater fish, contain substantial amounts of trimethylamine oxide (TMAO). Species can break down TMAO to trimethylamine (TMA), a tertiary amine. The reduction of TMAO to TMA involves a coupling reaction, the oxidation of lactate to acetic acid, CO_2 and H_2O through an activation step involving a 'triamine-oxidase'.

As the activity of spoilage bacteria increases after the death of a fish, a subsequent increase in the reduction of TMAO to TMA occurs. Thus the amount of TMA produced can be used as an index of bacterial spoilage. During spoilage of cod, TMAO decreases while there is a subsequent increase in TMA, it has been found that the increase in TMA is parallel to the rise in the bacterial population (Shewan, 1977). In low concentrations it produces a 'stale fish' smell. Bacteria first utilise lactic acid and sugars in the tissues when reducing TMAO (Beatty and Gibbons, 1937; Collins, 1941; Watson, 1939a and 1930b), and this reduction occurs primarily at the surface of the muscle tissue (Wood *et. al.*, 1942).

The muscle of most fish contains TMAO (Love, Lovern and Jones 1959). However, many species, particularly freshwater species, do not contain adequate amounts of TMAO for TMA to be an index of quality for these fish, an exception being land locked

salmon (Dyer and Monsway, 1945). Some marine species, flat fish, some pelagic fish and shell fish have such low levels of TMAO that, although there is an increase in TMA with spoilage, the increase is too small to be recorded due to the insensitive nature of the test.

At least 94% of TMA in spoiling fish originates from TMAO (Beatty, 1938). However, in many freshwater fish where TMAO is not generally formed, any TMA present in spoiled fish may originate from choline and similar compounds (Dyer and Wood, 1947).

The function of TMAO in marine fish has been suggested to be as an osmoregulater. However, there is little evidence to support this idea in teleost fish. In marine elasmobranchs, TMAO retention is known to be an adaptation in their physiology to osmoregulation. It is also thought to be a participant in other metabolic functions (Cohen, *et. al.* 1958). TMAO has the function of eliminating waste ammonia, which would otherwise become toxic (Baldwin, 1957). The tertiary amine oxide (TMAO) is thought to act as a methyl donor in the metabolism of elasmobranchs (Barrenscheen and Pantlitschke, 1949). Research has shown that elasmobranchs are also capable of producing TMAO, previously thought to be exogenous (Cohen *et. al.*, 1958).

TMAO is thought to be responsible for corrosion and maturation in the process of canning fish (Tarr, 1942; Ronald and Jakabsen, 1947), and causes 'greening' in canned fish (Kolzeims and Hasimoto, 1965).

Advantages of the TMA as an index of bacterial spoilage are similar to those of TVB-N. The index has widespread usefulness and correlates reasonably adequately with sensory changes during spoilage or deterioration e.g. in about 75% of cases, the taste panel and TMA results agree. The main advantage of using TMA as a spoilage index compared to the TVB-N index is that the substance (TMA) can be measured exactly.

Disadvantages include the problem that TMA analysis is often complex and costly. Different methods of TMA analysis appear to give inconsistent results. A source of variability includes the loss of soluble TMAO and TMA from fish during spoilage via leaching (Shewan and Jones, 1957). Dyer and Snow (1952) discovered that fish lose a

large portion of both TMAO and TMA by diffusion. Both TMAO and TMA have relatively small molecules, and thus probably diffuse as ions. A further source of variability arises through biological variations in the concentrations of precursors. The TMAO content of fish can vary depending upon the geographical distribution of the fish species e.g. Atlantic cod with values between 67-75% mg were found in English waters;, values varying between 50-92% were observed for Atlantic cod in Norwegian waters (Ronald and Jakobsen, 1947). Research evidence shows seasonal changes in TMAO levels (Lintzel *et. al.*, 1939; Lovern , 1952; Reay *et. al.*, 1950). Dyer and Monsway (1945) showed changes in the level of TMAO during, spawning and migration of Atlantic salmon. Concentrations of TMAO were low in January, increasing to a maximum in July and decreasing again towards the end of the year (Smith *et. al.* 1979).

There is evidence to suggest that the TMAO level in fish varies depending on the age of the fish, TMAO increasing with an increase in age (Shewan, 1951;). TMAO levels vary depending upon the species of fish. Elasmobranchs have the highest content of TMAO, amongst teleost fish, the amount of TMAO increasing from the lower to higher orders of fish (Dyer *et. al.* 1952). Fresh water fish contain little or no oxide.

Another problem in using TMA as an index of freshness is that if distribution in a fillet is not thought to be even e.g. TMA rapidly increases in nape tissues more than in other areas of fillet,' (Hardy *et. al.*, 1977). As TMA is produced by bacteria, and bacterial activity is directly effected by temperature so temperature gradients in individual fish can affect TMA levels (Horner, 1985).

The muscle of living fish, in general are approximately neutral in reaction and after harvesting pH decreases due to formation of lactic acid from glycogen by a series of reactions (Tarr, 1954). As spoilage advances, the pH value of the muscles rises first slowly and later quite rapidly. This is due to the accumulation of basic end product of bacterial spoilage which leads to a pH value of 7.5 – 8.0.

The measurement of pH was suggested by Wood *et. al.* (1942) a rapid test for detection of spoilage of fish. However, Farber (1965) doubted the reliability of this test and stated

that the factors affecting the pH value such as fishing methods, fishing grounds, species, season, bacterial load, type of bacteria etc. would require much more investigation.

2.5.3 Bacteriological Test

Castell *et. al.*, (1948) and Khalil (1964) have found that bacterial counts were valuable as a measure of degree of freshness of fish. Bacterial counting using total viable count is satisfactorily going on in research laboratories throughout the world. With some unavoidable difficulties this method is more or less suitable for routing testing of the quality of fish (Wittfozel,1961). The bacterial flora and its relation to fish spoilage has been studied by Tarr (1938), Shewan (1938) and Liston (1957).

Since bacteria are the main agents of spoilage, techniques have been developed to use their numbers as an index of fish quality. Lerke *et. al.*, (1967) were the most recent investigators to study this method. They developed a technique for the direct counting of bacteria, being able to differentiate between spoiled and unspoiled fish on numbers alone. More precise methods to estimate bacterial numbers have been developed, yet these methods tend to be time-consuming and expensive.

The main disadvantages of bacteriological methods are that bacterial counts are not directly related to sensory properties and cannot give information on the early stages of spoilage. Also, the sample which is being tested is normally destroyed.

MATERIALS AND METHODOLOGY

3 MATERIALS AND METHODOLOGY

3.1 Selection of the Fish

In selecting the test specimen, it was intended to look for certain sets of criteria, such as (a) availability of the species locally, (b) demand of the species by the consumers and (c) price of the species.

*Tilapia nilotica (*Tilapia), is small, fast growing, popular table food fresh water fish. The size ranging between 130 to 250g were used for organoleptic, biochemical and microbiological analysis.

Lates calcarifer (Bhetki), a brackish / marine water fish was another fish (650 – 1200g), which were also used in this experiment. It is also a fast growing, popular table food fish. Both species are found abundantly and possess high demand by the consumer due to its taste and low price compared to other species.

3.2 Collection of Fish

Tilapia nilotoca and *Lates calcarifer* both fishes were purchased from the local market (Gallamari market, Sandha market, Natun bazaar, Rupsha bazaar and Dolkhola bazaar under Khulne City Corporation). The actual post mortem history of the fish were not known. But according to fishermen's statement, tilapia was caught from the gher by 'tana jal' near Dumuria thana under Khulna District in early morning time and brought to the whole sale market in bamboo basket with some crushed ice. Bhetki were caught from the Passur River by 'Bhetki jal' during in late night and brought directly to the wholesale market early in the morning in bamboo basket with some crushed ice.

After purchasing the fish, they were brought immediately to the Quality Control laboratory of Fisheries and Marine Resource Technology Discipline of Khulna University in two insulated iceboxes with additional crushed ice and in bamboo basket with banana leaves. The insulated iceboxes with ice were kept at room temperature in the

laboratory. The freshness of the fish was evaluated by organoleptic assessment; biochemical and microbiological parameters intermittently.

Fish was kept in ice at a ratio of 1:1 in insulated boxes. During the experiment, ice was changed every day. In case of storage at ambient condition, fish were placed in bamboo baskets and covered with banana leaves.

3.3 Identification of Fish:

According to Talwar and Jhingran (1991), the classification of tilapia and Bhetki are as follows:

Table – 3 Classification of tilapia (a fresh water fish)

Local name	English name	Classification	
Nilotica	Cichlids	Phylum	Chordata
		Sub Phylum	Vertebrata
		Super class	Pisces
		Division	Gnathostomata
		Class	Chondrichthyes
		Sub class	Elasmobranchii
		Order	Perciformes
		Sub order	Percoidei
		Family	Cichlidae
		Genus	*Oreochromis*
		Species	*Oreochromis niloticus*

Table – 4 Classification of Bhetki (a marine/ brackish water fish)

Local name	English name	Classification	
Bhetki / Koral	Baramundi	Phylum	Chordata
		Sub Phylum	Vertebrata
		Super class	Pisces
		Division	Gnathostomata
		Class	Chondrichthyes
		Sub class	Elasmobranchii
		Order	Perciformes
		Sub order	Percoidei
		Family	Percidae
		Genus	*Lates*
		Species	*Lates calcarifer*

3.4 Techniques for Analysis of the Sample:

3.4.1 Organoleptic Analysis:

In case of storage condition, the iced store fish was presented directly to the taste panel members. The same procedure was followed for fish stored at ambient temperature.

Score sheet, specially prepared for this purpose was supplied to each of the judges. Care was taken to ensure that the panel members judged the product independently. A panel of three judges performed detail organoleptic tests. For both fresh and marine fish, organoleptic evaluation was carried out according to the procedure of Shewan and Ehrenbreg (1977). The score sheet was slightly modified related to scoring (Table – 5) to meet the requirement of the present investigation. Seven quality factors were considered to assess the organoleptic assessment of both raw fish (tilapia and bhetki). The factors were Eye, Pupil, Gill, Body Surface, Belly Wall, and Texture. Acceptability of the both fish were evaluated as per the characteristics described in Table - 5. Members of the taste panel who recorded their opinion on different characteristics used a 10 point scale. The score ranged between 5.5 to 6.4 arbitrarily were taken as the overall acceptability of the shelf life of *Tilapia nilotica* and *Lates Calcarifer*.

Table – 5 Organoleptic score sheet for fresh fish (Shewan and Ehreberg, 1977)

Eye Characteristics	Score
Bright, clear and dull	10
Slight dull	9
Moderately dull	8
Dull	7
Slightly sunken	6
Moderately sunken	4
Sunken	2

Pupil Characteristics	Score
Slight and transparent	10
Slight dull/ misty	8
Dull/slight milky white	7
Watery colour	6
Slightly misty	4
Pale white	2

Gill Characteristics	Score
Bright red	10
Slight dull red / slight pale	9
Slight pale/pale	8
Moderately pale	7
Pale	6
Brownish / yellowish	4
Dark Colour	2

Body surface Characteristics	Score
Shining bright	10
Slight loss of brightness	8
Loss of brightness	6
Slight dull	4
Dull and slight reddish of tail end	2

Belly wall Characteristics	Score
Normal fresh	10
Slightly discoloration	8
More discoloration	6
Slightly digested	4
More digested	2

Texture Characteristics	Score
Farm and Elastic	10
Rigor stage	8
Just post rigor stage	6
Slightly soft	4
Moderately soft	2

Odour Characteristics	Score
Fresh odour	10
Sweety odour	8
Slightly spoilage odour	7
Moderately spoilage odour	6
Spoilage odour	5
Slightly off odour	4
Moderately off odour	3
Off odour	2

Overall Acceptability Characteristics	Score	Modified score
Excellent (E)	10	9.4 - 10
Highly acceptable (HA)	9	8.5 - 9.4
Acceptable (A)	8	7.5 - 8.4
Moderately acceptable (MA)	7	6.5 - 7.4
Just acceptable (JA)	6	5.5 - 6.4
Just Unacceptable (JU)	5	4.5 - 5.4
Unacceptable (U)	4	3.5 - 4.4
More unacceptable (MU)	3	2.5 - 3.4

3.4.2 Biochemical Analysis:

TVN-N, TMA-N, TMAO-N and pH were determined according to the procedure stated in the manual of Siang and Kim (1992).

Solution and Reagents

- Inner ring solution- 1% boric solution containing indicator:

10g boric acid were weighed into a one-liter volumetric flask then added 200ml of ethanol. After dissolving the boric acid, 10 ml of mixed indicator solution was added, then made up to 1 liter with distilled water.

- Mixed indicator solution:

Bomocresol green (BCG 0.01g) and methyl red (MR) 0.02g were dissolved in to 10 ml of ethanol.

- 0.02 Hydrochloric acid, HCl

20ml of 1 (N) HCL standard solution were diluted with distilled water and made up to 1000ml.

- Standard potassium carbonate solution (K_2CO_3) solution.

60g of K_2CO_3 were weighed and then 50 ml of distilled water was added. It was boiled gently for 10 minutes. After cooling down, it was then filtered through filter paper.

- 4% Trichloroacetic acid (TCA, CCl_3COOH) solution.

40g of TCA were dissolved in 960ml of distilled water.

- Neutralized 10% formaldehyde solution

10gm of $MgCO_3$ were added to 100 ml of formalin (35% formaldehyde solution) and shaked in order to neutralize the acidity of formalin. Then diluted the filtrate 3 times with distilled water.

- 1% titanium Trichloride ($TiCl_3$) aqueous solution:

6.7ml of $TiCl_3$ were taken into 100ml volumetric flask and made up to 100ml with distilled water.

- Saturated potassium nitrate (KNO_3) solution:

55 g of KNO_3 were dissolved in 50 ml of distilled water

3.4.2.1 Estimation of Total Volatile Basic Nitrogen (TVB-N):

TVB-N, TMA-N and TMAO-N was determined according to the procedure of Conway (1953) as modified by Siang and Kim (1992).

Procedure:

Preparation of extract:

The extract was prepared by mixing 2 gm of the minced muscle with 8ml of 4% TCA in a 10 ml beaker and grind well. It left for 30 minutes at ambient temperature with occasional grinding. After then, it filtered through filter paper (Whitman no. 1). The filtered solution was kept in Mackerty bottle and was labelled. The filtered solution was also stored in a freezer at -20^0C (to prevent any further chemical, bacterial or enzymatic break down of the muscle).

Determination of TVB-N:

Three Conway's unit were taken which had been thoroughly cleaned with a neutral detergent to remove any containment. To the edge of the outer rim of each unit was applied the sealing agent (Vaseline). Using a micropipette, 1 ml of inner ring solution was pipetted into the inner ring of each unit. In to the outer ring of each unit, was pipetted 1 ml of the sample extract. 1 ml of saturated K_2CO_3 solution was carefully pipetted into the outer ring of each unit, carefully to prevent any entering the inner ring, and immediately the units were covered and closed with clip. The solutions in the units

were then mixed gently, to prevent any solution mixing from one ring to the other. After then, the units were placed in an incubator at 45^0C for 45 minutes. After this the units covers were removed and the inner ring solution, now a green colour, was titrated with 0.02N HCl using a burette (50ml) until green coloured solution, turned to pink.

An average titrated volume of HCl was found from the results of three titration for each fish species muscle sample. For each value the TVB-N values were calculated.
A blank test was also carried out using 1ml of 1% TCA, instead of sample extract.

3.4.2.2 Determination of Trimethyl Amine Nitrogen (TMA-N)

Trimethyl amine in fish muscle was determined by the Conway technique. Prior to addition of potassium carbonate, 1ml of 10% neutralised formalin was added to the extract to react with ammonia and thus allow only the TMA to diffuse over the unit. The calculation was done by the same formula as used in the Conway micro-diffusion technique for TVB-N. The calculation was done by the following formula:

Calculation:

TMA-N or **VB-N** (mg/100g) = Amt. of HCl used in titration × Amt. of ammonium nitrogen equivalent to 1 ml of 0.02N HCl × Ratio of the amt. of sample used to 100g muscle

$$= (V_S - V_B) \times (N_{HCl} \times A_N) \times \frac{[(W_S \times \frac{M}{100}) + V_E] \times 100}{W_S}$$

where,

V_S	=	Titration volume of 0.02N HCl for sample extract (ml)
V_B	=	Titration volume of 0.02N HCl for blank (ml)
N_{HCl}	=	Normality of HCl (= 0.02N × f, factor of HCl)
A_N	=	Atomic weigh of Nitrogen (14.00)
W_S	=	Weigh of muscle sample (g)
M	=	Percentage moisture of muscle sample
V_E	=	Volume of 4% TCA used in extraction

3.4.2.3 Determination of TMAO-N

Extract preparation

The extract preparation was same as used in the Conway micro-diffusion technique for TVB-N. In addition, the sample solution was taken a test tube and 1 ml of 15% $TiCl_3$ was added to make the solution violet colour. The solution was then allowed to stand in 80^0C water bath for 90 seconds to disappear the violet colour and cooled in running water. Finally the cooled solution was transferred in to a 10 ml volumetric flask and made up to 10ml with distilled water.

TMAO-N Determination

TMAO-N determination was same as the procedure TMA-N determination. The calculation of TMAO-N was done by the following formula-

TMAO-N (mg/100g) = (TMA-N after $TiCl_3$ reduction × 5) – (TMA-N before $TiCl_3$ reduction)

3.4.2.4 Determination of pH:

Procedure

The muscle of fresh fish was taken from anterior portion (avoiding the red meat portion). 10 gm sample was grinded and mixed well with 100 ml distilled water and grinded for 30 sec. The homogenate was measured in a electric pH meter with a glass electrode using a expanded scale.

3.4.2.5 Proximate Composition

Proximate composition (the percentage of moisture, protein, lipid and ash) of collected samples was analysed according to AOAC methods (1980).

3.4.2.5.1 Moisture

Procedure:

About 5 g of the macerated fish sample was taken in porcelain basin of known weight. The sample was weight accurately by using an electric balance and dried in an oven at 105^0C for 24 hours. Drying , cooling (in a desiccator) and weighing were continued for a constant final weight. The percentage of moisture content was calculated by using following equation:

Calculation:

$$\% \text{ of moisture} = \frac{\text{Weight (g) of the sample after drying}}{\text{Weight (g) of the sample before drying.}} \times 100$$

3.4.2.5.2 Ash:

Procedure:

5 gm of the sample was taken in porcelain crucible. The crucible was placed on heater and heated first over a low flame till the entiral was completely charred followed by heating in an Muffle Furnace at 550^0C for 5 hours till the residue become white. It was then cooled in a desiccator and weighed finally.

Calculation:

$$\text{Ash content (\%)} = \frac{W_2 - W_0}{W_1 - W_0} \times 100$$

was collected in 2% boric acid solution with indicator (mixed indicator) and finally titrated against N/10 HCl solution .

Calculation:

$$\%N = \frac{\text{ml of titrant} \times \text{strength of titrant} \times 0.014 \times 100}{\text{Weight of sample in gm}}$$

% of Crude Protein =%N x 6.25

3.4.2.5.4 Lipid:

Procedure:

10 gm of fish samples were taken from which moisture was first removed completely. The dries sample was grounded well and taken in to a thimble paper. The thimble with thimble paper was then placed in Soxlet apparatus and fat was extracted with acetone. Acetone was heated to its boiling temperature and continuous waster flow was maintained to condense evaporated acetone. Acetone extract was filtered in to a weighed cleaned beaker. It was heated on an electric hot plate to evaporate acetone and moisture and weighed.

Calculation:

$$\% \text{ of lipid} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

The appropriate dilutions were selected and for every dilution 1.0 ml aliquot was transferred into a sterile petridishes. 20.0 ml portion of molten Plate Count Agar (PCA) was poured into each of this sterile petridishes. The plates were then rotated 5 times clockwise, 5 times anticlockwise, 5 times back and forward. Care was taken not to splash agar on the lid of the dish. Plates were left to solidify The plates were inverted and incubated at 35^0 C± 2^0C for 48 hours. Plates with 30-300 colonies on the surface were only counted. In the present investigation, TVC value expressed in $\log_{10}$ values.

Calculation:

APC (Pour Plate Method) = X.d/s colony forming unit (cfu/gm)

d = dilution of which colonies are counted
x = average count of colonies
s = volume of aliquot

3.5 Methods of Data Analysis:

Assays for organoleptic chemical and microbiological parameters were performed in triplicate and results averaged. Statistical analysis for correlation of coefficient, Standard Deviation, Analysis of variance (ANOVA) and graphical representation were conducted on PC using Microsoft Excel (Version 5) program.

RESULTS

4 RESULTS

4.1 Quality Changes of Fish During Iced Storage

4.1.1 *Tilapia nilotica*

4.1.1.1 Organoleptic

Fig – 1 illustrates the organoleptic changes in tilapia during a period of 25 days of storage in ice. It is observed from the APPENDIX - 1 that the initial average score on the 1st day of the storage day was 9.08 as provided by the panellists. This score gradually decreased over the range of 25 days. The score ranged between 5.5 – 6.4 have been taken as 'just acceptable (JA)' from among the 7 parameters of a average score of 10. In this case, the score 6.25 is observed on the 10th day of iced storage. The score is further decreased steadily. On the 25th day, the score was only 3.86. The correlation between storage days and organoleptic score was found to be highly negative (r = -0.974) as shown in APPENDIX – 2a. It is clearly evident that there is a significant differences ($P \leq 0.05$) between the storage days and organoleptic score. This is shown in APPENDIX – 9a where the calculated value 'F' (7.9916) is higher than the tabulated value 'F' (2.0548).

4.1.1.2 Biochemical

4.1.1.2.1 Total Volatile Base Nitrogen (TVB-N)

Over a range of 25 days of storage trials in ice, TVB-N content in tilapia increased from a meagre value of 4.49±1.91 to 116.25±7.69 mg-N/100g. However, as tilapia was found to be 'just acceptable (JA)' on the 10th day, when the TVB-N showed a value of 20.64±0.94 mg-N/100g (Fig - 2). TVB-N content abruptly rose from 7.18±1.84 to 18.58±1.48 mg-N/100g between 3rd and 5th days of storage. Again, this value increased markedly from the 18th day onwards (APPENDIX - 1). The relationship between storage days and TVB-N was found to be highly positive (APPENDIX – 2a) while between

organoleptic score and TVB-N content , the correlation was found to be negative (APPENDIX – 2b). APPENDIX – 9b indicates that there is significant differences ($P \leq 0.05$) between storage days and TVB-N content as the calculated value 'F' (58.3584) was found to be higher than the tabulated value 'F' (2.0548).

4.1.1.2.2 Trimethylamine Nitrogen (TMA-N)

The result of the TMA-N is presented in Fig – 3 and APPENDIX - 1. Initially the value of TMA-N was low (1.10±0.01 mg-N/100g) which increased very slowly as the storage days progressed. On the 10th day, when the fish was 'just acceptable (JA)', TMA-N value was just 2.52±0.22 mg-N/100g. Even until the end of the storage period (25th day), TMA-N value increased only to 8.08±0.17 mg-N/100g. There has not been much abrupt changed in TMA-N content with increase of time. There has been excellent positive correlation between TMA-N value and storage time with 'r' value indicating 0.95 (APPENDIX – 2a). The correlation between organoleptic score and TMA-N content was found to be highly negative (r = -0.969) as shown in APPENDIX – 2b. Statistical analysis as observed in APPENDIX – 9b indicates that there is significant differences ($P \leq 0.05$) between storage day and TMA-N content where calculated value 'F' (8.0342) is higher than the tabulated value 'F' (2.0548).

4.1.1.2.3 Trimethylamine Oxide Nitrogen (TAMO-N)

The value of TMAO-N was found to 4.11±0.14 mg-N/100g at the beginning of the experiment. With the progress of the investigation , this value decreased slowly and at the 10th day of storage period, when the fish was 'just acceptable (JA)', the value was 2.23±0.1 mg-N/100g. This value slightly decreased to 1.98±0.06mg-N/100g on the 13th day. However, from the 14th day onward until the end of the experiment, TMAO-N could not be detected (Fig – 4 and APPENDIX – 1). The correlation between TMAO-N and storage time indicated a highly negative relationship (r = -0.97) as observed for a value from the 1st day until 13th days of storage as no value could be detected then in the later period (APPENDIX – 2a) and from APPENDIX – 2b, it is evident that organoleptic score showed a highly positive correlation (r = -0.99) with TMAO-N content. In the

similar manner, there is a non-significant differences (P≤0.05) between storage days and TMAO-N content as shown in APPENDIX – 9b.

4.1.1.2.4 pH

pH in tilapia was found to low (6.56±0.11) on the 1st day of iced storage (Fig – 1). This value increased progressively with time and was 6.97±0.01 on the 10th day of storage, when the fish was 'just acceptable (JA)'(APPENDIX - 1). This value of pH increased and was on the alkaline side. On the 25th days of storage, pH value was 7.41±0.13. There was also highly positive relationship (r = 0.95) between strong days and pH (APPENDIX – 2a) and from APPENDIX – 2b, the correlation between organoleptic score and pH was found to be highly negative (r = -0.99). Statistical analysis (APPENDIX – 9b) indicates a non-significant differences (P≤0.05) between storage time and pH as the calculated 'F' (1.2737) was lower than the tabulated value 'F' (2.0548).

4.1.1.3 Microbiological (Total Viable Count)

The initial (1st day) count of tilapia was $\log_{10}$5.33±0.07 cfu/gm (APPENDIX - 1). It increased to one log cycle ($\log_{10}$6.07±0.03 cfu/gm) on the 3rd day of storage while there was no distinct increased ($\log_{10}$6.08±0.03 cfu/gm) on the 15th days of storage trials (Fig-1). However on the 10th days of storage, when the fish was 'just acceptable (JA)', the value of TVC was found to be $\log_{10}$6.90±0.05 cfu/gm . This value gradually increased and was $\log_{10}$8.92±0.49 cfu/gm at the end of 22 days in ice. However, on the 25th day, TVC could not be detected as the plates were over populated. The correlation between TVC and storage days was found to be highly positive (r = 0.90) as only 22 days were counted for the relationship (APPENDIX – 2a). APPENDIX – 2b also indicates highly negative relationship (r = -0.948) between organoleptic score and TVC. In the similar manner, within the same storage time (22 days), it was observed that there is significant differences (P≤0.05) between TVC and storage trials (APPENDIX – 9c).

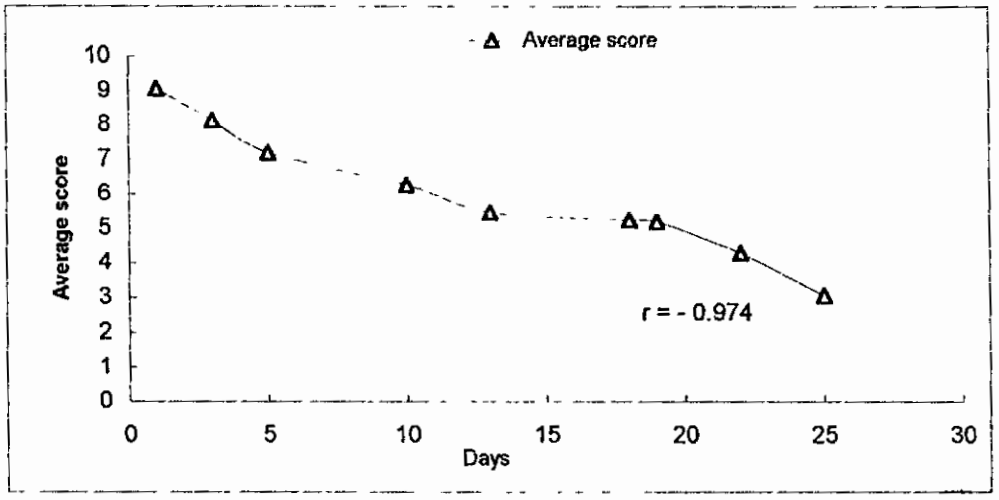


Fig. 1 Organoleptic score of *Tilapia nilotica* during iced storage

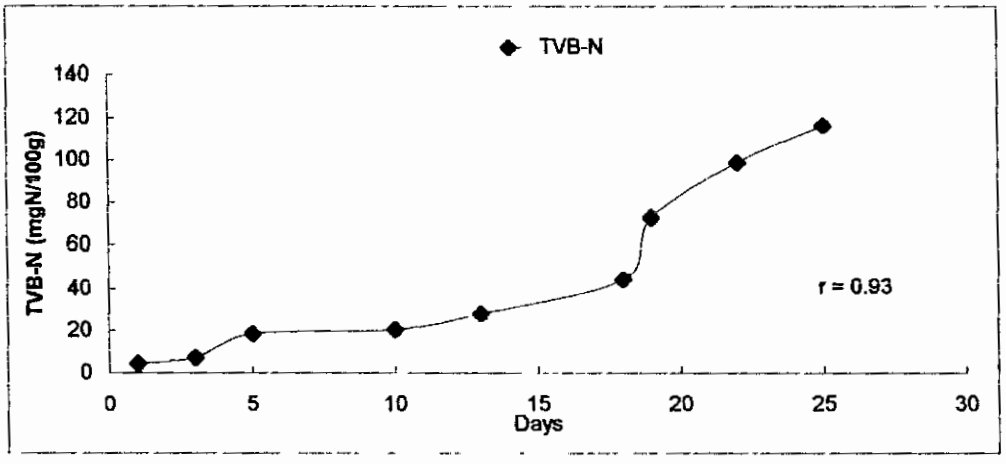


Fig. 2 TVB-N content of *Tilapia nilotica* during iced storage

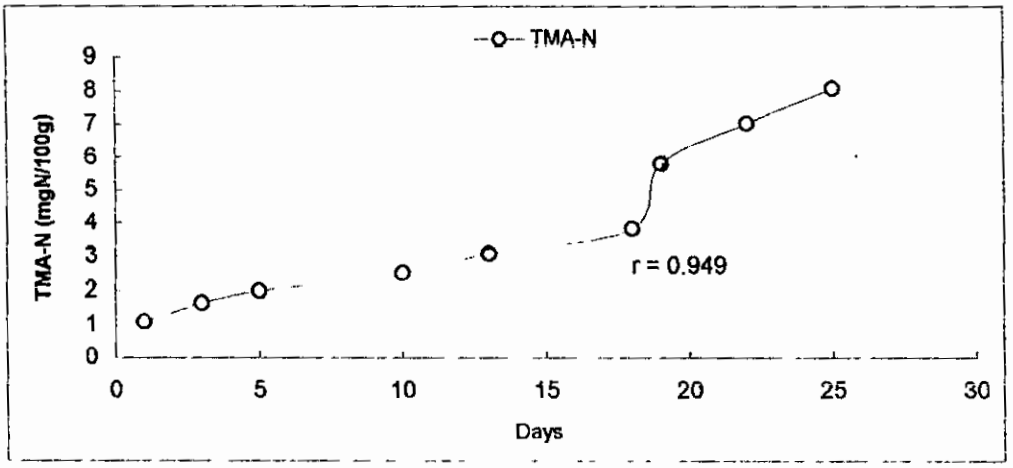


Fig. 3 TMA-N content of *Tilapia nilotica* during iced storage

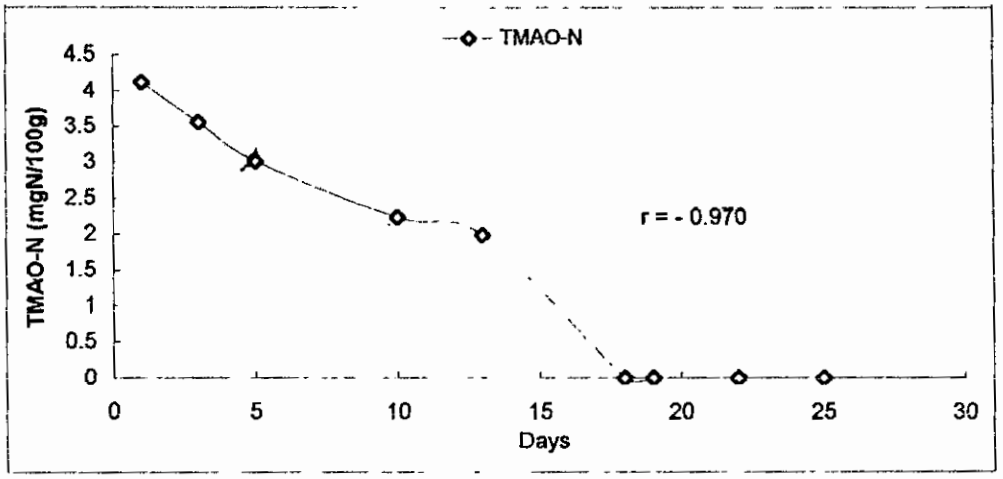


Fig. 4 TMAO-N content of *Tilapia nilotica* during iced storage

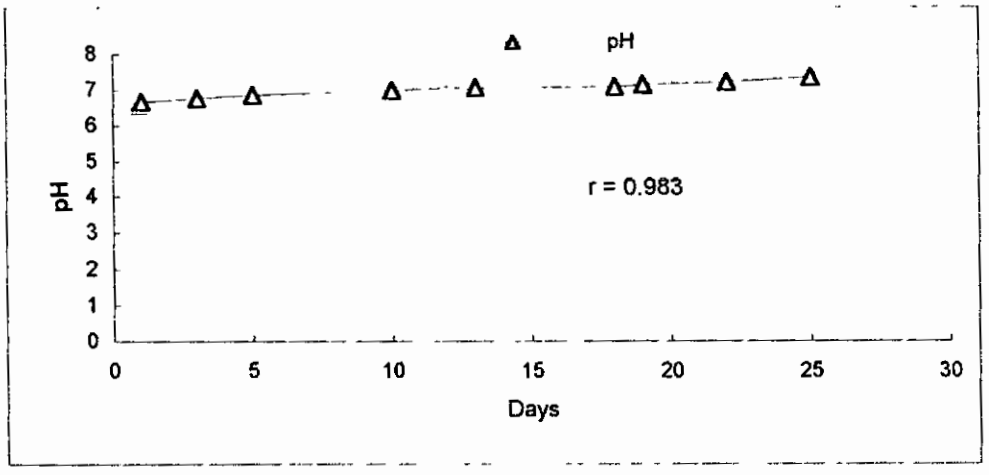


Fig. 5 pH in *Tilapia nilotica* during iced storage

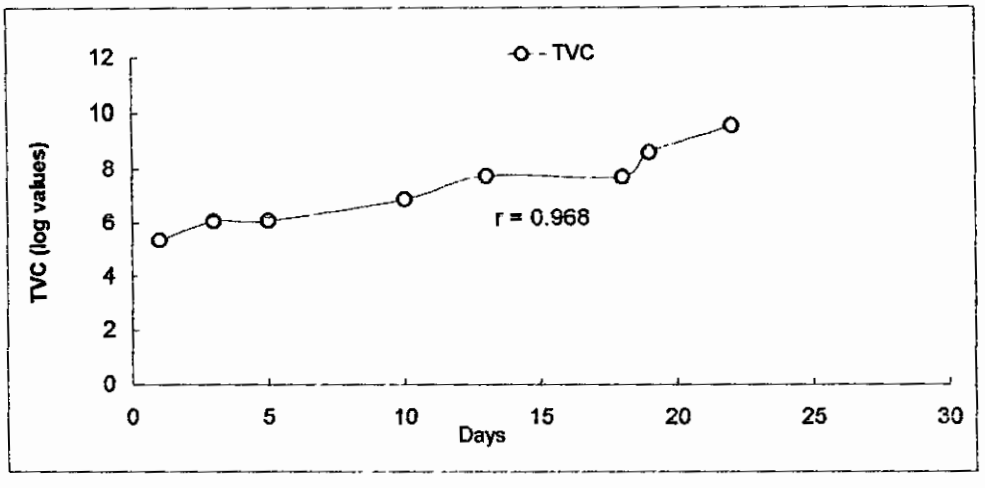


Fig. 6 Total Viable Count of *Tilapia nilotica* during iced storage

4.1.2 *Lates calcarifer*

4.1.2.1 Organoleptic

Fig – 7 and APPENDIX - 3 illustrates the result of organoleptic changes of bhetki during iced storage for a period of 25 days. The average score provided by the panellists on the 1st day of storage period was 8.67 which indicated 'highly acceptable (HA)' quality rating. This value slightly decreased with time until the 5th day when the score was 7.28. The score further decreased to 6.35 when bhetki was 'just acceptable (JA)'. It is very interesting to note that the pattern of changes in the score during the next 8 days remained with in a range which was recorded as 'just acceptable (JA)' by the panellists until 18th days of storage periods. Thus bhetki had a shelf life of 18 days stored in ice. From 18th day onwards, the fish became 'just unacceptable (JU)'. The correlation between storage days and organoleptic score was found to be highly negative (-0.98) as shown in APPENDIX – 4a. There is significant differences ($P \leq 0.05$) between storage day and organoleptic score. This is shown in APPENDIX – 10a where the calculated 'F' (6.0751) is higher than the tabulated value 'F' (2.0548).

4.1.2.2 Biochemical

4.1.2.2.1 Total Volatile Base Nitrogen (TVB-N)

The value of TVB-N in bhetki increased sharply from 4.24±0.02 to 32.90±1.88 mg-N/100g on the 18th day which was considered as 'just acceptable (JA)' (APPENDIX - 3 and Fig - 8). However, between the 10th and 18th day, when the quality rating was 'JA', TVB-N value increased from 21.02±0.50 to 32.90±1.88 mg-N/100g. Thereafter, TVB-N value increased enormously on the 25th days of storage when the value was 132.46±2.39 mg-N/100g. Positive correlation ($r = 0.87$) was observed between storage days and TVB-N content which is shown in APPENDIX – 4a and organoleptic score showed negative correlation ($r = -0.864$) with TVB-N content as shown in APPENDIX – 4b. APPENDIX – 10b indicates a significant differences ($P \leq 0.05$) between storage days and TVB-N where calculated value 'F' (65.3540) is higher than the tabulated value 'F' (2.0548).

4.1.2.2.2 Trimethylamine Nitrogen (TMA-N)

The pattern of spoilage in relation to TMA-N content in bhetki is shown APPENDIX - 3 and Fig – 9. TMA-N content increased almost six times (6.02±0.28 mg-N/100g) on the 5th day compared to TMA-N content on the 1st day (1.67±0.47 mg-N/100g). Until the 18th day when the fish was 'just acceptable (JA)' as judged by the panellists, TMA-N showed a value of 9.56±1.42 mg-N/100g. This value abruptly increased to 26.05±0.53 mg-N/100g on the 19th day, which was almost doubled (41.76±1.42 mg-N/100g) on the 22nd day. The value of TMA-N content on the 25th day was recorded as 61.84±5.1 mg-N/100g. The correlation between storage days and TMA-N content was found to be positive (r = 0.84) which is shown in APPENDIX – 4a and negative correlation (r = -0.846) was observed between organoleptic score and TMA-N content as shown in APPENDIX – 4b. Statistical analysis as shown in APPENDIX – 10b, indicates significant differences (P≤0.05) between storage days and TMA-N content as calculated value 'F' (151.40) is higher than the tabulated value 'F' (2.0548).

4.1.2.2.3 Trimethylamine Oxide Nitrogen (TMAO-N)

The value of TMAO-N reduced progressively as the days of storage increased (Fig - 10). On the 18th days of storage, TMAO-N value came down to 26.05±0.53 mg-N/100g from an initial value of 76.21±0.41 mg-N/100g. At the end of the storage trial, this value recorded 14.79±0.94 mg-N/100g (APPENDIX – 3). The correlation between storage days and TMAO-N was found to be highly negative (r = -0.98) as shown in APPENDIX – 4a, while between organoleptic score and TMAO-N, a highly positive correlation (r = 0.965) was observed from APPENDIX – 4b. There is clearly a significant differences (P≤0.05) observed between storage days and TMAO-N content where calculated value 'F' (23.1875) is higher than the tabulated value (APPENDIX – 10b).

4.1.2.2.4 pH

pH varied between 6.42±0.17 to 7.47±0.16 over a range of 25 days of storage trial (Fig - 11). Between the 10th and 18th days, the pH value varied between 6.91±0.92 to

7.03±0.14 (APPENDIX - 3). The pH indicated alkalinity from 19 day onwards. The correlation between storage days and pH was found to be highly positive (r = 0.98) which is evident from (APPENDIX – 4a) and, correlation between organoleptic score and pH was found to be highly negative (r = -0.982) as shown in APPENDIX – 4b. Statistical analysis as shown in APPENDIX – 10b, indicates a non-significant differences (P≤0.05) between storage days and pH where calculated value 'F' (1.4672) is lower than the tabulated value 'F' (2.0548).

4.1.2.3 Microbiological (Total Viable Count)

The value of TVC for bhetki fish stored in ice during period of 25 day is provided in Fig – 12 and APPENDIX - 3. It is observed that the initial value of TVC was relatively lower ($\log_{10}$5.26±0.04 cfu/gm). This increased slightly to $\log_{10}$6.06±0.06 cfu/gm on the 3rd day and remained almost within the same range on the 5th days of storage. However, on the 10th day, when the fish was acceptable organoleptically, TVC count indicated a value of $\log_{10}$6.75±0.04 cfu/gm. While on the 18th day, which was again recorded as 'just acceptable (JA)' by taste panellists, showed a value of $\log_{10}$6.77±0.08 cfu/gm. On the 22nd day, the TVC value was high as $\log_{10}$9.56±0.06 cfu/gm. However, TVC could not be counted on the 25th day because of enormous colonies. Positive correlation (r = 0.88) was found between storage days and TVC as shown in APPENDIX – 4a and, highly negative correlation (r = -0.95) between organoleptic score and TVC, which is evident from APPENDIX – 4b. Statistical analysis (APPENDIX – 10c) showed that there is significant differences (P≤0.05) between storage day and TVC as calculated value 'F' (4.7598) is higher than the tabulated value 'F' (2.0548).

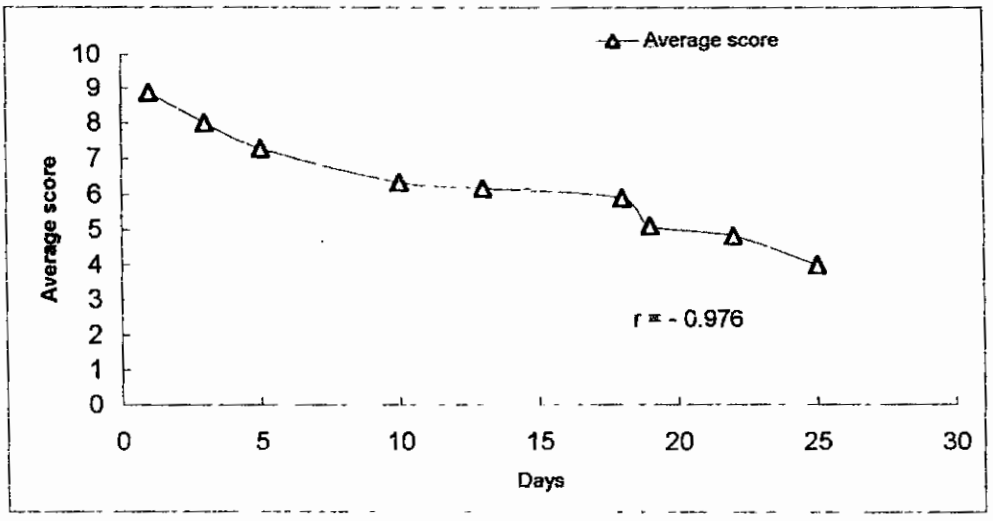


Fig. 7 Organoleptic score of *Lates calcarifer* during iced stoage

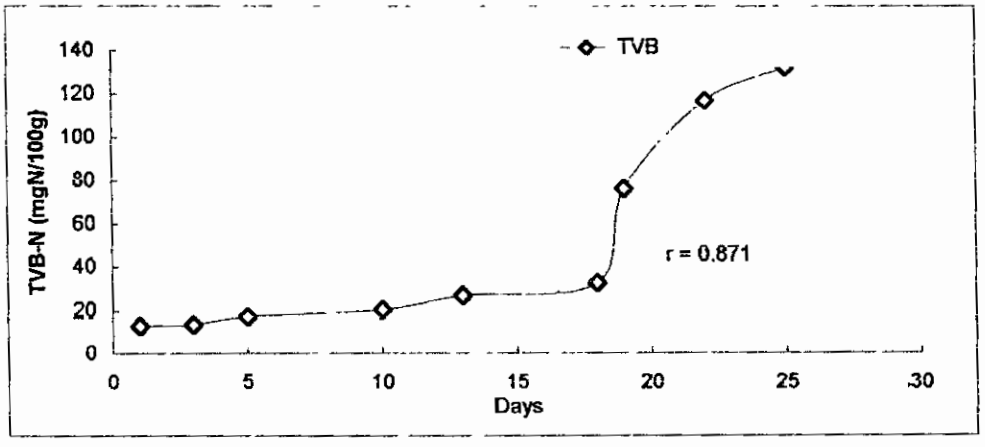


Fig. 8 TVB-N changes in *Lates calcarifer* during iced storage

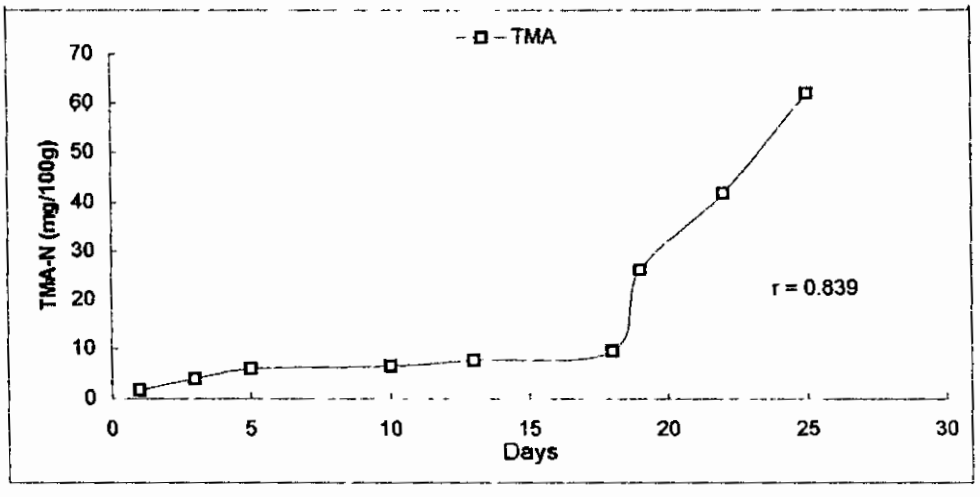


Fig. 9 TMA-N changes in *Lates calcarifer* during iced storage

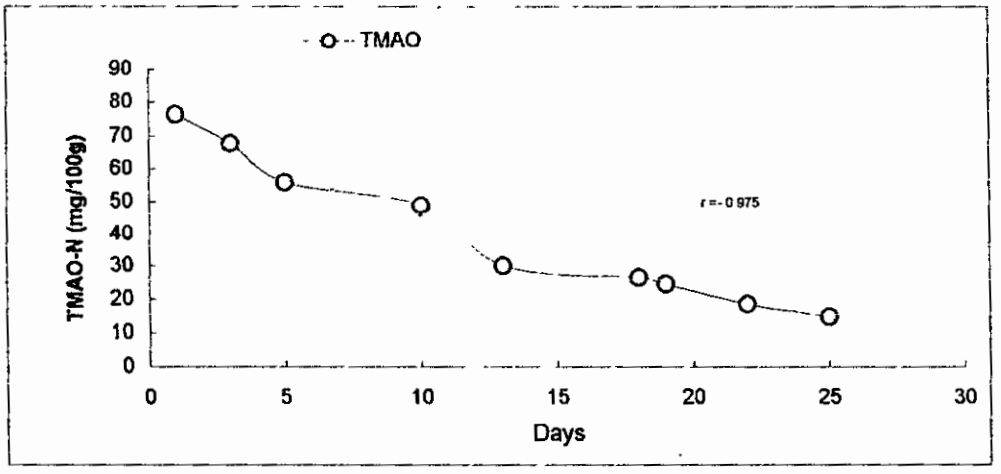


Fig. 10 TMAO-N changes in *Lates calcarifer* during iced storage

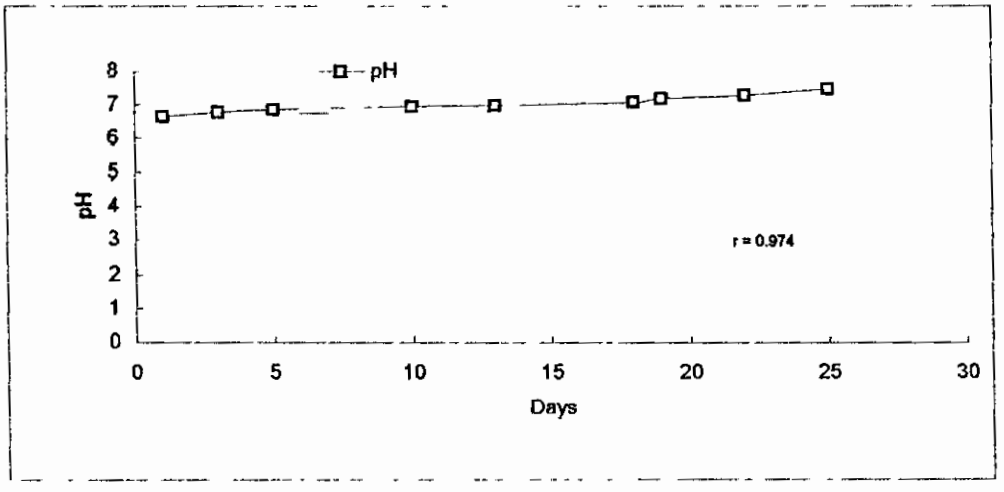


Fig. 11 pH in *Lates calcarifer* during iced storage

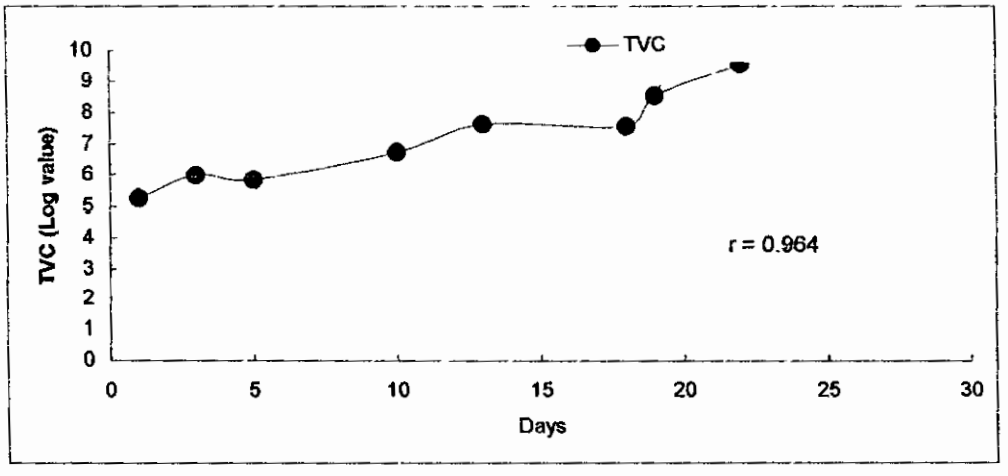


Fig. 12 Total Viable Count of *Lates calcarifer* during iced storage

4.2 Quality Changes of Fish During Storage at Ambient Temperature

4.2.1 *Tilapia nilotica*

4.2.1.1 Organoleptic

Quality deterioration of tilapia stored at ambient temperature in relation to organoleptic characteristics is shown in Fig – 13 and APPENDIX - 5. On an average score from quality characteristics, it is clearly evident that the score decreased steadily with time. Initially on the 0^{th} hour, tilapia was found to be very good quality. It was judged as 'highly acceptable (HA)' by the panellists who provided an average score of 9.11. As recorded by the panellists, tilapia, was 'just acceptable (JA)' till the 10^{th} hour of storage periods. On the 10^{th} hour, the average score was 6.27. At the end of storage trials, the score decreased to 3.37. The correlation between storage time and organoleptic score was found to be highly negative (r = -0.99) as evident from APPENDIX – 6a. Statistical analysis (APPENDIX – 11a) shows significant differences ($P \leq 0.05$) between storage days and organoleptic score where calculated 'F' (7.2344) is higher than the tabulated value 'F' (2.3860).

4.2.1.2 Biochemical

4.2.1.2.1 Total Volatile Base Nitrogen (TVB-N)

TVB-N content of tilapia was found to be in relatively smaller amount (3.58±1.34 mg-N/100g) on the 0^{th} hour, which further increased to 5.71±1.48 mg-N/100g on the 5^{th} hour. However, TVB-N content increased abruptly to 31.24±0.1 mg-N/100g on the 10^{th} hour, which was recommended as Acceptable time by the panellists as judged organoleptically. In the later period, TVB-N further increased and on the 24^{th} hour, tilapia was found to contain 114.47±3.81 mg-N/100g (APPENDIX - 5 and Fig - 14). Storage period showed a highly positive correlation (r = 0.95) with TVB-N content which is shown in APPENDIX – 6a while a highly negative correlation (r = -0.960) was observed between organoleptic score and TVB-N, as shown in APPENDIX – 6b. There

is clearly a significant differences (P≤0.05) between storage periods and TVB-N content (APPENDIX – 11b), where calculated value 'F' (66.7131) is higher than the tabulated value 'F' (2.3860).

4.2.1.2.2 Trimethylamine Nitrogen (TMA-N)

The value of TMA-N was found to be quite low in tilapia on the 0th hour (1.67± 0.07 mg-N/100g). It is reduced to meagre 2.02±0.08 mg-N/100g TMA-N content on the 10th hour, when the fish was 'just acceptable (JA)'. On the next 17th and 20th hour, TMA-N value increased slightly (2.27±0.07 mgN/100 and 3.81±1.21 mg-N/100g). On the 24th hour, this value almost doubled to 6.91±0.03 mg-N/100g (APPENDIX - 5 and Fig - 15). The correlation between storage periods and TMA-N was found to be positive (r = 0.89) as shown in APPENDIX – 6a and highly negative correlation (r = -0.910) between organoleptic score and TMA-N which is shown in APPENDIX – 6b. From APPENDIX – 11b, there is significant differences (P≤0.05) between storage periods and TMA-N where calculated value 'F' (5.3789) is higher than the tabulated value 'F' (2.3860).

4.2.1.2.3 Trimethylamine Oxide Nitrogen (TMAO-N)

The result of TMAO-N of tilapia is listed in APPENDIX - 5 and Shown in Fig – 16. On the initial hour (0th hour), TMAO-N was 4.14± 0.78 mg-N/100g, which reduced further to a value of 1.26±0.19 mg-N/100g on the 10th hour. This was the time for the limit of acceptability judged by the panellists. It is very interesting to note that TMAO-N could not be further detected form the 10th hour onwards. Highly negative correlation (r = -0.93) was observed between storage periods and TMAO-N which is shown in APPENDIX – 6a and the correlation between organoleptic score and TMAO-N was found to be positive (r = 0.876) as shown in APPENDIX – 6b. From APPENDIX – 11b, statistical analysis indicates a significant differences (P≤0.05) between storage periods and TMAO-N content.

4.2.1.2.4 pH

The value of pH did not shift much between 0^{th} and 5^{th} hour (6.43±0.05 and 6.51±0.08). On the 10^{th} hour (limit of acceptability), pH value was found to be 6.59±0.06. It then continued to increased at a steady rate and indicated a value of 7.57±0.05 on the 24^{th} hours of storage (APPENDIX - 5 and Fig- 17). APPENDIX – 6a indicated a highly positive correlation (r = 0.92) between storage periods and pH while negative correlation (r = -0.898) was observed between organoleptic score and pH (APPENDIX – 6b). There is clearly a non-significant differences (P≤0.05) between storage periods and pH as shown in APPENDIX – 11b, where calculated value 'F' (1.6553) is lower than the tabulated value 'F' (2.3860).

4.2.1.3 Microbiological (Total Viable Count)

The result of TVC is shown in APPENDIX - 5 and Fig – 18. Initially the TVC was low ($\log_{10}$4.38±0.04 cfu/gm) which increased to only one log cycle higher ($\log_{10}$5.71±0.06 cfu/gm) on the 10^{th} hour, the time that was considered 'just acceptable (JA)'. In the next 17^{th} hour, this value increased to $\log_{10}$6.94±0.04 cfu/gm. However, on the 20^{th} and 24^{th} hour, TVC drastically increased to a value $\log_{10}$8.83±0.18 cfu/gm and $\log_{10}$9.73±0.14 cfu/gm respectively. APPENDIX – 6a indicated a highly positive correlation (r = 0.95) between storage periods and TVC while highly negative correlation (r = -0.95) was observed between organoleptic score and TVC which is shown in APPENDIX – 6b. However, from APPENDIX – 11c , statistical analysis shows a significant differences (P≤0.05) between storage periods and TVC, where calculated value 'F' (10.7792) is grater than the tabulated value 'F' (2.3860).

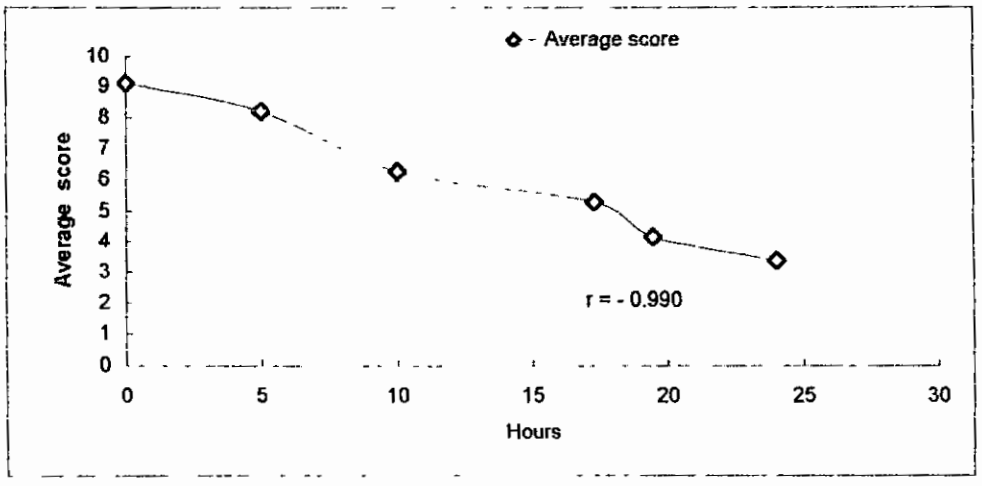


Fig. 13 Organoleptic score of *Tilapia nilotica* stored at ambient temperature

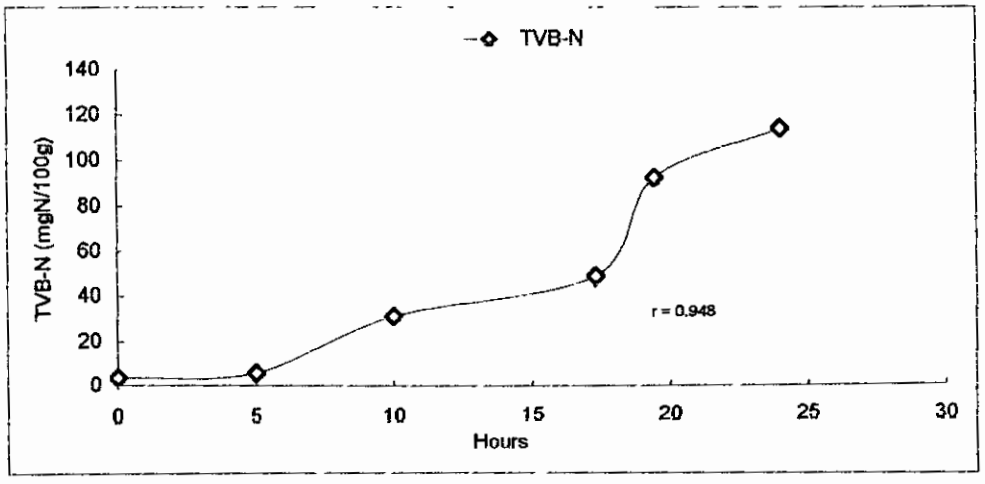


Fig. 14 TVB-N content of *Tilapia nilotica* stored at ambient temperature

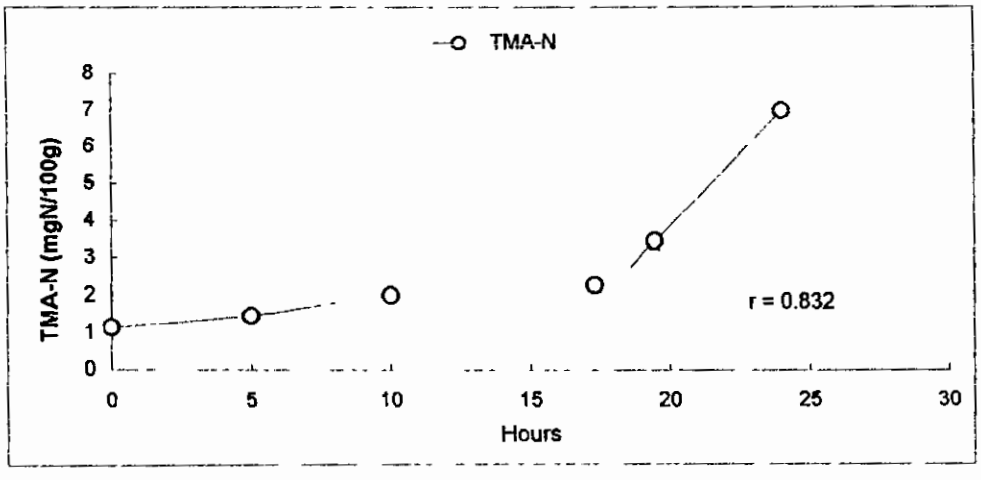


Fig. 15 TMA-N content of *Tilapia nilotica* stored at ambient temperatire

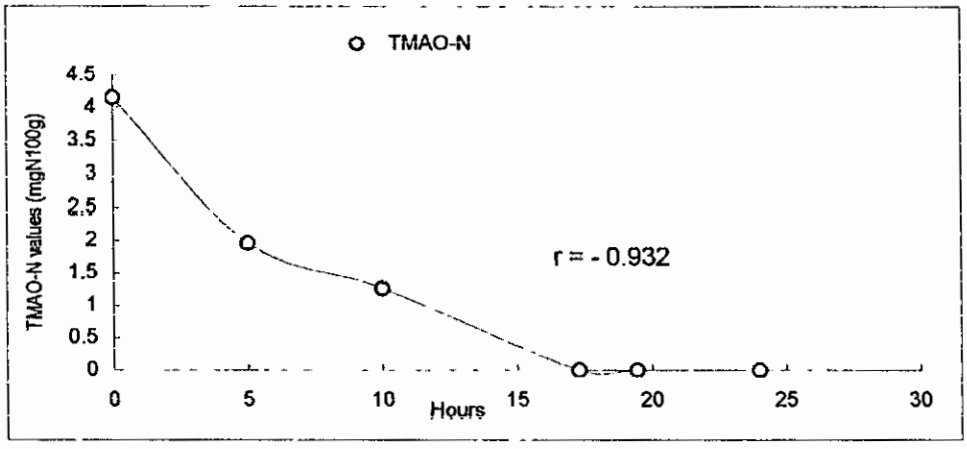


Fig. 16 TMAO-N content of *Tilapia nilotica* stored at ambient temperature

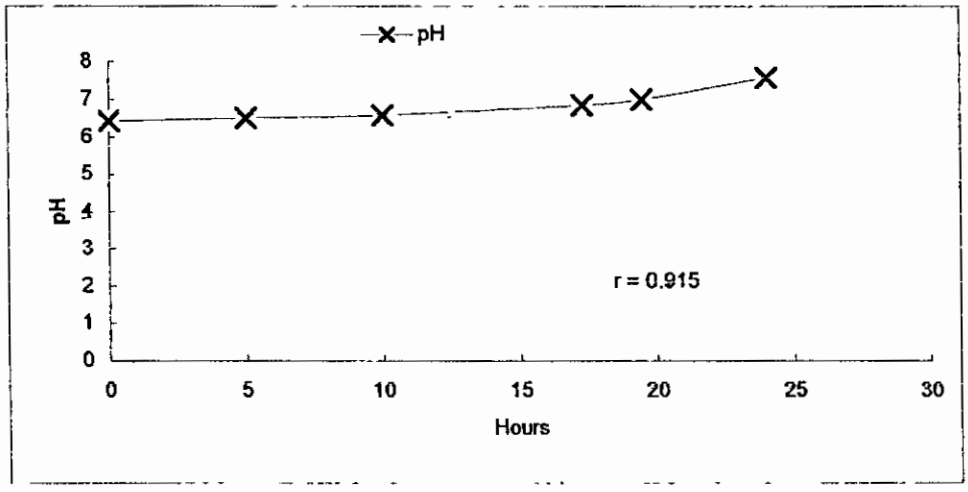


Fig. 17 pH of *Tilapia nilotica* stored at ambient temperature

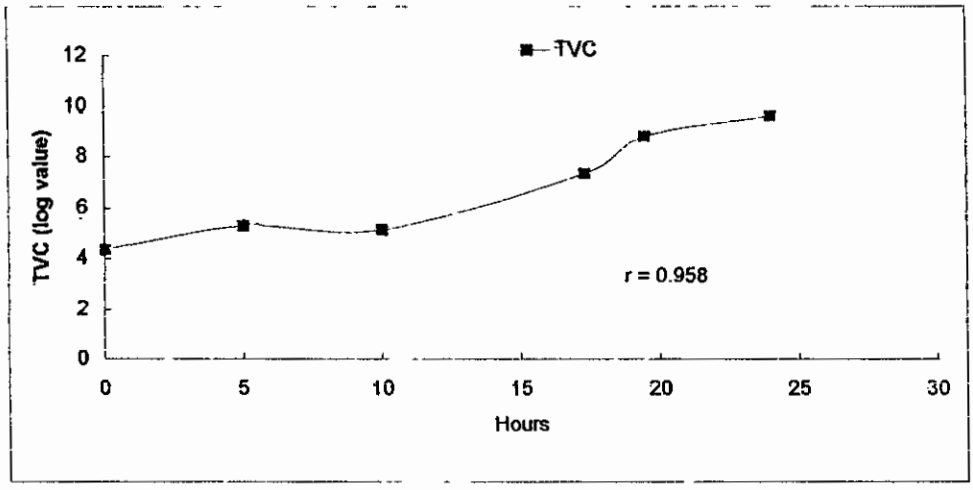


Fig. 18 Total Viable Count of *Tilapia nilotica* stored at ambient temperature

4.2.2 *Lates calcarifer*

4.2.2.1 Organoleptic

Very fresh bhetki was examined on the 0th hour for organoleptic changes. The taste panellists recommended the fish to be highly acceptable thus provided an average score of 9.16 on a 10 point scale. It is quite interesting to note that the shelf life bhetki remained acceptable (Just Acceptable) at the end of the 17th hour, when the score was 6.27 (APPENDIX - 7 and Fig - 19). The correlation between storage periods and organoleptic score was found to highly negative (r = -0.99) which is shown in APPENDIX – 8a, while statistical analysis indicates a significant differences (P≤0.05) between storage periods and organoleptic scores as shown in APPENDIX – 12a.

4.2.2.2 Biochemical

4.2.2.2.1 Total Volatile Base Nitrogen (TVB-N)

There has been a wide range of variation in the value of TVB-N in bhetki stored at ambient temperature (APPENDIX - 7 and Fig - 20). The value initially started with 4.42±1.1 mg-N/100g and increased almost twice the value until the 10th hour. On the 17th hour, TVB-N value was 27.88±1.41 mg-N/100g. This drastically increased to greater than 100 mg on the 20th and 24th hour sample. Storage time showed a positive correlation (r = 0.86) with TVB-N which is shown in APPENDIX - 8a and from APPENDIX – 8b, the correlation between organoleptic score and TVB-N was found to be highly negative (r = -0.915). APPENDIX – 12b indicates a significant differences (P≤0.05) between storage period and TVB-N.

4.2.2.2.2 Trimethylamine Nitrogen (TMA-N)

Initially the TMA-N value was found in the lower range (1.52±0.71 mg-N/100g) on the 0th hour. It then increased (9.41±0.04 mg-N/100g) steadily until 17th hour of storage at

ambient temperature, this was the time for the limit of acceptability. However, on the 20th hour, the value drastically increased to 40.75±2.50 mg-N/100g (Fig - 21). At the end of the experiment (24th hour), this value increased to 48.96±2.32 mg-N/100g (APPENDIX - 7). APPENDIX – 8a indicated a positive correlation (r = 0.85) between storage periods and TMA-N while the correlation between organoleptic score and TMA-N was found to be highly negative (r = -0.910) which is shown in APPENDIX – 8b. Statistical analysis shows a significant differences (P≤0.05) between storage periods and TMA-N as shown in APPENDIX – 12b.

4.2.2.2.3 Trimethylamine Oxide Nitrogen (TMAO-N)

The result of TMAO-N is presented in APPENDIX - 7 and shown in Fig – 22. From an initial value of 68.30±0.58 mg-N/100g, it decreased to a level of 13.70±2.00 mg-N/100g at the end of the investigation. On the 17th hour, when bhetki was just accepted, TMAO-N value was 24.97±0.28 mg-N/100g. The correlation between storage periods and TMAO-N content was found to be highly negative (r = -0.98) which is shown in APPENDIX – 8a while APPENDIX – 8b indicated a highly positive correlation (r = 0.968) between organoleptic score and TMAO-N. It is evident from the APPENDIX – 12b that there is clearly significant differences (P≤0.05) between storage period and TMAO-N where calculated 'F' value (25.6570) is higher than the tabulated value 'F' (2.3860).

4.2.2.2.4 pH

pH ranged from 6.83±0.05 to 7.53±0.05 in total storage periods (Fig - 23). During the initial period of storage, the value increased steadily which on the 17th hour (JA), was found to be 7.10±0.05 (APPENDIX - 7). The correlation between storage periods and pH (APPENDIX – 8a) was found to highly positive (r = 0.94) where highly negative (r = -0.964) correlation was observed between organoleptic score and pH (APPENDIX – 8b). Statistical analysis shows a significant differences (P≤0.05) between storage periods and pH as shown in APPENDIX – 12b.

4.2.2.3 Microbiological (Total Viable Count)

From a very low initial value of $\log_{10}4.40\pm0.05$ cfu/gm , the TVC increased to $\log_{10}6.91\pm0.05$ cfu/gm on the 17th hour sample, the time where panellists recommended the fish was just acceptable (Fig – 24 and APPENDIX - 7). At the end of the storage trial, TVC showed a value of $\log_{10}8.73\pm0.11$ cfu/gm. From APPENDIX – 8a, highly positive correlation (r = 0.97) was observed between storage periods and TVC. The correlation between organoleptic score and TVC was found to be highly negative (r = -0.971) which is shown in APPENDIX – 8b. APPENDIX – 12c indicates a significant differences between storage period and TVC where calculated value 'F' (6.81) is higher than the tabulated value 'F' (2.3860).

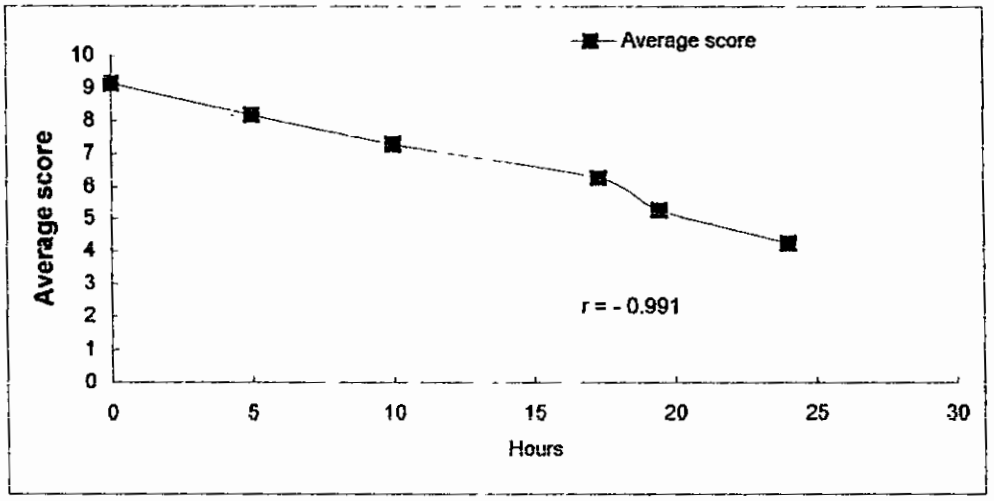


Fig. 19 Organoleptic score of *Lates calcarifer* stored at ambient temperature

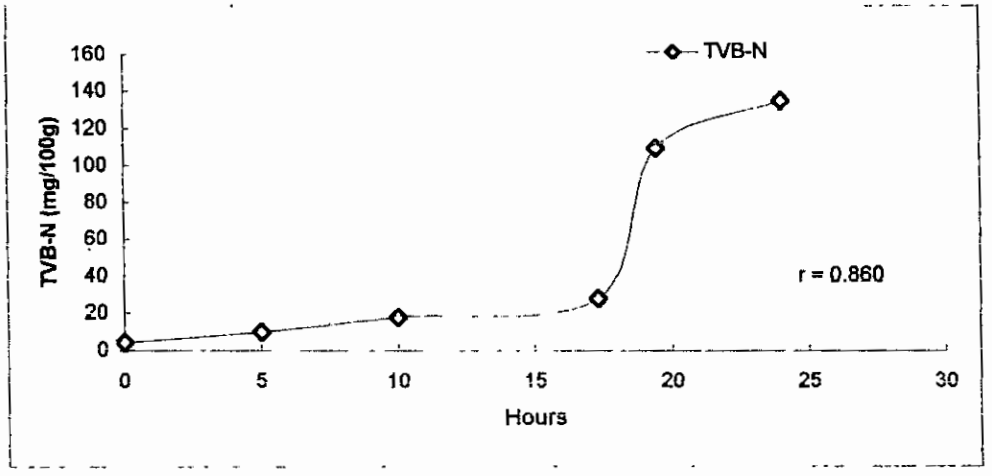


Fig. 20 TVB-N changes in *Lates calcarifer* stored at ambient temperature

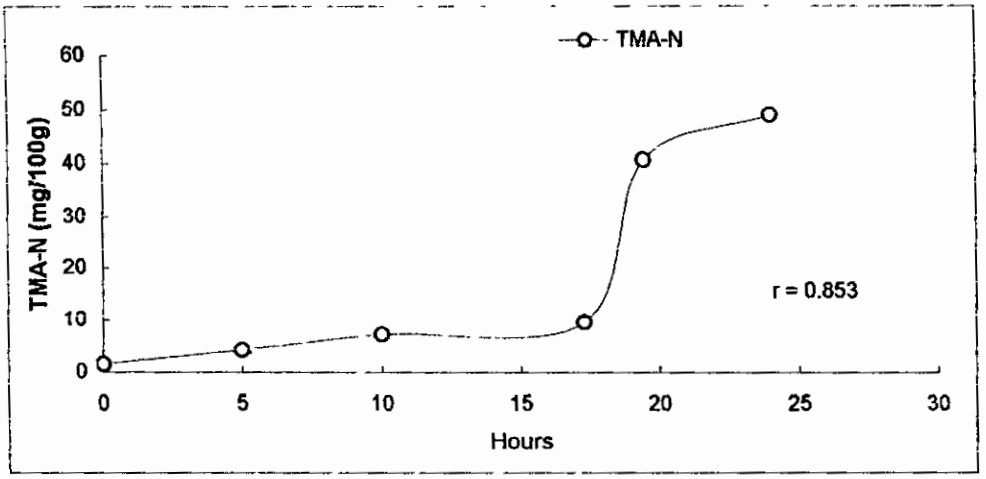


Fig. 21 TMA-N changes in *Lates calcarifer* stored at ambient temperature

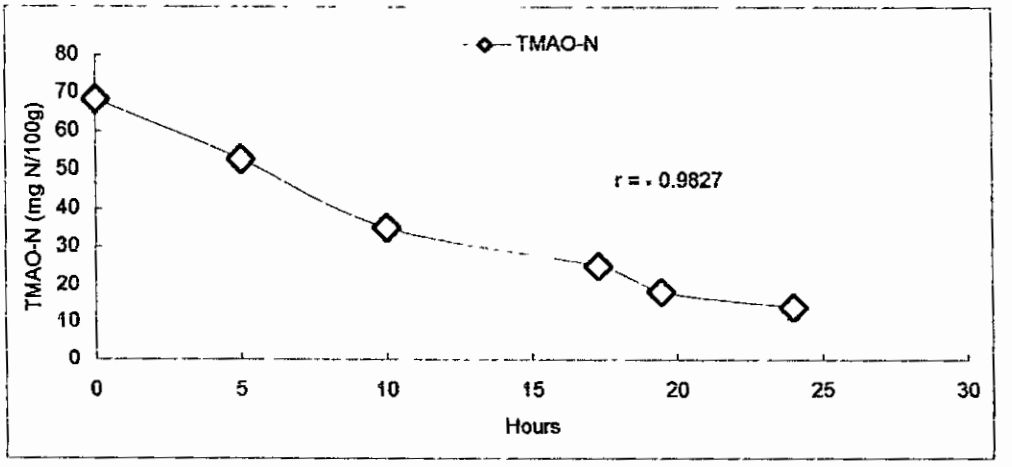


Fig. 22 TMAO-N changes in *Lates calcarifer* stored at ambient temperature

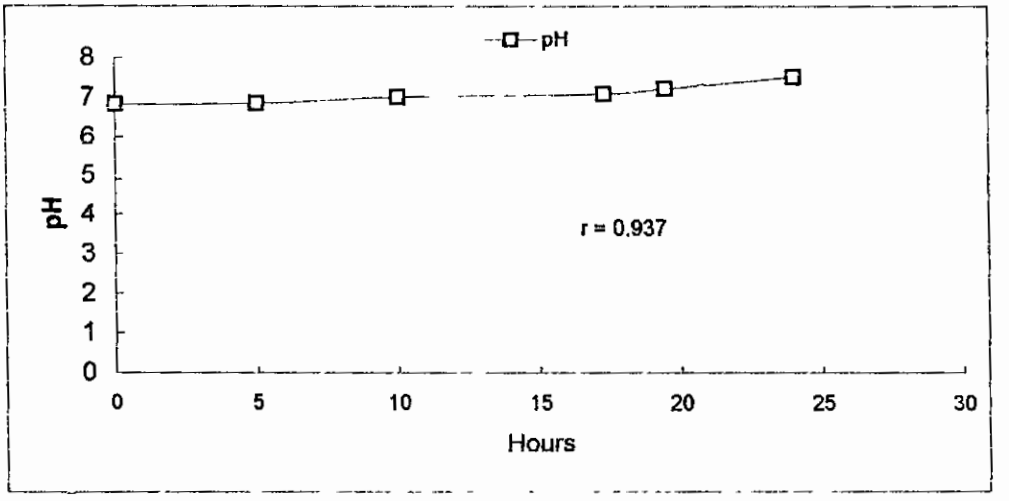


Fig. 23 pH in *Lates calcarifer* stored at ambient temperature

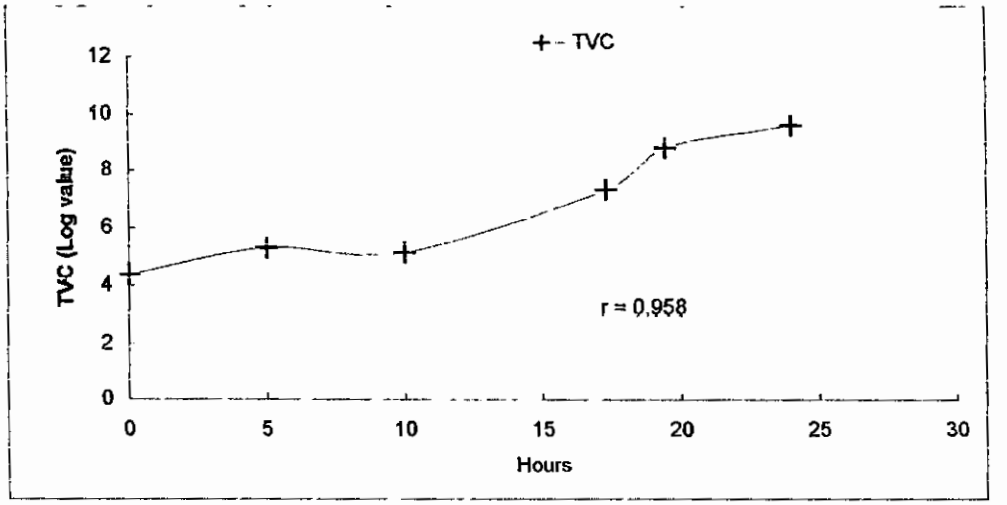


Fig. 24 Total Viable Count in *Lates calcarifer* stored at ambient temperature

4.3 Comparative Study of Quality Changes of *Tilapia Nilotica* and *Lates Calcarifer* During Iced Storage

4.3.1 Organoleptic

Fig – 25 illustrates the comparative organoleptic changes in *Tilapia nilotica* and *Lates calcarifer* during 25 days of iced storage trial. The average score of the both species , provided by the panellists on the 1st day of storage period was 9.08 and 8.87 which indicated 'Highly Acceptable (HA)' quality. These score decreased gradually in the same pattern over the range of 25 days iced storage trials. However, on the 10th day, the score for tilapia was 6.25 which on the 13th day decreased to 5.46. This marked the shelf life of tilapia which lied between 10 to 13 days while for bhetki, the score was 5.92 on the 18th day which then reduced to 5.10 indicating that the shelf life of bhetki fell between 18 to 19 days.

4.3.2 Biochemical

4.3.2.1 Total Volatile Base Nitrogen (TVB-N)

Spoilage pattern in relation to TVB-N content changes between tilapia and bhetki was shown in Fig – 26. The pattern of changes is clearly different. Initially the value of TVB-N in tilapia and bhetki were 4.49±1.91 mg-N/100g and 4.24±0.02 mg-N/100g. These values increased similarly up to 13 day which was almost same in both the species. But on the 18th day, TVB-N value in tilapia increased drastically to 44.64±1.91 mg-N/100g while for bhetki, the value was 32.90±1.88 mg-N/100g. From 18th day onwards, the value increased to a large content as shown in Fig – 26.

4.3.2.2 Trimethylamine Nitrogen (TMA-N)

TMA-N values of both tilapia and bhetki were found to be smaller amount (1.10±0.01 mg-N/100g and 1.67±0.47 mg-N/100g) on the initial day. As earlier, organoleptically it was observed that tilapia had a shelf life lied between 10 to 13 days, the corresponding

TMA-N value ranged between 2.52±0.22 to 3.01±0.13 mg-N/100g. In the same manner, thc shelf life for bhetki lied between 18 – 19 days. The TMA-N value within this storage time ranged between 6.59±0.14 to 9.56±1.42 mg-N/100g. It is evident from the Fig – 27 that the formation of TMA-N content is much higher in bhetki than tilapia as the storage time progressed.

4.3.2.3 Trimethylamine Oxide Nitrogen (TMAO-N)

During 25 days of iced storage trials, TMAO-N content was found to be much lower in tilapia than bhetki. In case of tilapia, TMAO-N value decreased slowly and on the 10^{th} day, the value was found to be only 2.23±0.10 mg-N/100g. However, from 13^{th} day onwards, no TAMO-N was detected (Fig - 28). For bhetki, initially TMAO-N value was found to be very high (76.21±0.42 mg-N/100g) which decreased progressively as the storage time increased. On the 18^{th} day, when the bhetki was judged 'just acceptable (JA)' by the panellists, the value of TMAO-N was 26.45±0.53 mg-N/100g. At the end of the investigation, the value of TMAO-N was reduced to 14.79±0.94 mg-N/100g.

4.3.2.4 pH

Quality changes of tilapia and bhetki during iced storage trial in relation to pH changes is shown in Fig – 29. The pattern of pH change is insignificant in both the species . The pH value ranged from 6.97±0.1 to 7.05±0.11 when the shelf life of tilapia had lied between 10 to 13 days as panellists on that storage time judged the fish 'just acceptable (JA)'. However, when the bhetki was judged 'just acceptable (JA)' by the panellists, the value of pH ranged from 7.03±0.14 to 7.21±0.16. The result of pH showed much higher value during iced storage.

4.3.3 Microbiological (Total Viable Count)

Fig – 30 illustrates the change of TVC of tilapia and bhetki during iced storage. The pattern of changes in both the species were almost same up to 10 days of storage trial. It was observed that when tilapia was judged 'just acceptable (JA)' by the panellists, TVC

ranged between $\log_{10}6.90\pm0.05$ cfu/gm and $\log_{10}7.72\pm o.05$ cfu/gm as shelf life lied between 10 – 13 days. However, between 10 to 18th day, when bhetki was found within limit of acceptability, the value of TVC ranged between $\log_{10}6.75\pm0.04$ to $\log_{10}6.77\pm0.08$ cfu/gm.

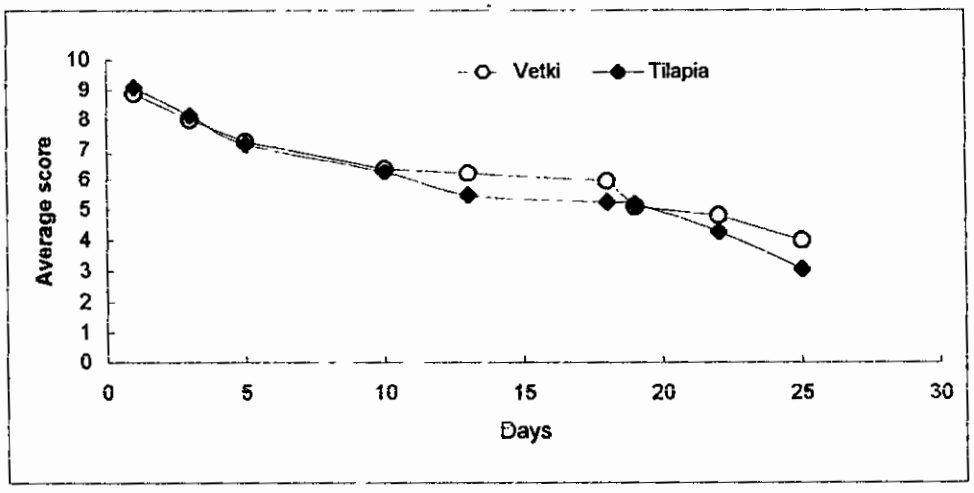


Fig. 25 Comparative study of organoleptic score of *Tilapia nilotica* and *Lates calcarifer* during iced storage

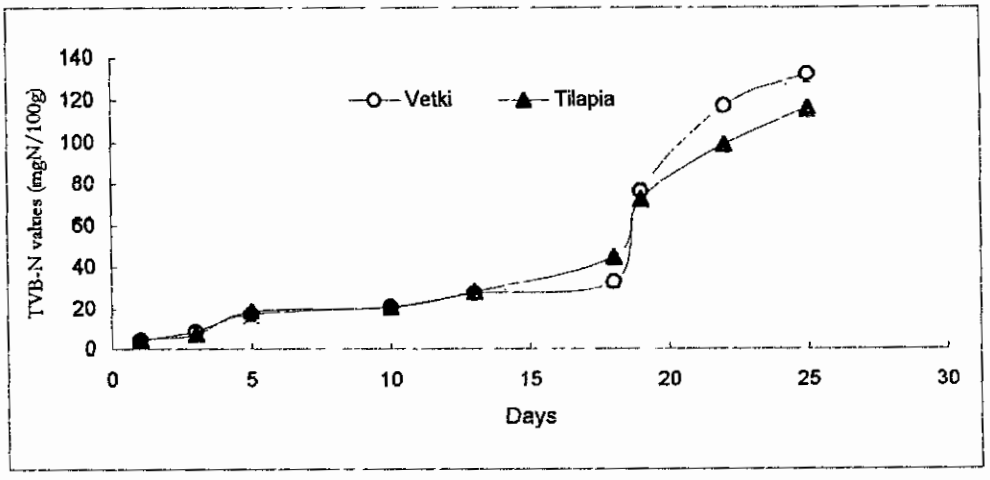


Fig. 26 Comparative study of TVB-N value in *Tilapia nilotica* and *Lates calcarifer* during iced storage

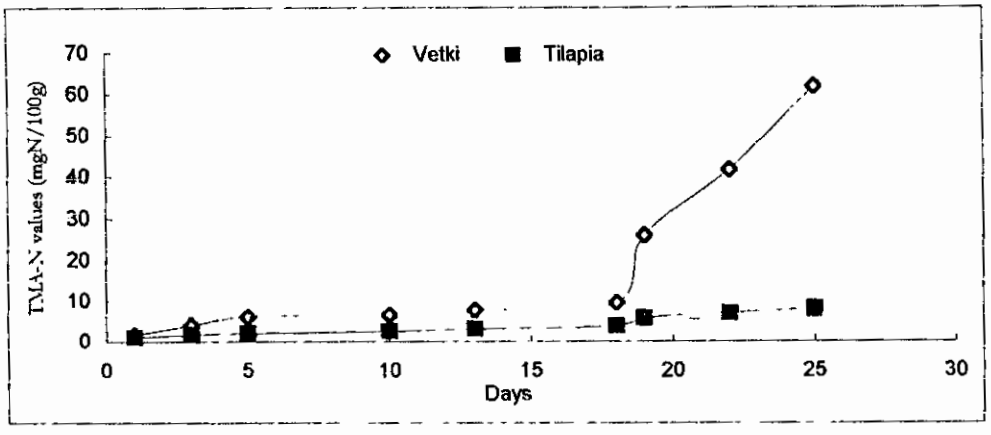


Fig. 27 Comparative study of TMA-N value in *Tilapia nilotica* and *Lates calcarifer* during iced storage

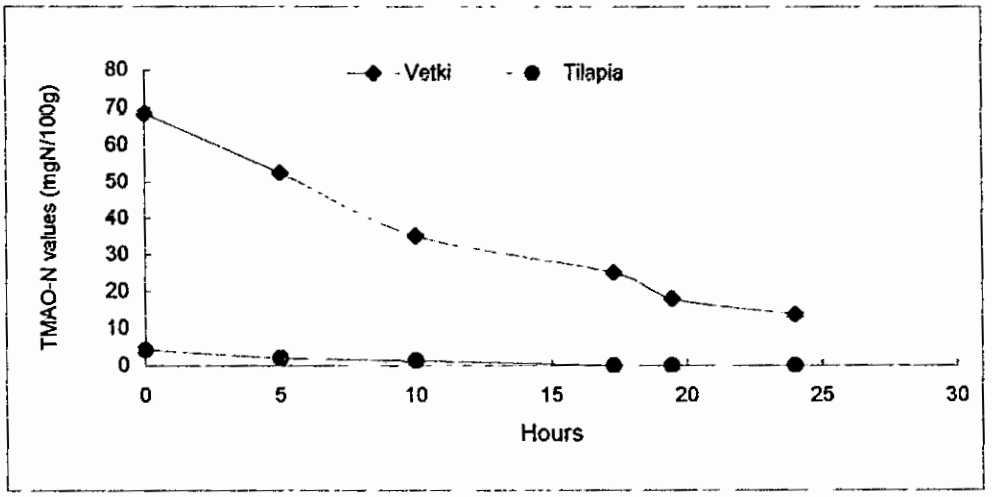


Fig. 34 Comperative study of TMAO-N value in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature

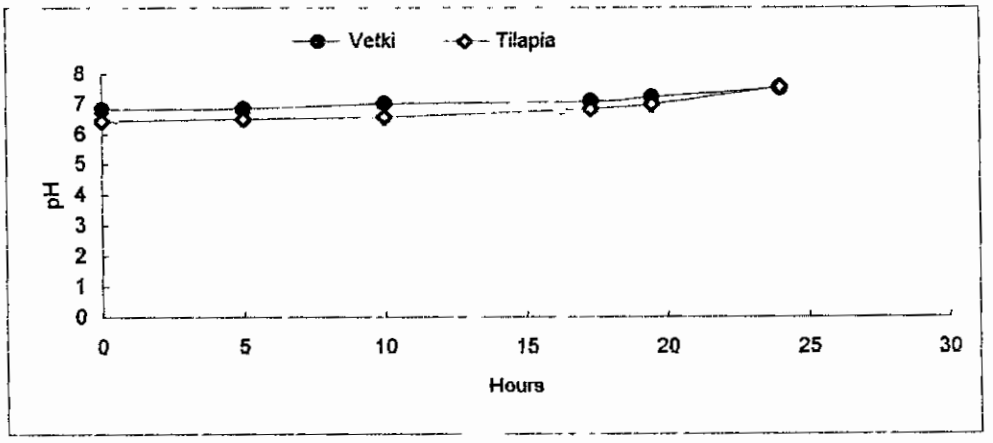


Fig. 35 Comperative study of pH in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature

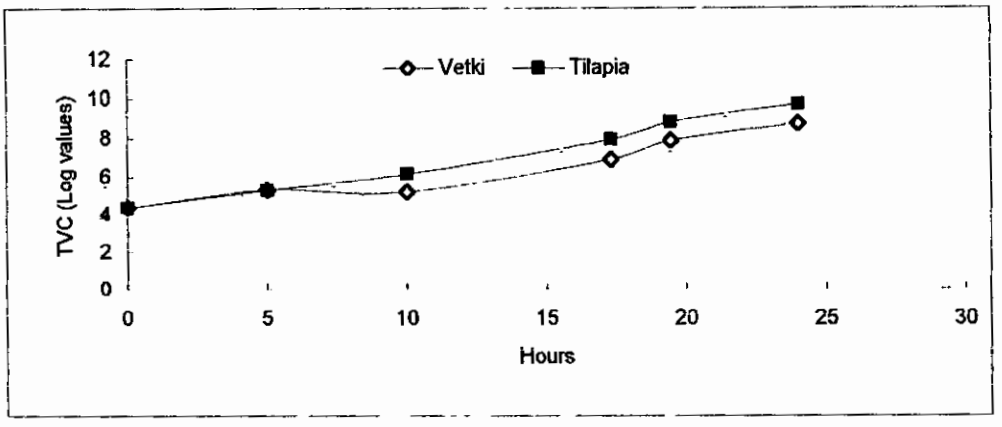


Fig. 36 Comperative study of Total Viable Count in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature.

Comparative study of tilapia and bhetki between different parameters (Organoleptic score, TVB-N, TMA-N and TVC) during iced storage

The result obtained from the comparative study between different parameters (Organoleptic score, TVB-N, TMA-N and TVC) is presented in Table - 6. Initially (on the 1st day) when tilapia and bhetki were found to be in very good quality (9.08 and 8.87), the corresponding TVB-N value was 4.49±1.91 mg-N/100g and 4.24±0.02 mg-N/100g respectively, while TMA-N value recorded as 1.1±0.01 mg-N/100g and 1.67±0.47 mg-N/100g and TVC ranged between $\log_{10}$5.33±0.07 cfu/gm and $\log_{10}$5.26±0.04 cfu/gm. As earlier, it is organoleptically evident that tilapia had a shelf life lied between 10 to 13 days when organoleptic score were between 6.25 to 5.46. At the same storage time, the score for bhetki was slightly higher (6.35 and 6.19). The value of TVB-N varied between 20.64±0.94 mg-N/100g and 28.03±1.42 mg-N/100g, which was close the value of TVB-N in bhetki (21.02±0.50 mg-N/100g and 27.31±1.78 mg-N/100g). The corresponding TMA-N value was lower (2.52±0.22 to 3.01±0.13 mg-N/100g, while for bhetki, 6.59±0.14 to 7.74±0.19 mg-N/100g . At the same storage time, TVC changes between tilapia and bhetki did not show any definite pattern of changes. For bhetki, from 10th day to 18th day, when the fish was judged as 'just acceptable (JA)' by the panellists, the organoleptic score in bhetki ranged between 6.35 to 5.92 where the value for tilapia varied between 6.25 to 5.24. During this storage periods, the change of TVB-N value was much higher in tilapia (20.64±0.94 to 44.64±1.91 mg-N/100g) than bhetki (21.02±0.50 to 32.90±1.88 mg-N/100g). From the 18th day onwards, this value drastically increased to 72.62±2.91 mg-N/100g and 76.51±3.07 mg-N/100g on the 19th day for both tilapia and bhetki. The value of TMA-N at the same storage time (10 to 18th day) were found to be much higher (6.59±0.14 to 9.56±1.42 mg-N/100g) in bhetki than tilapia (2.52±0.22 to 3.83±1.21 mg-N/100g). With regard to TVC, the value crossed the acceptable limit at the same storage periods (10 to 18th day).

The shelf life of tilapia and bhetki during iced storage trial with acceptable limit in relation to different parameters (organoleptic score, TVB-N, TMA-N and TVC) showed in Table - 7. In case of tilapia, when judged as 'just acceptable (JA)' by the panellists, the fish had a shelf life lied between 10 –13 days with corresponding organoleptic score

ranged between 6.25 to 5.42, TVB-N, TMA-N and TVC value ranged between 20.64±0.94 to 28.03±1.42, 2.52±0.22 to 3.01±0.13 mg-N/100g and $\log_{10}$6.90±0.05 to $\log_{10}$7.72±0.05 cfu/gm respectively. On the other hand for bhetki, the shelf life lied between 18 – 19 days during iced storage trial. Within this time, the organoleptic score ranged between 6.35 to 5.92, TVB-N value varied between 32.90±1.88to 76.51±3.05 mg-N/100g, TMA-N value extended between 9.56±1.42 to 26.05±0.2 mg-N/100g with corresponding TVC value ranged between $\log_{10}$6.77±0.08 to $\log_{10}$7.91±0.45 cfu/gm.

Table - 6 Comparative study of *Tilapia nilotica* and *Lates calcarifer* between different parameters (Organoleptic score, TVB-N TMA-N and TVC) during iced storage.

Storage day	Organoleptic score		TVB-N (mg-N/100g)		TMA-N (mg-N/100g)		TVC (log_{10}cfu/gm)	
	Tilapia	Bhetki	Tilapia	Bhetki	Tilapia	Bhetki	Tilapia	Bhetki
1	9.08	8.87	4.49 ±1.91	4.24 ± 0.02	1.10 ±0.01	1.67 ± 0.47	5.33 ±0.07	5.26 ± 0.04
3	8.14	8.00	7.18 ± 1.84	8.64 ± 0.21	1.66 ± 0.03	3.94 ± 1.11	6.07 ± 0.03	6.06 ± 0.06
5	7.19	7.28	18.58 ± 1.48	17.37 ± 1.14	2.01 ± 0.08	6.02 ± 0.28	6.08 ± 0.03	5.87 ± 0.06
10	6.25	6.35	20.64 ± 0.94	21.02 ± 0.50	2.52 ± 0.22	6.59 ± 0.14	6.90 ± 0.05	6.75 ± 0.04
13	5.46	6.19	28.03 ± 1.42	27.31 ± 1.78	3.01 ± 0.13	7.74 ± 0.19	7.72 ± 0.05	6.67 ± 0.06
18	5.24	5.92	44.64 ± 1.91	32.90 ± 1.88	3.83 ± 1.21	9.56 ±1.42	8.78 ± 0.12	6.77 ± 0.08
19	5.19	5.10	72.62 ± 2.91	76.51 ± 3.07	5.80 ± 0.19	26.05 ± 2.6	8.30 ± 0.49	7.91 ± 0.45
22	4.29	4.81	98.78 ± 5.62	117.19 ± 3.28	7.02 ±0.12	41.76 ±1.42	8.92 ± 0.49	9.56 ± 0.06
25	3.06	4.0	116.25 ± 7.64	132.46 ± 2.39	8.08 ± 0.17	61.84 ± 5.1	Uncoun table	Uncoun table

Table – 7 Shelf life of tilapia (*Tilapia nilotica*) and bhetki (*Lates calcarifer*) during iced storage.

Species	Acceptability	Days	Organoleptic score	TVB-N (mg-N/100g)	TMA-N (mg-N/100g)	TVC (log_{10}cfu/gm)
Tilapia nilotica	Just Acceptable	10 - 13	6.25 – 5.46	20.64±0.94 to 28.03±1.42	2.52±0.22 to 3.01±0.13	6.90±0.05 to 7.72±0.05
Lates calcarifer	Just Acceptable	18– 19	5.92 – 5.10	32.90±1.88 to 76.51±3.05	9.56±1.42 to 26.05±0.2	6.77±0.08 to 7.91±0.45
	Just Acceptable		Just Acceptable score: 5.5 - 6.4	**Standard limit** Fresh: 30 – 40 Marine: up to 30	**Standard limit** Fresh: negligible Marine: 9 - 15	**Standard limit** Fresh and Marine : 10^6/g

4.4 Comparative Study of Quality Changes of *Tilapia Nilotica* and *Lates Calcarifer* During Storage at Ambient Temperature

4.4.1 Organoleptic

Initially (0^{th} hour) both tilapia and bhetki were found to be very good quality as the panel score very high for the species (9.11 and 9.16). However, on the 10^{th} hour, the score for tilapia was 6.27 which later on the 10^{th} hour decreased to 5.29. This indicated that tilapia had a shelf life lied between 10 to 17 hours while the score for bhetki was 6.27on the 17^{th} hour which then reduced to 5.27 indicating that the shelf life of bhetki fell between 17 to 20 hour (Fig - 31).

4.4.2 Biochemical

4.4.2.1 Total Volatile Base Nitrogen (TVB-N)

There is clear differences in the value of TVB-N between tilapia and bhetki stored at ambient temperature . With storage time, TVB-N increased in both the species. However, the pattern of changes were clearly different. On the 0^{th} hour, TVB-N value of both tilapia and bhetki were 3.38±1.34 mg-N/100g and 4.42±1.34 mg-N/100g . For bhetki, this increased to 9.81±1.89 mg-N/100g while in case of tilapia, there was slight increased value (5.71±1.48 mg-N/100g). But on the 10^{th} hour, TVB-N value in tilapia increased drastically to 31.24±0.1 mg-N/100g while on the same time, TVB-N value for bhetki was only 17.81±1.34 mg-N/100g. On the 17^{th} hour, TVB-N value for bhetki was 27.88±1.41 mg-N/100g , while for tilapia it was as high as 48.80±0.24 mg-N/100g . But on the 20^{th} hour, TVB-N value increased to a large extent in both the species as shown in Fig – 32.

4.4.2.2 Trimethylamine Nitrogen (TMA-N)

The initial value of TMA-N in both tilapia and bhetki stored at ambient temperature were almost same (1.16±0.07 mg-N/100g and 1.52±0.71 mg-N/100g). The shelf life of tilapia

as observed organoleptically earlier lied between 10 and 17th hour for which the value vary between 2.02±0.08 to 2.27±0.07 mg-N/100g . In the similar manner, the shelf life of bhetki lied between 17 and 20 hour and the value of TMA-N ranged between 9.41±0.05 to 40.75±2.50 mg-N/100g . It is apparent that bhetki showed much higher value of TMA-N in comparison to tilapia as the line of storage time increased when both fish stored at ambient temperature (Fig - 33).

4.4.2.3 Trimethylamine Oxide Nitrogen (TMAO-N)

The value of TMAO-N was much lower in tilapia in compare to bhetki. The low value in tilapia decreased sharply as the day of storage progressed (Fig - 34). On the 10th hour, the TMAO-N value in tilapia was found to be only 1.26±0.19 mg-N/100g. Thereafter, no detection of TMAO-N was observed during the storage trial. But in case of bhetki, a marine species, TMAO-N was very high (68.3±0.58 mg-N/100g) on the 0th hour which decreased progressively with time of storage. Between 17th and 20th hour, when bhetki was with in the limit of acceptability as judged by the panellists, the value of TMAO-N ranged between 17.98±0.62 to 24.97±0.28 mg-N/100g . At the end of the experiment, the value of TMAO-N was 13.70±2.0 mg-N/100g .

4.4.2.4 pH

The change of pattern in the value of pH were not significantly different between tilapia and bhetki when stored at ambient temperature (Fig - 35). Between the 10th and 17th hour of storage, pH in tilapia were 6.59± 0.06 and 6.86±0.08 respectively while between 17th and 20th, the pH of bhetki ranged from 7.02±0.04 to 7.1±0.05. There were the two distinct time when both tilapia and bhetki were judged as 'just acceptable' by the panellists. However, pH in both the species were high when investigated on the 24th hour of storage time

4.4.3 Microbiological (Total Viable Count)

The result of TVC is shown in Fig – 36. The pattern of TVC in tilapia and bhetki were almost similar until 17th hour of storage. When tilapia was found to be the limit of acceptability between 10 and 17 hour, the value of TVC were $\log_{10}5.17\pm0.16$ cfu/gm and $\log_{10}6.94\pm0.05$ cfu/gm respectively. On the other hand, when bhetki was judged as 'just acceptable (17 – 20 hours of storage)', the value of TVC ranged between $\log_{10}6.91\pm0.04$ to $\log_{10}7.87\pm0.56$ cfu/gm.

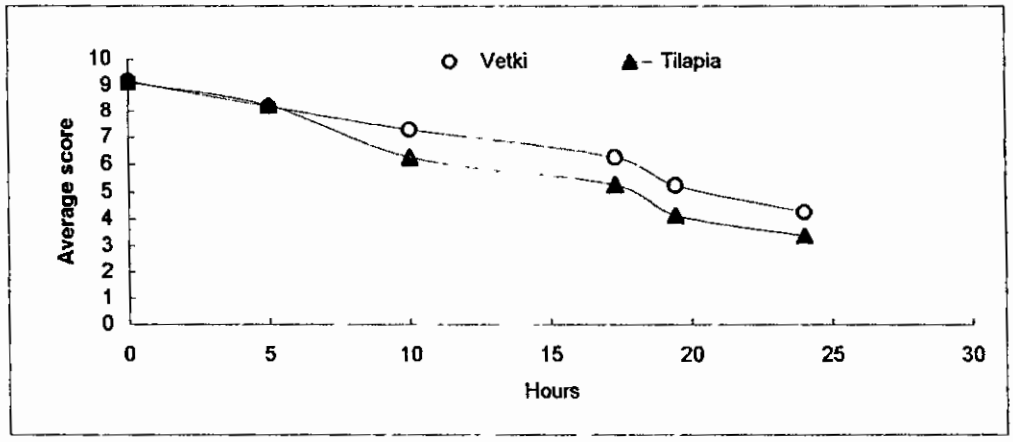


Fig. 31 Comperative study of organoleptic score in *Tialpia nilotica* and *Lates calcarifer* stored at ambient temperature

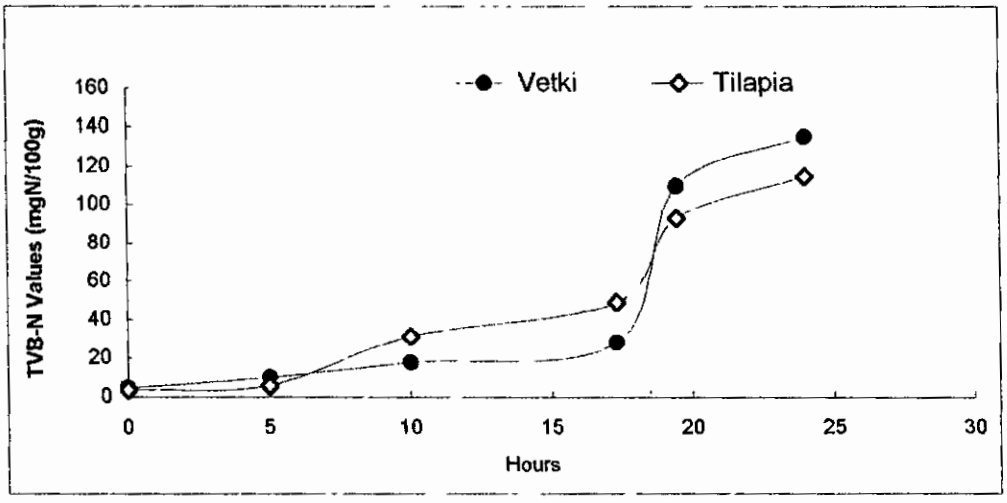


Fig. 32 Comparative study of TVB-N value in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature

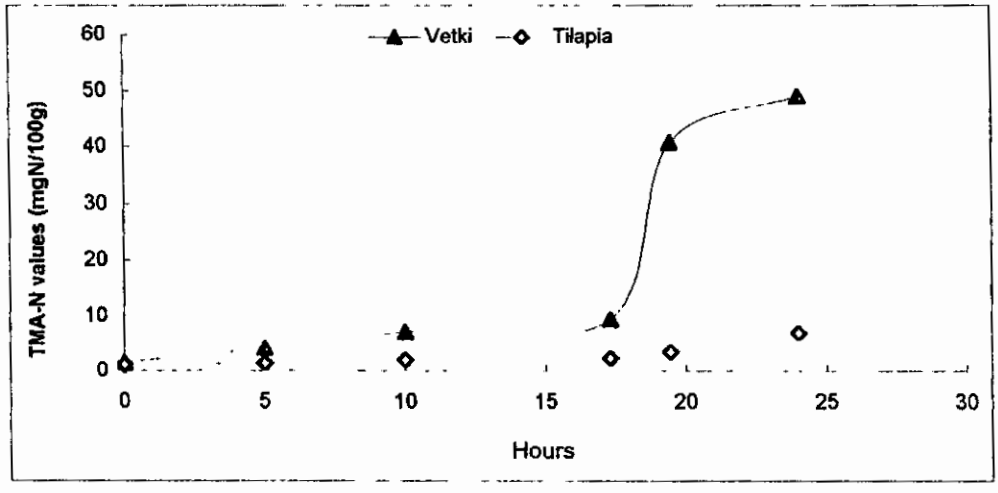


Fig. 33 Comperative study of TMA-N value in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature

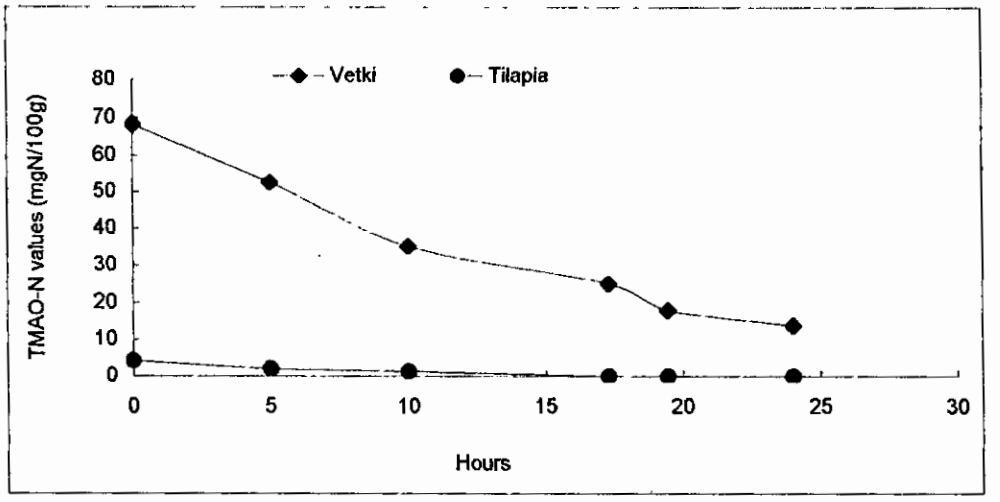


Fig. 34 Comperative study of TMAO-N value in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature

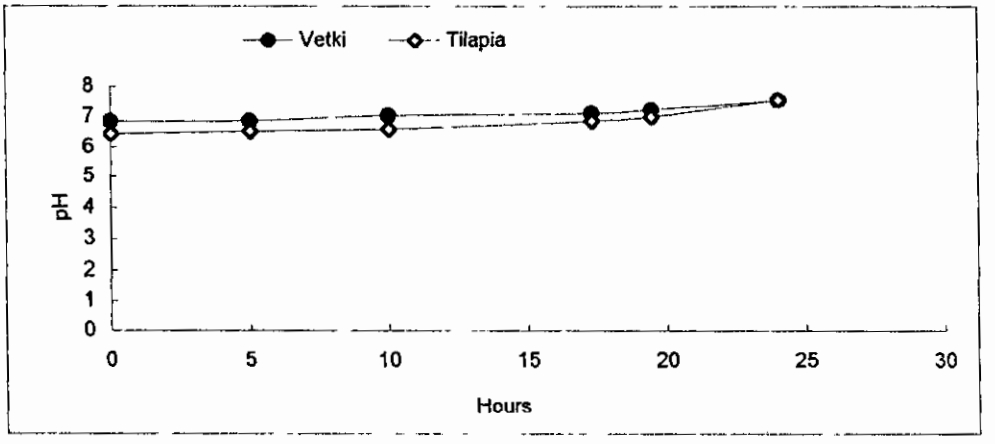


Fig. 35 Comperative study of pH in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature

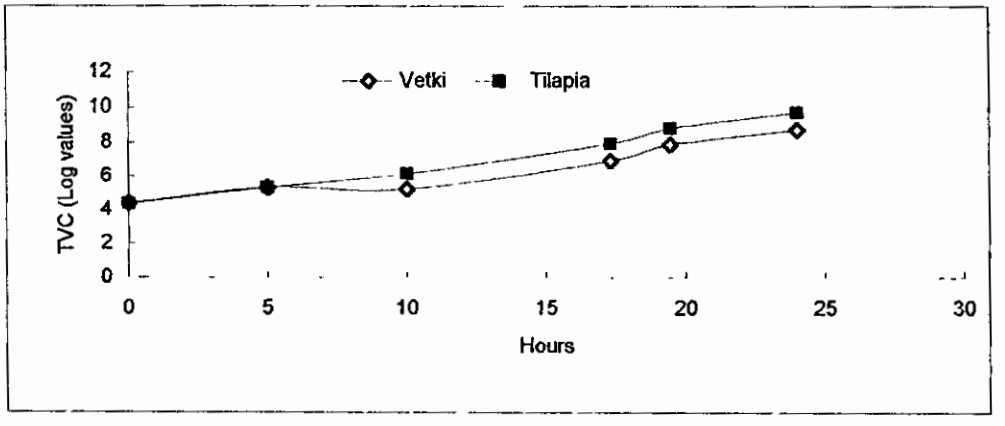


Fig. 36 Comperative study of Total Viable Count in *Tilapia nilotica* and *Lates calcarifer* stored at ambient temperature.

Comparative study of tilapia and bhetki between different parameters (Organoleptic score, TVB-N, TMA-N and TVC) stored at ambient temperature

A complete picture of the comparative study of tilapia and bhetki is presented in Table - 8. On the 0^{th} hour, when tilapia and bhetki were fresh (9.11 and 9.16), the vary of TVB-N was recorded as 3.58±1.34 mg-N/100g and 4.42±1.13 mg-N/100g respectively while TMA-N ranged between 1.16±0.07 mg-N/100g and 1.52±0.71 mg-N/100g and TVC varied between $\log_{10}$4.38±0.04 cfu/gm and $\log_{10}$4.40±0.05 cfu/gm. The shelf life tilapia as already stated earlier lied between 10 and 17^{th} hour when the organoleptic score were between 6.27 to 5.29. During the same period, the score for bhetki was much higher 7.29 and 6.27. TVB-N value during this period in tilapia ranged between 31.24±0.1 to 48.80±0.24 mg-N/100g which was much higher for bhetki (17.81±1.34 to 27.88±1.41 mg-N/100g). However, at the same time, the value of TMA-N was much lower (2.02±0.08 to 2.27±0.27 mg-N/100g), while for bhetki, TMA-N ranged between 7.14±1.33 to 9.41±0.05 mg-N/100g. Again on the same period, the value of TVC between two species did not differ much.

When considering bhetki judged by the panellists, the shelf life lied between 17 – 20 hour. During this time, organoleptic score in tilapia were lower (5.29 to 4.14) in comparison to bhetki (6.27 to 5.27). The value of TVB-N was much higher (48.80±0.24 mg-N/100g) in tilapia on the 17^{th} hour when compared to bhetki (27.88±1.41 mg-N/100g). This value drastically increased on the 20^{th} hour for both tilapia and bhetki (92.83± and 109.54±2.96 mg-N/100g). The scenario for TMA-N in both the species during the same periods (17 – 20^{th} hour) were completely different. In tilapia, TMA-N content was very low and ranged between 2.27±0.07 to 3.81±1.21 mg-N/100g while in bhetki, this value varied between 9.41±0.05 to 40.75±2.50 mg-N/100g. Considering the TVC, the value in both the species completely crossed the limit of acceptability.

In Table - 9 , the shelf life of tilapia, stored at ambient temperature with the limit of acceptability related to different parameters (organoleptic score, TVB-N, TMA-N and pH) is presented. For tilapia, the limit of acceptability (just acceptability) as judged by the panellists indicated that the fish had a shelf life (ambient temperature) which lied

between 10 – 17 hours. When organoleptic score were between 6.27 to 5.29, the vary of TVB-N and TMA-N ranged between 31.24±0.1 to 48.80±0.24 and 2.02±0.08 to 2.27±0.07 mg-N/100g. While for TVC, the value varied between $\log_{10}$5.17±0.06 to $\log_{10}$6.94±0.05 cfu/gm.

For bhetki, the shelf life at ambient temperature lied between 17^{th} – 20^{th} hours. During this time, organoleptic score were between 6.27 – 5.27, TVB-N 27.88±1.41 to 109.54±2.96 , TMA-N 9.41±0.05 to 40.75±2.50 mg-N/100g, and TVC $\log_{10}$6.91±0.04 to $\log_{10}$7.87±0.56 cfu/gm respectively.

Table - 8 Comparative study of *Tilapia nilotica* and *Lates calcarifer* between different parameters (Organoleptic score, TVB-N TMA-N and TVC) during storage at ambient temperature.

Storage Periods (hours)	Organoleptic Score		TVB-N (mg-N/100g)		TMA-N (mg-N/100g)		TVC (log_{10}cfu/gm)	
	Tilapia	Bhetki	Tilapia	Bhetki	Tilapia	Bhetki	Tilapia	Bhetki
0	9.11	9.16	3.58 ± 1.34	4.42 ± 1.13	1.16 ± 0.07	1.52 ± 0.71	4.38 ± 0.04	4.40 ± 0.05
5	8.21	8.17	5.71 ±1.48	9.81 ± 1.89	1.46 ± 0.7	4.16 ± 1.41	5.32 ± 0.04	5.33 ± 0.03
10	6.27	7.29	31.24 ± 0.1	17.81 ± 1.34	2.02 ± 0.08	7.14 ± 1.33	5.17 ± 0.06	5.23 ± 0.04
17	5.29	6.27	48.80 ± 0.24	27.88 ± 1.41	2.27 ± 0.07	9.41 ± 0.05	6.94 ± 0.05	6.91 ± 0.04
20	4.14	5.27	92.83 ± 3.22	109.54 ± 2.96	3.81 ± 1.21	40.75 ± 2.50	8.83 ± 0.10	7.87 ± 0.56
24	3.37	4.25	114.47 ± 3.81	134.95 ± 3.90	6.94 ± 0.33	48.96 ± 2.32	9.73 ± 0.14	8.73 ± 0.11

Table – 9 Shelf life of tilapia (*Tilapia nilotica*) and bhetki (*Lates calcarifer*) during storage at ambient temperature .

Species	Acceptability	Hours	Organoleptic Score	TVB-N (mg-N/100g)	TMA-N (mg-N/100g)	TVC (log_{10}cfu/gm)
Tilapia nilotica	Just Acceptable	10 - 17	6.25 – 5.46	31.24±0.1 to 48.80±0.24	2.02±0.08 to 2.27±0.07	5.17 ±0.06 to 6.94±0.05
Lates calcarifer	Just Acceptable	17 - 20	6.35 – 5.92	27.88±1.41 to 109.54±2.96	9.41±0.05 to 40.75±2.50	6.91±0.04 to 7.87± 0.56
	Just Acceptable		Just Acceptable score: 5.5 - 6.4	**Standard limit** Fresh: 30 – 40 Marine: up to 30	**Standard limit** Fresh: negligible Marine: 9 - 15	**Standard limit** Fresh and Marine : 10^6/g

4.5 Proximate Composition of *Tilapia Nilotica* and *Lates Calcarifer* in Relation to Different Anatomical Portions, Sizes and Sexes.

4.5.1 Proximate composition: in relation to different anatomical portion (head, middle and tail portion)

Table – 10 illustrates the proximate composition of bhetki along the body length of bhetki. The total length of the fish has been categorised into three portion i. e. head, middle and tail. The protein content within the anatomical portion varied between 18.56% to 20.77%. The protein content in middle and head portion was almost same. Near the tail portion protein content reduced to 18.56%. Lipid content was found to be higher in head portion (1.80%) while in the other two portion (middle and tail), lipid content was 1.70% and 1.55% respectively. Moisture on the three anatomical portion was found to be almost same.

The proximate composition of tilapia in the different anatomical portion is summarised in Table – 11. Protein was found to be higher in the head portion of tilapia (17.21%) while in the tail and middle portion, protein content were almost same. Amount of lipid was higher in head in comparison to tail and middle portion. Ash in tilapia was higher in tail (1.09%) than head (0.26%) and middle (0.13%) portion. Moisture content remained within 81%.

Comparative proximate composition of tilapia and bhetki between different portions (Head, Middle and Tail)

Comparative proximate composition of the two species (tilapia and bhetki) in relation to different anatomical portion (Head, Middle and Tail) is shown in Table – 12. Protein was found to be much higher in bhetki (20.77%) compared to tilapia (17.21%) in the head portion. Almost similar quantity of lipid was observed in both the species in head. While ash percentage was higher (1.26%) in bhetki than tilapia (0.26%) in the head portion. Moisture remained high (80.92%) in tilapia compared to bhetki (78.82%) in the head portion. With regard to head portion, protein content was much higher (20.55%) in

bhetki than tilapia (16.28%). There was a meagre differences in the lipid percentage of thc two species in the middle portion. Ash was found to be higher in bhetki (1.47%) than tilapia (0.13%) while moisture was higher in tilapia (81.02%) than bhetki (78.99%).

As with the result of tail and middle portion, the protein content in the head portion was higher (20.77%) in bhetki compared to tilapia (17.21%). Lipid was slightly higher (1.80%) in bhetki compared to tilapia (1.02%) in the tail portion, while ash was found to be very high (1.67%) in bhetki than tilapia (0.13%) in the tail portion. In line with the result of head and middle, moisture content was much higher (80.67%) in tilapia than bhetki (77.45%) in the tail portion.

Table - 10 Proximate composition of *Lates calcarifer* in relation to different anatomical portions

Species	Portion of the body	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Lates calcarifer	Head	20.77	1.80	1.26	78.82
	Middle	20.55	1.70	1.47	78.99
	Tail	18.56	1.55	1.67	77.45

Table - 11 Proximate composition of *Tilapia nilotica* in relation to different anatomical portions

Species	Portion of the body	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Tilapia nilotica	Head	17.21	1.36	0.26	80.92
	Middle	16.28	1.03	0.13	81.02
	Tail	16.5	1.02	1.09	80.67

Table: 12 Comparative study of Proximate composition of *Lates calcarifer* and *Tilapia nilotica* in relation to different anatomical portions

Portion	*Lates calcarifer*				*Tilapia nilotica*			
	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Head	20.77	1.80	1.26	78.82	17.21	1.36	0.13	80.92
Middle	20.55	1.70	1.47	78.99	16.28	1.03	0.26	81.02
Tail	18.56	1.55	1.67	77.45	16.5	1.02	1.09	80.67

4.5.2 Proximate composition : in relation to different sizes (large, medium and small)

The result presented in Table – 13 – 15 indicated a considerable variation in proximate composition of different sizes (large, medium and small) of tilapia and bhetki. The fish were categorised into large, medium and small sizes according to their weight. In tilapia, above 150g were considered as large, 100-150g as medium and below 100g were considered as small fish. On the other hand, in case of bhetki, above 1kg was considered as large fish, 500-below 1kg as medium and below 500g recorded as small fish.

Table – 13 illustrates the proximate composition of bhetki in relation to different sizes of fish i. e. large, medium and small. Protein content of different sizes of fish ranged between 17.29% to 19.55% respectively. Lipid content was found to be higher in large size fish (2.55%) while in other two sizes (medium and small), lipid content was 2.05% and 1.88%. Moisture content of large, medium and small sized fish were 77.02%, 78.22% and 79% respectively. Ash content in different sizes of fish were almost same.

From Table – 14, it is evident that moisture content of the large, medium and small sized fish varied between 80.66% to 81.55%. Moisture content in large and medium sized fish (80.66% and 80.75%) were almost same but in small sized fish, moisture were much higher (81.55%). Small sized fish had less protein (15.76%) while large and medium sized fish contained almost same. Ash content was almost same in large to small sized fish. Large sized fish contained more fat (1.83%) than medium (1.49%) and small size (1.01%) tilapia.

<u>Comparative proximate composition of tilapia and bhetki in relation to different sizes (large, medium and small)</u>

Results presented in Table – 15 shows the comparative proximate composition of two species (tilapia and bhetki) in relation to different sizes (large, medium and small).

Moisture was found to be much higher in tilapia (81.55%) compared to bhetki (79%) in small sized fish. Lipid content was observed high in bhetki (1.88%) than tilapia (1.01%)

while ash percentage were almost same in both the species. Protein was found to be much higher in bhetki (17.29%) than tilapia (15.76%) in small size fish. With regard to medium size, protein content was found to be higher in bhetki (17.88%) compared to tilapia (17.21%). Similar percentage of ash were observed in both the species while lipid content was higher in bhetki (2.05%) than tilapia (1.49%). Moisture content was found to be much higher in tilapia (80.75%) than bhetki (78.22%). Protein percentage was found to be higher in bhetki (19.55%) than tilapia (17.91%) in case of large sized fish. Moisture content remained high in tilapia (80.66%) than bhetki (77.02%) while lipid content was low in tilapia (1.83%) than bhetki (2.55%). Ash content was found to higher in bhetki (1.89%) than tilapia (1.46%).

In comparison to different sizes of fish, protein content in large size fish was found to be much higher (19.55%) in bhetki than tilapia (17.91%). Moisture content was observed to be higher (81.55%) in tilapia than bhetki (79%) in case of small size fish. With regard to lipid content, the value was found to be much higher in bhetki (2.55%) than tilapia (1.49%) and ash content was also high in bhetki (1.89%) than tilapia (1.46%) in case of large sized fish.

Table: 13 Proximate composition of *Lates calcarifer* in relation to different sizes

Species	Size of fish	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Lates calcarifer	Large (above 1kg)	19.55	2.55	1.89	77.02
	Medium (500-1kg)	17.88	2.05	1.68	78.22
	Small (up to 500g)	17.29	1.88	1.58	79

Table: 14 Proximate composition of *Tilapia nilotica* in relation to different sizes.

Species	Size of fish	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Tilapia nilotica	Large (above 150g)	17.91	1.33	1.46	80.66
	Medium (100-150g)	17.21	1.09	1.29	80.75
	Small (up to 100g)	15.76	1.01	1.23	81.55

Table: 15 Comparative study of Proximate composition of *Lates calcarifer* and *Tilapia nilotica* in relation to different sizes

	Lates calcarifer				*Tilapia nilotica*			
Size	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Large (above 150g)	19.55	2.55	1.89	77.02	17.91	1.33	1.46	80.66
Medium (100-150g)	17.88	2.05	1.68	78.22	17.21	1.09	1.29	80.75
Small (below 100g)	17.29	1.88	1.58	79	15.76	1.01	1.23	81.55

4.5.3 Proximate composition : in relation to different sexes (male and female)

The result obtained from the proximate composition in relation to different sex is presented in Table – 16 – 18. Moisture content was found to be higher (77.98%) and protein content was lower (18.21%) in female bhetki than male (moisture- 76.37% and protein- 19.21%). Lipid content was higher in male (2.35%) while in the female, lipid content was low (1.98%). The ash content in both male and female bhetki was almost same (Table – 16).

The proximate composition of tilapia in relation to different sex is summarised in Table – 17. Moisture content was found to be higher in female (81.09%) than the male (80.78%) while protein content in male was higher (17.98%) than the female (16.82%). Lipid and Ash content were almost same.

Comparative proximate composition of tilapia and bhetki in relation to different sexes (male and female)

Comparative proximate composition of the two species obtained from the different sexes (male and female) is presented in Table – 18.

Protein content was higher in male bhetki (19.91%) than the male tilapia (17.98%). Moisture remained much high (80.78%) in tilapia in comparison to male bhetki (76.37%). Lipid content was found to be higher (2.35%) in male bhetki than the male tilapia (1.98%). Ash content in both the species (male) was almost same.

Moisture content was found to be much higher in female tilapia (81.09%) than the female bhetki (77.98%) while protein content remained much high in female bhetki (18.21%) than the female tilapia (16.82%). Lipid and ash content in both the species (female) were almost similar.

Between the two species in relation to different sex, protein content was found to be higher in male (19.91% in bhetki) in comparison to female (18.21% in bhetk). While in tilapia, protein content remained high in male (17.98%) than the female (16.82%). Lipid

content was found to be higher in male (2.35%) than the female (1.98%) in bhetki while in tilapia, lipid content was almost same. Moisture content was found to be higher in female (77.98%) than the male (76.37%) in bhetki while in case of tilapia moisture content was low in male (80.78%) than the female (81.09%). The outlined result in relation to both sexes of the both species expressed that the ash content remained almost same in both the species.

Table: 16 Proximate composition of *Lates calcarifer* in relation to different sexes

Species	Sex	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Lates calcarifer	Male	19.91	2.35	1.06	76.37
	Female	18.21	1.98	1.21	77.98

Table: 17 Proximate composition of *Tilapia nilotica* in relation to different sexes

Species	Sex	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Tilapia nilotica	Male	17.98	1.67	1.22	80.78
	Female	16.82	1.55	1.1	81.09

Table: 18 Comparative study of Proximate composition of *Lates calcarifer* and in *Tilapia nilotica.* in relation to different sexes

Sex	***Lates calcarifer***				***Tilapia nilotica***			
	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)
Male	19.91	2.35	1.06	76.37	17.98	1.67	1.22	80.78
Female	18.21	1.98	1.21	77.98	16.82	1.55	1.1	81.09

DISCUSSION

5 DISCUSSION

5.1 Quality Changes during Iced Storage

5.1.1 Organoleptic

Organoleptic methods are universally accepted for estimating the quality of fish (Shewan, 1977). The basic method of evaluating the quality and shelf life is by periodic sensory testing which is based on the characterisation and differentiation of the various sensory perceptible factors such as appearance and odour of the raw fish and odour, flavour, texture etc. of the cooked fish which affect the sensory judgement of the buyers and consumers (Anon, 1989).

At the start of the investigation, both species were considered of highly acceptable. The eyes were clear and pupils were bright and transparent, the gills were bright red, and body surface lost its brightness slightly. The belly wall were normal fresh. The texture were just in rigor stage followed by sweetty odour. The eyes maintained their clarity for only a few days being judged moderately dull on the 5^{th} day and definitely dull and slightly sunken on the 10^{th} in tilapia and 18^{th} in bhetki. The colour of the gills changed rapidly and it became pale on the 10^{th} day. The texture remained just in rigor stage on the tenth day and by day 13, the texture were just in post rigor stage. The odour remained good through 10 days in tilapia and 18 day in bhetki but sign of incipient spoilage were detected on the 13 day in tilapia and 19 day in bhetki.

The most obvious indicator of change for fish, threadfin bream (*Nemipterus peronii*), long spine seabream (*Argyrops spinifer*), painted sweetlip (*Plectorynchus pictus*) and a snapper like fish (*Lutjanus vittus*) in ice were the appearance of the eyes (their clarity and shape) followed by odour development and colour changes in the gills (Barile *et.al.*, , 1989). Thus these changes were most obvious in parts of the fish not normally eaten. It is assumed that concomitant changes are occurring in the edible flesh. This assumption is inherent in all non destructive scoring schemes which evaluated external characteristics (Breamner, *et. al.* 1985). Barile *et. al.* (1985) observed that Faughn's mackerel

(*Rastrelliger faughni* Matsui) were rejected by the taste panellist was mainly characterised by rancid to strong rancid, sour and fishy odours and very soft texture. The general appearance was rated below the acceptable limit based on sunken, opaque eyes with yellowish, reddish and slightly greenish cornea and faded characteristic colour of the skin with brownish discoloration on the ventral part of the body. Similar results were observed in the present investigation for both species.

Studies on organoleptic assessment was carried out on tilapia and bhetki during iced storage. It was evident that tilapia was acceptable between 10 – 13 days while bhetki between 18 – 19 days . Thus the shelf life of ~~tilapia~~ bhetki was much higher compared to tilapia.

The shelf life of some freshwater species, pejerry (*Basilichthys bonariensis*), carp (*Cyprinus carpio*) , trout (*Salmo gairdneri*) and Sangara in crushed ice were 15, 20, 15 – 16 and 15 days respectively (Poulter and Nicolaides, 1985; Mlay *et. al.*, 1982). These freshwater species indicated longer shelf life compared to tilapia in the present investigation. The longer shelf life observed in the present study may be attributed to the possible effect of the catching region and variation between species to species (Ababouch *et. al.*, 1996). However, Sumner and Orejana, (1985) found that storage life of tilapia to vary between 21 and 27 days. The variation between the shelf life of the same species are probably due to geographical location, catching methods , species differences etc. (Ababouch *et. al.*, 1996).

The shelf life of some marine species in ice, *Mugil cephalus* , *Liza subviridis*, and *Valamugil seheli* were 16 –24, 28 – 29 and 21- 22 days (Sumner and Orejana, 1985). Brackish water prawn (*Penaeus monodon*) spoiled within 17 days when stored in ice. (Reilly *et. al.*, 1985). The findings of the above mentioned researchers are in concurrence with the results of the present investigation. However these results differed from the work of Carrol *et. al.*,(1980) and Ababouch *et. al.*, (1996) as they had found much shorter shelf life of 8 – 9 days in Goldlined seabream (*Rhabdossargos sarba*) and 8 – 11 days in Sardine *(Sardina pilchardus)*. These difference were probably due to inherent species difference, fat content, size, shape,season and feeding ground. (Lima Dos Santos, 1978).

5.1.2 Biochemical

5.1.2.1 Total Volatile Base Nitrogen (TVB-N)

Total Volatile Base (TVB-N) in which ammonia ,Trimethylamine and other basic substances are aerated or distilled from a midley alkaline fish extract , collected in standard acid and estimated (Stansby and Olcott, 1963). Total Volatile Nitrogen or base test is quite generally applicable; it usually gives reasonable good correlation with organoleptic freshness. Volatile bases are produced in increasing concentration during spoilage of fish. TVB-N value cannot be used to estimate the degree of freshness of fish in the early stages of storage but only the degree of spoilage in the later stages (Shewan, 1977).

The TVB-N of content tilapia and bhetki on the 1st day of iced storage was found to be 4.49±1.91 mg-N/100g and 4.24±0.24 mg-N/100g. The initial low` TVB-N (4 –7 mg)percentage was also observed for pjerry, carp and trout (Poulter and Nicolaides, 1985). A slight increase in TVB-N amount initially was found in *Chrysichthys* (9.4mg) *Bagrus* (8.4 mg) and *Tilapia* (7.6 mg) by Adebona (1982). However, Azam *et. al.* (1997) recorded a very high TVB-N value (17.03 mg-N) initially for tilapia. Similarly Mlay *et. al.* (1985) also observed very high value of TVB-N (51.8 mg). The low value of TVB-N initially is an indication of quality of fresh fish while the high value of TVB-N may be due to action of autolytic enzymes and spoilage bacteria which might have passed their lag phase (Adebon, 1982). It also may be attributed to geographical difference, catching methods, fat content and size of fish (Ababouch *et. al.*, 1996).

TVB-N value increased progressively with increased storage time for both tilapia and bhetki. However, with increased storage time, in some species, TVB-N content was found to be decrease (Mlay *et. al.*, 1985). The explanation for this can be that volatile bases were leached out due to melting during storage. Boury (1936) demonstrated that if the leaching could be prevented, TVB-N value would show regular increase with storage tome observed in the present study. As well, leaching could be prevented using plastic foil.

At the time of rejection, TVB-N value on both tilapia and bhetki were 31.24±0.1 mg-N/100g and 28.03±1.42 mg-N/100g as observed in the present investigation. However, this result differed with the findings of Putro *et. al.* (1985) who found that rabbit fish stored at chilled temperature had a TVB-N content of 24.20 mg % when approaching the border line of acceptance. These values were lower than TVB-N content of iced Skipjack when rejected by the panellists (25.30 – 27.06 mg %) (Saleh *et. al.*, 1984). According to Connell (1980), TVB-N content in fish varies between 30 – 40 mg % when rejected. The results of the present study for both the species are almost in agreement of Connell's statement.

Connell (1975) stated that TVB-N content in fresh fish has highly positive and highly negative correlationship with storage time indicating that TVB-N is a good indicator of spoilage. The result of the present study is in absolute agreement with the findings of Connell (1975). However, other researchers did not find TVB-N as a good indicator spoilage (Nandanie *et. al.*, 1985 and Barile, 1985).

5.1.2.2 Trimethylamine Nitrogen (TMA-N)

The most commonly used chemical method for assessing fish quality is the estimation of TMA. This is one of the volatile basic compounds which is found in very low amounts in fresh fish but accumulates in spoiling marine fish as a result of mainly bacterial reduction of trimethylamine oxide (TAMO). This means that analysis does not give any information about early autolytic changes or degree of freshness but only about the much later bacterial changes or degree of spoilage. Trimethylamine is a volatile compound and has a very low odour threshold (Botta and Shaw, 1976). Trimethylamine could serve as an index of spoilage for marine fish. Fresh water fish cannot be judged by either TMA values because they do not contain the precursor, TMAO-N, in sufficient amount (Bligh, 1971).

The TMA values of *Sardina sirm* during iced storage did not indicate actual spoilage pattern of fish and did not seem to be useful as index of freshness (Nandanie *et. al.* , 1985). When fish (*Restrallier faughni*) spoiled during iced storage, TMA values ranged

from 1.44 – 1.82 mg-N/100g and showed no significant correlation with storage time and scnsory results (Barile *et. al.* (1985). This findings differed from the present investigation for both the species as TMA-N showed highly positive correlation with storage time and highly negative correlation with organoleptic score. Statistical analysis also supported the significance of TMA-N as a good spoilage indicator in the present investigation.

TMA values of goldline seabream, stored in ice increased very quickly so that by day 10, when the taste panel rejected the fish, the value was 34 mg % (Poulter and Nicolaides, 1985). TMA value in sardine (*Sardina pilchards*) during iced storage was 13.1 mg % (Ababouch *et. al.*, 1996). The value is well above the recommended level for TMA (10 – 15 mg-N/100g) suggested by Connell (1975). The suggested value (Connell, 1975) is closed to the acceptable value (9.56 to 26.05) of TMA-N in bhetki during the present investigation.

Beatty and Gibbons (1937) suggested the acceptable limit of TMA was 4 – 6 mg-N/100g. There was also wide variation in acceptable values suggested for individual species i. e. 5 – 7 mg % for herring and 1 – 5 mg % for haddock (Singurdsson, 1955; Castell and Triggs, 1955). On rejection, the TMA-N value found to range between 2.52 to 3.01 mg % in the present investigation. The value is well agreement with the above mentioned findings.

During iced storage, the TMA value of three different species of bele fish (*Glossogobius guiris*) ranged between 1.20 to 9.4 mg % of the fish muscle. The variation in the value of TMA in to three different species may be due to the effect of species (Khuda *et. al.*, 1960). In the present investigation, on rejection , the TMA-N value in tilapia ranged between 2.52 to 3.01 mg % while in bhetki the value varied between 9.56 to 26.05 mg % during iced storage. The presence of TMA-N in fresh water fish (tilapia) muscle was very low than marine (bhetki) species (Castell and Triggs, 1955) and TMAO present in the fresh water fish was very little (Shewan and Jones, 1957; Labire and Gibbons, 1937).

5.1.2.3 Trimethylamine Oxide Nitrogen (TMAO-N)

TMAO-N is a part of body's normal buffer system and also a part of system used for osmoregulation of marine fishes (Farber, 1965; Love, 1966). It is non protein nitrogen fraction. TMAO-N is not found in fresh water fish to any great extent and those species of marine fish that can migrate into fresh water lowering TMAO-N content to almost zero when in fresh water (Martin, 1982).

TMAO present in freshwater fish was very little (Shewan and Jones, 1957; Habire and Gibbons, 1937). TMAO exists in negligible amount in freshwater fish (Dyer, 1952; Groninger, 1959; Ruiter, 1971; Harada, 1975). Freshwater fish did not contain any significant amount of TMAO in their muscle because they excreted TMA promptly (Yamada, 1967). The mentioned finding are true for tilapia (2.23 mg-N/100g) in this investigation.

TMAO is the only amine that shows a decrease value during ice storage (Oehlenschlagler, 1996). TMAO and related compounds are encountered in a wide variety of marine species and highest value were observed in Elasmobranch tissue (500-1500 mg-N/100g), followed by gadoid and squid muscle (Yamada, 1967). The pattern of decrease during iced storage is concurrence with the findings of Oehlenschlagler (1996.)

Gadoid muscle averages 88 mg % and cod 95 mg % (Dyer, 1952). TMAO-N value was found to be 76.21 mg % in the present investigation. The variation in the value due to season, size and age of fish as well as environmental condition to which the animal is subjected (Martin, 1982).

5.1.2.4 pH

The muscle of living fish, in general, are approximately neutral in reaction and after catching, the pH value decreases, due to formation of lactic acid from glycogen by series of reaction (Tarr, 1954). As spoilage advances, the pH value of the muscle rises first slowly and later quite rapidly. This is due to the accumulation of the basic end product of

bacterial spoilage and pH rises up to 7.5 to 8.0 (Wood *et. al.* , 1942).

pH value of pejerry (*Basilichthys bonariensis*), carp (*Cyprinus carpio*) and trout (*Salmo gairdneri*) increased during 17 – 20 days of iced storage. The initial pH of the muscles of these fish were in the range of 6.3 – 6.4. The value obtained for trout was exceptionally erratic and would not provide a useful index of fish freshness with this species (Poulter and Nicolaides (1985). Similar results were observed in the present investigation for both species.

The pH value of the *Penaeus monodon* ranged from 7.1 – 8.1 stored in ice (Reilly *et. al.*, 1985). pH in sardine (*Sardina pilchards*) during iced storage increased between 6.35 – 6.77 (Ababouch *et. al.* 1996). pH increased rapidly from 6.2 to 7.5 by day 14 in goldline seabream during iced storage (Carrol *et. al.*, 1980). The value indicated the faster rate of spoilage in iced storage. However, in this experiment, the pH value reached 7.41 in tilapia and 7.47 in bhetki at the end of 25 days storage in ice. It may be due to alkalinity of ammonia and amino compounds formed during bacterial proteolysis which increase with the length of storage period (Poulter, 1978).

Abdullah and Yean (1985) reported that pH had little influence on the textural deterioration of chub mackerel under storage in ice.. This is in agreement with the present investigation for both the species but in contrast with the findings of Lowie and Little (1966).

5.1.3 Microbiological (TVC)

The spoilage of fish held in ice is a bacteriological phenomenon and chemical changes that take place are mainly due to bacterial enzymes. It is well known that during post mortem period bacteria present on the surface and in the gut multiplied rapidly and gradually invade the flesh which provide nutrition for their growth and multiplication. The bacterial population in fish greatly influence the quality of fish and fishery product. There were some recommended limits of bacteria load which the quality of fish and fishery products has been judged under various storage conditions. Investigation on the

changes in the bacterial flora in fish during iced storage could provide useful information on the spoilage pattern of fishes (Barile *et. al.*, 1985). For the bacterial spoilage, the most commonly used test is the total viable count. With some unavoiding difficulties this method is more or less suitable for routing testing of quality of fish (Whittsfogel, 1961).

The bacterial count for Indian mackerel (*Restralliger kanagurta*), Indian oil sardine (*Sardinella lonjiceps*), pink perch (*Nemipterus japonicus*) and croaker (*Johneops* sp.) during 1st day of iced storage were found to be 3.05×10^5 , 3.55×10^4 and 3.4×10^4 respectively (Devaraju and Setty, 1985). The initial bacterial counts for bay trout (*Arripis trutta*), bream (*Acanthopagrus butchernia*) and mullet (*Aldrichetta forsteri*) during iced storage ranged between $10^3 - 10^5$/g and rose to $10^8 - 10^9$/g (Gorczgyca and Len, 1985), similar to those established for other temperate marine fish (Gillespie and Mocrae, 1975; Shewan, 1977) and also for trōpical marine and freshwater fish (Estrada *et. al.* 1985; Putro *et. al.* 1985; Saleh *et. al.* 1985). The initial bacterial load of the Furu (*Haplochromis* sp.), Kamongo (*Protopterus ethiopicus*) and Sangara (*Lates* sp.) during iced storage ranged between $10^4 - 10^5$/g and increased to $10^6 - 10^7$/g when they were considered to be spoiled (7 days after storage) (Mlay *et. al.*, 1982). The initial bacterial count of the sardine (*Sardinell pilchards*) was similar to the level found in cold water fish but much lower than observed in tropical fish that reached $10^9 - 10^{10}$/g (Gram, 1989). Poulter and Nicolaides (1985) reported the initial bacterial loads of pejerry (*Basilichthys bonariensis*), carp (*Cyprinus carpio*) and trout (*Salmo gairdneri*) ranging between $10^2 - 10^4$/g of muscle on day 1. These level exceeded 10^7/g between 17 – 20 days of iced storage which is the maximum microbiological limit for fresh fish recommended by ICMSF (ICMFS, 1978). However, the result of the present investigation are in agreement with the above mentioned findings for both species.

There is an overall increase in the TVC over the 15 days iced storage periods (Azam *et. al.*, 1997). The increased value was not linear as TVC decreased around 5th day of the iced storage. This findings contrasted with the present investigation as TVC increased progressively during the 25 days iced storage trial. This variation may be explained by a decrease in metabolic activity of the mesophills followed by gradual assertion of the psychrophibilic microflora due to melting ice (Liston, 1980; Acuff *et. al.* 1984).

Bacterial levels in *Mugil cephalus* ranged from $10^3 - 10^4$/g to $10^7 - 10^9$/g. Rejection levels of $10^7 - 10^9$/g were typical for *Liza subviridis* and *V. seheli* . Initially bacterial levels in tilapia was $10^3 - 10^4$/g and rose to $10^6 - 10^7$/g at rejection (Sumner and Orejana, 1985). Bacterial counts during iced storage remained within the range between $10^6 - 10^9$/g on rejection of *Penaeus monodon* reported by Reilly *et. al*, 1985). Similar results were observed in the present investigation for both species.

5.2 Quality Changes During Storage at Ambient Temperature

5.2.1 Organoleptic

The oldest and still most wide spread means of evaluating the acceptability and edibility of fish are the sense, smell and sight supplemented by taste and touch. The reason for the preferential use of sensory test are obvious : no special laboratory equipment is needed; the fish can be examined whatever they happened to be; the test can be carried out quickly and many samples can be evaluated in a relatively short time (Farber, 1965). In spite of certain limitation, organoleptic methods are universally accepted for assessing the quality of fish (Shewan, 1976).

At ambient temperature, in both the species judged in the present investigation, the most noticeable characteristic to changes first was the decrease in the stiffness as the fish passed through rigor. This was followed by the development of the odour and mucus of the gill. These were also observed by Bremner *et. al.* (1985) on *Argyrops spinifer*, *Latjanus vittus* and in *Plectorynchus pictus.*

Whiting (*Sillago maculata*) stored at ambient temperature spoiled within 12 hour. When rejected by the taste panellists, fish had a bitter flavour and mushy texture with sour to ammonicol odour. Tilapia (*Oreochromis nilotica*) which was rejected on the 16.5 hours at ambient temperature had a slightly putrid, bitter and itchy flavour. No softening of the texture of the raw fish was observed (Estrada *et. al.*, 1985). When mackerel (*Rastrelliger faughni*) spoiled at ambient temperature, the fish had bitter, sour, rancid flavours with an itchy after taste and sulphide fishy odours. The texture was dry and fibrous (Barile *et. al.*, 1985). Prawn (*Penaeus monodon*) held at ambient temperature were rejected after 16 hours due to discoloration with a pinkish cooked appearance, strong ammonicol odours and very soft textures. Prawns lost up to 15% of their body weight after 16 hours. It is interesting to note that sulphide odours were not detected in sample stored at ambient temperature (Reilly *et. al.*, 1985). At ambient temperature, *Haplochromis* sp. spoiled after 20 hours, *Protopterus pictus* after 3 days and *Lates* fillets after 2 days. In case of *Haplochromis* sp. when the fish was found definitely spoiled, it gave off a strong odour

and showed soft texture (Mlay *et. al.*, 1982). However, in the present study of the two species, stored at ambient temperature, tilapia had a shelf life lied of 10 – 17 hours and bhetki 17 an 20 hours. The rejection was observed in relation to sunken eyes, watery coloured pupil, pale colour gill, body surface with no brightness, belly wall with more discoloration , slightly soft texture and spoilage odour.

Storage of mackerel (*Rastrelliger faughni*) at ambient temperature resulted in spoilage after 7 hours storage. Strong belly burst took place during spoilage with prolonged exposure to ambient temperature (Gildberg and Roa, 1980). The findings is similar to the result observed in tilapia. Strong belly burst took place probably due to digestive enzymes present in the contents of the gut.

Rabbit fish (*Siganus* sp.) kept at ambient temperature were rejected by the taste panel after 12 hour. The raw odours and general appearance of the gill deteriorated rapidly. Based on textural quality, rabbit fish can be stored for 18 hour because of its hard and compact skin (Putro *et. al.*, 1985). Bhetki in the present investigation could be stored for a longer period because of its compact and hard skin.

5.2.2 Biochemical

5.2.2.1 Total Volatile Base Nitrogen (TVB-N)

The total volatile basic substances in fish is mainly composed of ammonia, TMA and DMA. The level of TVB-N increases after spoilage begins (both enzymic and bacterial). It does not distinguish the origin nor the component of these volatile compounds, hence its use is more general (Siang and Kim, 1992).

Estrada *et. al.* (1985) recorded the TVB-N content of whiting (*Sillago maculatus*) and tilapia (*Oreochromis nilotica*) were 52 and 15 mg % respectively at ambient temperature. At ambient temperature, TVB was found to increase in sardine (*Sardina pilchardus*) to reach 57.3 mg % of fish at spoilage (Ababouch *et. al.*, 1996). However, in the present investigation, TVB-N value reached 114.47±3.81 in tilapia and 134.95±3.90 mg % in

bhetki respectively. These value were higher than observed by other researchers. It may be attributed to rapid autolytic and bacterial spoilage (Azam *et. al.*,1997).

TVB-N content of mackerel (*Rastrelliger faughni*) varied between 18.05 – 21.29 mg % when stored at ambient temperature (Barile *et. al.*, 1985). During storage of furu (*Haplochromis* sp.) at room temperature, TVB value rose within one day from 51.8 to 91.4 mg % (Mlay *et. al.* 1982).

However, in the present investigation the value of TVB-N increased from 3.38 to 114.47 mg % in tilapia and 4.42 to 134.95 mg % in bhetki. The differences among storage trials and with other fish species confirms that TVB-N cannot be used as universal quality indicators with a specific set of criteria and standards applicable to all fish species (Ababouch *et. al.* , 1996).

Reilly *et. al.*, 1985) reported that TVB value was doubtful as quality indices of some tropical fish and shell fish. The value fluctuated during ambient temperature. At ambient temperature the TVB-N value of both species (tilapia and bhetki), did not fluctuate and therefore served as good indicator of spoilage. TVB-N highly correlated with storage time and organoleptic score (Table 6a-b and 8a-b). TVB-N value increased due to autolytic and bacterial spoilage (Reilly *et. al.*, 1985).

5.2.2.2 Trimethylamine Nitrogen (TMA-N)

The use of TMA-N as an index of fish freshness was first proposed by Beatty and Gibbons (1936). This was based on the observation that the production of TMA was dependent on the bacterial activity as well as from endogenous enzyme. In recent years, there were opinions that TMA itself may not be a very suitable freshness index. This is because the TMA-N content in a fish may vary with season, and also the distribution of TMA within a piece of fillet may not be uniform. Under the local conditions, TMA was found to be a good indicator of freshness for white pomfret, Chinese pomfret and grouper. TMA is not a good indicator of freshness for lizard fish. Instead, DMA and FA are suitable indices (Siang and Kim, 1992).

For whiting (*Sillago maculatus*) and tilapia (*Oreochromis nilotica*) stored at ambient temperature, TMA contents were 13 and 15 mg % respectively (Estrada *et. al.*, 1985). When fish (*Rastrelliger faughni*) was rejected by the taste panel, TMA value ranged between 1.22 to 2.89 mg % at ambient temperature (Barile *et. al.*, 1985). Similar results were observed in the present investigation for both the species.

TMA content of brackish water prawn (*Penaeus monodon*) fluctuated during storage at ambient temperature and were regarded as poor indices of quality (Reilly *et. al.*, 1985). The value of TMA was found to be questionable use as indices of spoilage for mackerel (*Rastrelliger faughni*) (Barile *et. al.*, 1985). The findings differed with the result of the present investigation as TMA-N value did not fluctuate and showed good correlation with storage time and organoleptic score. Probably, the value of TMA-N for both the species increased gradually due to continuous autolytic and bacterial action (Azam *et. al.*, 1997).

Trimethylamine Nitrogen (TMA-N) accumulated rapidly in sardine (*Sadina pilchardus*) at ambient temperature to reach 112.2 mg % at rejection times (Ababouch *et. al.*, 1996). The value was too high compared to value observed in the present (48.96 mg %) for bhetki. The higher value may be due to the presence of high concentration of TMAO-N in sardine compared to bhetki.

5.2.2.3 Trimethylamine Oxide Nitrogen (TMAO-N)

Trimethylamine oxide is a nitrogenous compound commonly present in marine organisms. It has been suggested that TMAO-N functions as an osmoregulatory in these animals. The degradation of TMAO-N to simpler compounds such as trimethylamine, dimethylamine and formaldehyde depends on the enzyme present in the tissue. Generally, TAMO breaks down to TMA in marine fishes, either by endogenous enzyme, bacterial enzyme or both (Siang and Kim, 1992).

Many workers have found no TMAO in freshwater fishes (Hope-Seyer, 1930; Kutscher, and Ackerman, 1933; Smith, 1936, Bettey, 1939; Dyer, 1952; Jhonson, 1951). Shewan

(1951) noted that TMAO often occurred in fresh water fish but the amount of TMAO is much smaller in freshwater species than in marine species. The findings are in agreement with the present investigation where tilapia contained very low or negligible amount of TMAO-N. While bhetki, a marine species contained significant amount of TMAO-N (Love *et. al.*, 1959).

Highest TMAO value were observed in elasmobranch tissue (500-1500mg/100g) followed by squid and gadoid (88mg/100g) (Yamada and Harda, 1967). Cod showed an average value of 95mg % (Dyer, 1952). A compilation of data up to 1975 by Harda (1975) showed the differences in the average content of TMAO by family: from highest to lowest, Selachii (ca. 190 mg %), Rajida (ca. 99 mg %), Gadida (83 mg %), Cottida (54 mg %), Lophiida (47 mg %), Pleuronctida (47 mg %), Clupeida (31 mg %), Percida (28 mg %), Tetraodontida (23 mg %) and Belonida (19 mg %). TMAO-N content in bhetki as found in the present study was 68.3 mg %. TMAO content is not uniform. It variation depended on geographical location, fishing method, landings etc. (Ababouch *et. al.*,, 1996).

TMAO-N decreased with the increase of storage time at ambient condition (Magnusson and Martinsdottir, 1995). Similar pattern was also observed by Oehlenschlagler (1996) and Edmondson (1994). However, the study in the present investigation are in agreement with the above mentioned findings.

5.2.2.4 pH

The measurement of pH was suggested by Wood *et. al.* (1942) as a rapid test for detection of spoilage of fish. However, Farber (1965) doubted the reliability of this test and stated that the factors affecting the pH value such as fishing methods, fishing grounds, species, season, bacterial load, types of bacteria etc. would require much more investigation (Anon, 1988).

The pH value of the brackish water prawn (*Penaeus monodon*) ranged from 7.1 to 8.1 at ambient temperature (Reilly *et. al.*, 1985). At ambient condition in sardine (*Sardina*

pilchadus), the muscle pH increase from 6.15 to 6.85 (Ababouch *et. al.*, 1996). Similar results were observed in the present investigation. The increase in pH values can be attributed to the higher levels of volatile compounds produced by microbial and tissue enzymes in fish (Reilly *et. al.*, 1985).

5.2.3 Microbiological (TVC)

For the bacterial spoilage the most commonly used method is the total viable count (Rahman, 1980). Castell *et. al.* (1948) and Khalil (1964) have found that bacterial counts were valuable as a measure of degree of freshness of fish.

The initial and rejection of bacterial count of whiting (*Sillago maculata*) and tilapia (*Oreochromis nilotica*) at ambient temperature were within the range reported for temperate species of $10^3 - 10^8$/g (Shewan, 1976). Gorczyca *et. al.* (1985) reported the total bacterial count of the 'wet' fish ranging from $10^3 - 10^8$ /g when stored at ambient temperature. At ambient temperature, there was a rapid increase in bacterial numbers from $10^4 - 10^8$ /g with 16 hours and bacterial counts were within the range $10^6 - 10^9$/g on rejection (Reilly *et. al.*, 1985). Barile *et. al.* (1985) had carried out an experiment on the spoilage pattern of mackerel (*Rastrelliger kanagurta*, Matsui) at ambient temperature and reported that when the fish was rejected by the taste panel, the bacterial load was 10^7/g. However, in the present investigation for both species (tilapia and bhetki), the initial and rejection bacterial count were almost same as observed by the above authors.

5.3 Proximate Composition of Tilapia (*Tilapia nilotica*) and Bhetki (*Lates calcarifer*) in Relation to Anatomical Portion, Size and Sex.

Fish are utilised chiefly as a food, primarily for human consumption, but to lesser extent, in the nutrition of animals as well. A knowledge of chemical composition of fish along with its nutritive value is important in order that fish can compete with other sources of animal protein food (Stansby and Olcott, 1963). Processing method is dependent on the biochemical composition of the fish and its effect on the nutrient content. Chemical composition is also of importance to housewife and to the dietician since different fish cooking methods are applicable to the species of varying composition and to provide suggestion about diets to the patients. Chemical composition of fish are affected by numerous factors either of intrinsic or extrinsic. The chemical composition of fish can be expected to vary largely from one species to another and even within the same specimen (Jacquot, 1961). Sexual stage of development also influenced the composition of fish (Khuda *et. al.*, 1962).

5.3.1 Proximate in relation to anatomical portion (Head, Middle and Tail)

Studies on the proximate composition of tilapia and bhetki in relation to anatomical portion (head, middle and tail) showed that higher ash content was found in tail region. It is due to higher proportion of bones in this region (Rubbi *et. al.*, 1985).

Protein and lipid content was found to be higher in head region. But moisture content among different parts of the body did not vary considerably. The variation in protein content was not pronounced in bele fish (*Glossogobius guiris*) (Anon, 1983). The ventral portion showed highest fat content and lower moisture content in compared to other portions.

Highest amount of moisture content was found in the dorsal region (77.16%) and lowest in the ventral region (74.55%). Highest fat content(5.6%) was in the ventral region lowest in the dorsal region (2.2%). Protein and ash content did not vary considerably in *Catla catla*, (Anon, 1989).

Moisture and fat contents were inversely related. The greater fluctuation in moisture and fat with small variation in case of protein content was reported by Khuda *et. al.* (1960), Claude and Thurston (1958) and Stansby (1963).

Highest amount of moisture content was found in tail part (81.36%) but fat content was highest in ventral part (0.96%). Protein content was highest in the dorsal part and lower in the tail portion. Ash content did not show any significant pattern of variation (Anon 1983).

5.3.2 Proximate composition in relation size (Large, Medium and Small)

The result of the proximate composition of tilapia and bhetki in relation to size (large, medium and small) indicated that small sized fish contained more protein than large sized fish. Juvenile fish contained more protein but less fat than the adults in the same season. Such variation in composition was observed by Rubbi *et. al.* (1985). In the early stage, the growth rate is rapid and a higher rate of protein anabolism takes places in younger ones. Food taken by younger fish is diverted to the building of new cell i. e. mainly to protein (Rubbi *et. al.*, 1985). As fish grows bigger (juvenile to adult), the percentage of many constituents increases (Anderson and Fellers, 1952).

In the present investigation, lipid content was found to be higher in large sized fish while small size fish contained lower lipid percentages. Sesa (1953) reported that smaller fish contained more moisture but less fat. Khuda *et. al.* (1962) observed decreased in moisture but increase in the fat percentage with the increase of age in case of carps.

Ash content did not vary considerably in all sized fish i.e. almost same in the present investigation. Variation in ash and fibre contents between large and small sized catla were insignificant and these two body constituents remain close to constant in salmon stated by Stansby (1963).

The moisture content of the large and small sized fish were 74.26% and 77.47% respectively. Similar result were observed in the present investigation for both species as

small sized fish contained more moisture (Sesa, 1953).

5.3.3 Proximate composition in relation sex (Male and Female)

Analysis of proximate composition of tilapia and bhetki in relation to sex (male and female) in the present investigation showed that protein and fat content were found to be high in male than the female. Similar pattern of findings were observed by Rahman (1980) . However, this result differed with the findings of Rubbi *et. al.* (1985) and Anon (1983) who found that female had higher protein and lipid content. This may be due to the fact that during egg formation, fish spend great deal of energy, the principal source of which is fat and protein. The two components are mobilised for gonad formation (Anon, 1983).

SUMMARY AND CONCLUSION

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The investigation focused on the shelf life studies of *Tilapia nilotica* and *Lates calcarifer* stored in ice and at ambient temperature. The study was also extended to measure the proximate composition of *Tilapia nilotica* and *Lates calcarifer* in relation to different anatomical portions, sizes and sexes. Iced storage trial was carried out for a period of 25 days while fish were stored at ambient temperature for 24 hours.

In case of *Tilapia nilitica* stored in ice, the fish were organoleptically of very good quality initially. However, the quality of fish decreased with storage time. The shelf life thus lied between 10 and 13 days when the score were between 6.25 to 5.46. The correlation between storage days and organoleptic score was found to be highly negative ($r = -0.974$). While there were significant differences ($P \leq 0.05$) between storage days and organoleptic score.

TVB-N content in *Tilapia nilotica* increased from 4.49±1.91 to 116.5±7.4 mg-N/100g. At the time of rejection, TVB-N value was 28.03±1.92 mg-N/100g. The relationship between storage days and TVB was positive ($r = 0.93$) but negative ($r = -0.876$) with organoleptic score. Statistical analysis indicated significant differences ($P \leq 0.05$) between storage days and TVB-N value.

TMA-N value varied between 2.52±0.22 to 3.01±0.13 mg-N/100g at the time of rejection. Storage days and TMA-N indicated positive ($r = 0.95$) correlation and negative ($r = -0.969$) with organoleptic score. Significant statistical differences ($P \leq 0.05$) were observed between time and TMA-N.

When *Tilapia nilotica* was just acceptable, TMAO-N value ranged between 4.11±0.14 to 1.98±0.06 mg-N/100g. From 14th day onward TMAO-N could not be detected. Correlation between storage time and TMAO-N was found to be highly negative ($r = -0.97$) and highly positive ($r = 0.99$) with organoleptic score. No significant differences ($P \leq 0.05$) were observed between storage time and TMAO-N content.

The pH of *Tilapia nilotica* was 7.05±0.11 when the fish was rejected organoleptically. Relationship between storage days and pH were highly positive (r = 0.98) and highly negative (r = -0.99) with organoleptic score. No significant differences (P≤0.05) were observed.

TVC of *Tilapia nilotica* increased from $\log_{10}$5.33±0.07 to $\log_{10}$8.92±0.49 cfu/gm. The correlation between storage days and TVC was found to be highly positive (r = 0.90) and organoleptic score indicated negative correlation (r = -0.948) with TVC. Statistically significant differences (P≤0.05) were observed between storage days and TVC.

For stored in ice, the shelf life of *Lates calcarifer* lied between 18 and 19 days when the score was found to vary between 5.92 to 5.10. The correlation between storage days and organoleptic score was found to be highly negative (r = -0.98). Significant differences (P≤0.05) were observed between storage days and organoleptic score.

At the time of rejection, TVB-N value was 76.51±3.07 mg-N/100g. Storage days showed positive (r = 0.87) correlation with TVB-N while organoleptic score and TVB-N indicated a negative (r = -0.864) relationship. Significant statistical differences (P≤0.05) were observed between storage days and TVB-N.

The TMA-N value of *Lates calcarifer* increased from 1.67±0.47 to 61.84±5.1 mg-N/100g. When the fish was just acceptable as judged by the panellists, the TMA-N value ranged between 9.56±1.42 to 26.05±0.53 mg-N/100g. Correlation between storage days and TMA-N content was found to be positive (r = 0.84) and negative (r = -0.846) with organoleptic score. Significant differences (P≤0.05) were observed between storage days and TMA-N content.

Initially TMAO-N value was found to be 76±0.41 mg-N/100g in *Lates calcarifer*. At the time of rejection, the TMAO-N was 24.45±0.53 mg-N/100g. Storage days indicated a highly negative (r = -0.98) correlation with TMAO-N while highly positive (r = 0.965) correlation was observed between organoleptic score and

TMAO-N content. Statistical analysis indicated a significant differences ($P \leq 0.05$) between storage days and TMAO-N.

pH of *Lates calcarifer* increased from 6.42±0.17 to 7.47±0.16. When the fish was just acceptable as judged by the panellists the pH varied between 7.03±0.14 to 7.21±0.16. The correlation between storage days and pH indicted a positive ($r = 0.98$) relation but organoleptic score showed a highly negative ($r = -0.982$) correlation with pH. Statistically non significant differences ($P \leq 0.05$) were observed between storage days and pH.

When the fish was just acceptable organoleptically, TVC ranged between $\log_{10}6.77 \pm 0.08$ to $\log_{10}7.91 \pm 0.45$ cfu/gm. TVC could not be detected at the end of the investigation because of enormous number of colonies. Positive ($r = 0.88$) correlation was found between storage days and TVC. Organoleptic score indicated a highly negative ($r = -0.95$) correlation with TVC. While statistically significant differences ($P \leq 0.05$) were observed between storage days and TVC.

Initially at ambient temperature, *Tilapia nilotica* was found to be of very good quality as judged by the taste panellists who provided an average score of 9.11. However, the quality of fish deteriorated with time and were rejected after 10^{th} hour onwards. The shelf life thus lied between 10 and 17 hours when the score were between 6.27 to 5.29. The correlation between storage time and organoleptic score was highly negative ($r = -0.99$). Significant differences ($P \leq 0.05$) were observed between storage time and organoleptic score.

TVB-N value of *Tilapia nilotica* was 48.80±0.24 mg-N/100g at the time of rejection. Storage period and TVB-N indicated a highly positive ($r = 0.95$) correlation where as organoleptic score showed highly negative correlation ($r = -0.96$) with TVB-N. Statistically significant differences ($P \leq 0.05$) were observed between storage time and TVB-N.

TMA-N content increased from 1.67±0.07 to 6.91±0.03 mg-N/100g at ambient temperature. When the fish was just acceptable organoleptically, the value of TMA-N varied between 2.02±0.08 to 2.27±0.03 mg-N/100g. Positive correlation (r = 0.89) was found between storage time and TMA-N but organoleptic score indicated a negative (r = -0.91) relation with TMA-N. Statistical analysis indicated significant differences (P≤0.05) between storage time and TMA-N.

Initially TMAO-N of *Tilapia nilotica* was 4.14±0.78 mg-N/100g at ambient temperature. This value reduced further to 1.26±0.19 mg-N/100g on the 10^{th} hour when the fish was 'just acceptable' TMAO-N could not be detected from the 10^{th} hour onwards. The correlation between storage time and TMAO-N was found to be highly negative (r = -0.93). However, organoleptic score showed positive correlation (r = 0.876) with TMAO-N. Significant differences (P≤0.05) were observed between storage time and TMAO-N.

pH varied between 6.59±0.06 to 6.85±0.08 at the time of rejection. Storage periods and pH indicated a highly positive (r = 0.92) correlation and organoleptic score showed negative (r = -0.898) correlation with pH. While there was significant differences (P≤0.05) between storage time and pH.

The TVC of *Tilapia nilotica* was between $\log_{10}5.17\pm0.16$ to $\log_{10}6.94\pm0.05$ cfu/gm when the fish was judged as 'just acceptable' by the panellists. Storage time showed a highly positive (r = 0.95) correlation with TVC while organoleptic score showed highly negative (r = -0.95) correlation. Significant statistical differences (P≤0.05) were observed between storage period and TVC.

At ambient temperature, the shelf life of *Lates calcarifer* lied between 17 and 20 hour organoleptically. Initially taste panellists recorded the fish as 'highly acceptable' with an average score of 9.16 on a 10 point scale. At the time of rejection, when the fish judged was 'just acceptable', the score varied 6.27 to 5.27. Highly negative (r = -0.99) correlation was found between storage time and organoleptic score where as statistical analysis indicated significant differences (P≤0.05) between storage time and organoleptic score.

TVB-N content of *Lates calcarifer* increased from 4.42±1.1 to 134.95±3.90 mg-N/100g at ambient temperature. When the fish was rejected organoleptically, the TVB-N value was 109.54±2.96 mg-N/100g. Storage periods and TVB value indicated a positive correlation (r = 0.86) but organoleptic score showed a highly negative correlation (r = -0.915) with TVB-N. Statistical analysis indicated significant differences (P≤0.05) between storage period and TVB-N content.

At the time of rejection, TMA-N of *Lates calcarifer* was 40.75±2.50 mg-N/100g. Storage time showed a positive (r = 0.85) correlation with TMA-N content. But correlation between organoleptic score and TMA-N content was highly negative (r = -0.91). Statistically significant differences (P≤0.05) were observed between storage period and TMA-N value.

The initial value of TMAO-N was 68.30±0.78 mg-N/100g which decreased to 13.70±2.00 mg-N/100g at the end of the investigation. The correlation between storage period and TMAO-N value was found to be highly negative (r = -0.988). Organoleptic score showed highly positive (r = 0.968) correlation with TMAO-N. While significant differences (P≤0.05) were observed between storage time and TMAO-N.

pH of *Lates calcarifer* varied between 7.10±0.05 to 7.23±0.05 at the time of rejection. Correlation between storage time and pH was highly positive (r = 0.94) where as organoleptic score showed highly negative (r = -0.964) correlation with pH. Significant differences (P≤0.05) were observed between storage periods and pH.

When the fish was just acceptable as judged by the panellists, TVC ranged between $\log_{10}$6.91±0.04 to $\log_{10}$7.87±0.56 cfu/gm. Storage period showed highly positive (r = 0.97) correlation with TVC where as organoleptic score indicated highly negative (r = -0.910) correlationship. Statistically significant differences (P≤0.05) were observed between storage time and TVC.

The comparative study between *Tilapia nilotica* and *Lates calcarifer* in ice indicated that tilapia had a shelf life which lied between 10 to 13 days and bhetki 18 to 19 days respectively. Initially, both species were considered very good quality. At the time of rejection, the value of TVB-N, TMA-N, TMAO-N and pH indicated a range between 20.64±0.10 to 28.03±1.42 mg-N/100g and 32.90±1.88 to 76.51±3.07 mg-N/100g, 2.52±0.22 to 3.01±0.13 mg-N/100g and 9.56±1.42 to 26.05±2.6 mg-N/100g, 2.23±0.10 to 1.98±0.06 mg-N/100g and 26.45±0.53 to 24.45±2.42 mg-N/100g and 6.97±0.10 to 7.05±0.11 and 7.03±0.14 to 7.21±0.16 in *Tilapia nilotica* and *Lates calcarifer*. The corresponding organoleptic score and TVC were between 6.25 to 5.46 and 5.92 to 5.10 and $\log_{10}$6.90±0.05 to $\log_{10}$7.72±0.05 cfu/gm and $\log_{10}$6.77±0.04 to $\log_{10}$7.91±0.08 cfu/gm in tilapia and bhetki.

Comparative study at ambient temperature indicated that *Tilapia nilotica* had a shelf life which lied between 10 to 17 hours and *Lates calcarifer* 17 to 20 hours. Initially both the species were found to be very good quality (9.11 and 9.16). During this time, the value of TVB-N, TMA-N, TMAO-N and pH was 3.38±1.34 and 4.42±1.13, 1.16±0.07 and 1.52±0.71, 4.14±0.78 and 68.3±0.58 mg-N/100g, and 6.43±0.05 and 6.83±0.05 in tilapia and bhetki. TVC indicated a value of $\log_{10}$4.28±0.04 and $\log_{10}$4.93±0.05 cfu/gm initially in both the species (tilapia and bhetki). At the end of the investigation, TVB-N, TMA-N, TMAO-N and pH was 114.47±3.81 and 134.95±3.90, 6.94±0.33 and 48.96±2.32, not detected and 13.70±2.00 mg-N/100g, 7.57±0.05 and 7.53±0.05, and $\log_{10}$9.73±0.14 and $\log_{10}$8.73±0.11 cfu/gm respectively in both the species (tilapia and bhetki). The corresponding organoleptic score were 3.37 and 4.28.

Protein and lipid content was found to be high in the head portion of *Lates calcarifer* (20.77% and 1.8%) compared to *Tilapia nilotica.* Ash content did not vary much in both the species. Moisture remained high (80.92% to 81.02%) in all the portion of *Tilapia nilotica* than *Lates calcarifer* in (77.45% - 78.99%).

Large size of *Lates calcarifer* had higher percentage of protein (19.55%) and lipid (2.55%) compared to *Tilapia nilotica* (17.91% and 1.33%). Ash content was almost same in both the species. Small size *Tilapia nilotica* contained higher moisture than *Lates calcarifer*.

With regard to different sexes (Male and Female), moisture was found to be much higher in *Tilapia nilotica* (80.78% - 81.09%) in comparison to *Lates calcarifer* (76.37% - 77.98%). Protein and lipid content varied between 16.82% - 19.91% and 1.55% - 2.35% respectively in both the species. Ash content was almost similar.

The present investigation provides some data on the organoleptic, biochemical (TVB-N, TMA-N, TMAO-N and pH) and microbiological (TVC) changes that took place during the storage of tilapia and bhetki in ice and at ambient temperature. It was confirmed that spoilage pattern of marine species (bhetki) were not always same to that of fresh water fish (tilapia). From the results obtained in the present study, the following conclusions could be drawn:

1) Tilapia had a shorter shelf life of 10 – 13 days while bhetki had relatively longer shelf life of 18 –19 days when stored in ice.

2) At ambient temperature (28 – 30^{0}C) both the species spoiled very quickly. Again in line with iced storage trial, keeping time of tilapia was short (10 –17 hours). But bhetki had a longer keeping time (17 – 20 hours).

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APPENDIX

APPENDIX - 1 Quality (Organoleptic, Biochemical & Microbiological) of *Tilapia nilotica* during iced storage.

Storage condition	Storage period	Acceptability	Average score	TVB-N (mgN/100g)	TMA-N (mgN/100g)	TMAO-N (mgN/100g)	pH	TVC (Log_{10} values) Cfu/gm
Iced	1	HA	9.08	4.49 ±1.91	1.10 ± 0.01	4.11 ±0.14	6.56 ± 0.11	5.33 ±0.07
	3	A	8.14	7.18 ± 1.84	1.66 ± 0.03	3.56 ± 0.03	6.67 ± 0.14	6.07 ± 0.03
	5	MA	7.19	18.58 ± 1.48	2.01 ± 0.08	3.02 ± 0.07	6.86 ± 0.15	6.08 ± 0.03
	10	**JA**	6.25	**20.64 ± 0.94**	**2.52 ± 0.22**	**2.23 ± 0.10**	**6.97 ± 0.1**	**6.90 ± 0.05**
	13	**JU**	5.46	**28.03 ± 1.42**	**3.01 ± 0.13**	**1.98 ± 0.06**	**7.05 ± 0.11**	**7.72 ± 0.05**
	18	U	5.24	44.64 ± 1.91	3.83 ± 1.21	Nd*	7. 13 ± 0.1	8.78 ± 0.12
	19	U	5.19	72.62 ± 2.91	5.80 ± 0.19	Nd*	7.2 ± 0.1	8.30 ± 0.49
	22	MU	4.29	98.78 ± 5.62	7.02 ± 0.12	Nd*	7.24 ± 0.09	8.92 ± 0.49
	25	MU	3.06	116.25 ± 7.64	8.08 ± 0.17	Nd*	7.41 ± 0.13	Uncountable

Nd* = Not detected

APPENDIX - 2a Correlation between Storage period (days) and Organoleptic score, TVB-N, TMA-N, TMAO-N, pH) and TVC in *Tilapia nilotica* during iced storage.

Storage condition	**Relationship**	**Coefficient of correlation (r value)**
Iced storage	Storage day Vs Organoleptic score	- 0.97
	Storage day Vs TVB-N	0.93
	Storage day Vs TMA-N	0.95
	Storage day Vs TMAO-N	- 0.97 (up to 13th day)
	Storage day Vs pH	0.98
	Storage day Vs TVC	0.90 (up to 22nd day)

APPENDIX - 2b Correlation between Organoleptic score and TVB-N, TMA-N, TMAO-N, pH and TVC in *Tilapia nilotica* during iced storage.

Storage condition	**Relationship**	**Coefficient of correlation (r value)**
Iced storage	Organoleptic score Vs TVB-N	- 0.876
	Organoleptic score Vs TMA-N	- 0.969
	Organoleptic score Vs TMAO-N	0.99 (up to 13th day)
	Organoleptic score Vs pH	- 0.99
	Organoleptic score Vs TVC	- 0.948 (up to 22nd day)

APPENDIX - 3 Quality (Organoleptic, Biochemical & Microbiological) of *Lates calcarifer* during stored in ice.

Storage condition	Storage period	Acceptability	Average score	TVB-N (mgn/100 g)	TMA-N (mgn/100 g)	TMAO-N (mgn/100 g)	pH	TVC (Log values) Cfu/gm
Iced	1	HA	8.87	4.24 ± 0.02	1.67 ± 0.47	76.21 ± 0.42	6.42 ± 0.17	5.26 ± 0.04
	3	A	8.00	8.64 ± 0.21	3.94 ± 1.11	67.80 ± 0.83	6.54 ± 0.21	6.06 ± 0.06
	5	MA	7.28	17.37 ± 1.14	6.02 ± 0.28	55.73 ±1.03	6.71 ± 0.15	5.87 ± 0.06
	10	JA	6.35	21.02 ± 0.50	6.59 ± 0.14	48.80 ± 0.24	6.91 ± 0.12	6.75 ± 0.04
	13	JA	6.19	27.31 ± 1.78	7.74 ± 0.19	29.98 ± 0.26	7.01 ± 0.11	6.67 ± 0.06
	18	**JA**	**5.92**	**32.90 ± 1.88**	**9.56 ±1.42**	**26.45 ± 0.53**	**7.03 ± 0.14**	**6.77 ± 0.08**
	19	**JU**	**5.10**	**76.51 ± 3.07**	**26.05 ± 2.6**	**24.45 ± 2.42**	**7.21 ± 0.16**	**7.91 ± 0.45**
	22	U	4.81	117.19 ± 3.28	41.76 ±1.42	18.02 ± 0.85	7.27 ± 0.17	9.56 ± 0.06
	25	U	4.0	132.46 ± 2.39	61.84 ± 5.1	14.79 ± 0.94	7.47 ± 0.16	Uncountable

APPENDIX - 4a Correlation between Storage periods (days) and Organoleptic score, TVB-N, TMA-N, TMAO-N, pH and TVC in *Lates calcarifer* during iced storage.

Storage condition	Relationship	Coefficient of correlation (r value)
	Storage day Vs Organoleptic score	- 0.98
	Storage day Vs TVB-N	0.87
Iced storage	Storage day Vs TMA-N	0.84
	Storage day Vs TMAO-N	- 0.98
	Storage day Vs pH	0.98
	Storage day Vs TVC	0.88 (up to 22nd day)

APPENDIX - 4b Correlation between Organoleptic score and TVB-N, TMA-N, TMAO-N, pH and TVC in *Lates calcarifer* during iced storage.

Storage condition	Relationship	Coefficient of correlation (r value)
	Organoleptic score Vs TVB-N	- 0.864
Iced storage	Organoleptic score Vs TMA-N	- 0.846
	Organoleptic score Vs TMAO-N	0.965
	Organoleptic score Vs pH	- 0.982
	Organoleptic score Vs TVC	- 0.950 (up to 22nd day)

APPENDIX - 5 Quality (Organoleptic, Biochemical & Microbiological) of *Tilapia nilotica* stored at ambient temperature.

Storage condition	Storage period	Acceptability	Total score	TVB-N (mgn/100g)	TMA-N (mgn/100g)	TMAO-N (mgn/100g)	pH	Colony (Log values) Cfu/gm
Stored at ambient temperature	0	HA	9.11	3.58 ± 1.34	1.16 ± 0.07	4.14 ± 0.78	6.43 ± 0.05	4.38 ± 0.04
	5	A	8.21	5.71 ±1.48	1.46 ± 0.7	1.96 ± 0.28	6.51 ± 0.08	5.32 ± 0.04
	10	JA	6.27	31.24 ± 0.1	2.02 ± 0.08	1.26 ± 0.19	6.59 ± 0.06	5.17 ± 0.06
	17	JU	5.29	48.80 ± 0.24	2.27 ± 0.07	Nd*	6.86 ± 0.08	6.94 ± 0.05
	20	U	4.14	92.83 ± 3.22	3.81 ± 1.21	Nd*	7.00 ± 0.06	8.83 ± 0.10
	24	MU	3.37	114.47 ± 3.81	6.94 ± 0.33	Nd*	7.57 ± 0.05	9.73 ± 0.14

Nd* – Not detected

APPENDIX - 6a Correlation between Storage periods (hours) and Organoleptic score, TVB-N, TMA-N, TMAO-N, pH and TVC in *Tilapia nilotica* stored at ambient temperature.

Storage condition	**Relationship**	**Coefficient of correlation (r value)**
At ambient temperature	Storage periods (hours) Vs Organoleptic score	- 0.99
	Storage periods (hours) Vs TVB-N	0.95
	Storage periods (hours)Vs TMA-N	0.83
	Storage periods (hours)Vs TMAO-N	- 0.93 (up to 10^{th} hour)
	Storage periods (hours)Vs pH	0.92
	Storage periods (hours)Vs TVC	0.95

APPENDIX - 8a Correlation between Storage periods (hours) and Organoleptic score, TVB-N, TMA-N, TMAO-N, pH and TVC in *Lates calcarifer* stored at ambient temperature.

Storage condition	Relationship	Coefficient of correlation (r value)
	Storage periods (hours) Vs Organoleptic score	- 0.99
At ambient	Storage periods (hours) Vs TVB-N	0.86
temperature	Storage periods (hours)Vs TMA-N	0.85
	Storage periods (hours)Vs TMAO-N	- 0.98
	Storage periods (hours) Vs pH	0.94
	Storage periods (hours) Vs TVC	0.97

APPENDIX - 8b Correlation between Organoleptic score and TVB-N, TMA-N, TMAO-N, pH and TVC in *Lates calcarifer* stored at ambient temperature.

Storage condition	Relationship	Coefficient of correlation (r value)
	Organoleptic score Vs TVB-N	- 0.915
At ambient	Organoleptic score Vs TMA-N	- 0.910
temperature	Organoleptic score Vs TMAO-N	0.968
	Organoleptic score Vs pH	- 0.964
	Organoleptic score Vs TVC	- 0.971

APPENDIX - 9 Analysis of Variance (ANOVA) between Storage days and Organoleptic score, TVB-N, TMA-N, TMAO-N, pH and TVC in *Tilapoa nilotica* during iced storage.

APPENDIX - 9a Storage day versus Organoleptic Score

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	8250.8	8	1031.35	**7.9916	2.0548
Within groups	10453.3	81	129.05		
Total	18704.1	89			

APPENDIX - 9b Storage day versus Biochemical (TVB-N, TMA-N, TMAO-N and pH)

TVB-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	111138	8	13892.25	**58.3584	2.0548
Within groups	19282.08	81	238.05		
Total	130420	89			

TMA-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	760.59	8	95.07	**8.0342	2.0548
Within groups	958.52	81	11.83		
Total	1719.11	89			

TMAO-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	4.314114	4	1.078528	*0.275047	2.575737
Within groups	176.4565	45	3.921254		
Total	180.7706	49			

pH:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	99.38	8	12.42	*1.2737	2.0548
Within groups	790.02	81	9.75		
Total	889.4 0	89			

APPENDIX - 9c Storage day versus Microbiology (TVC)

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	521.28	8	65.16	**5.5981	2.0548
Within groups	959.96	81	11.85		
Total	1481.24	89			

SS= Sum of Square, MS= Mean of Square, df= degree of freedoms, stat.= statistical
** = Significant, * = Non significant

APPENDIX - 10 Analysis of Variance (ANOVA) between Storage days and Organoleptic score, TVB-N, TMA-N, TMAO-N, pH and TVC in *Lates calcarifer* during iced storage.

APPENDIX - 10a Storage day versus Organoleptic core

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	6383.29	8	797.91	**6.0751	2.0548
Within groups	10638.5	81	131.34		
Total	17021.79	89			

APPENDIX - 10b Storage day versus Biochemical (TVB-N, TMA-N, TMAO-N and pH)

TVB-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	146255.3	8	18281.92	**65.3540	2.0548
Within groups	22658.69	81	279.74		
Total	168914	89			

TMA-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	30305.49	8	3788.19	*151.46	2.0548
Within groups	2025.89	81	25.01		
Total	32331.33	89			

TAMO-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	2994.2	8	3742.81	**23.1875	2.0548
Within groups	130.75	81	161.41		
Total	43017	89			

pH:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	113.68	8	14.21	*1.4672	2.0548
Within groups	784.42	81	9.68		
Total	898.1	89			

APPENDIX - 10c Storage day versus Microbiology (TVC):

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	469.47	8	58.68	**4.7593	2.0548
Within groups	998	81	12.33		
Total	1468.22	89			

SS= Sum of Square, MS= Mean of Square, df= degree of freedoms, stat.= statistical
** = Significant, * = Non significant

APPENDIX - 11 Analysis of Variance (ANOVA) between Storage days and Organoleptic score, TVB-N, TMA-N, TMAO-N, pH and TVC in *Tilapoa nilotica* stored at ambient temperature.

APPENDIX - 11a Storage day versus Organoleptic score

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	5752.97	5	1150.59	**7.2344	2.3860
Within groups	8588.34	54	159.04		
Total	14341.32	59			

APPENDIX - 11b Storage day versus Biochemical (TVB-N, TMA-N, TMAO-N and pH)

TVB-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	88146.77	5	176.29	**66.7131	2.3860
Within groups	14269.84	54	264.26		
Total	1024166.6	59			

TMA-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	384.35	5	76.87	**5.3789	2.3860
Within groups	771.71	54	14.29		
Total	1156.06	59			

TMAO-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	15.74918	2	7.87459	*2.162159	3.354131
Within groups	98.3341	27	3.642004		
Total	114.0833	29			

pH:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	81.35	5	16.27	*1.6553	2.3860
Within groups	530.71	54	9.83		
Total	612.05	59			

APPENDIX - 11c Storage day versus Microbiology (TVC):

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	393.74	5	78.75	**10.7797	2.3860
Within groups	394.48	54	7.31		
Total	788.22	59			

SS= Sum of Square, MS= Mean of Square, df= degree of freedoms, stat.= statistical
** = Significant, * = Non significant

APPENDIX - 12 Analysis of Variance (ANOVA) between Storage days and Organoleptic score, TVB-N, TMA-N, TMAO-N, pH and TVC in *Lates calcarifer* stored at ambient temperature.

APPENDIX - 12a Storage day versus Organoleptic score

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	5646.69	5	1129.34	**7.0174	2.3860
Within groups	8690.35	54	160.93		
Total	1433.7	59			

APPENDIX - 12b Storage day versus Biochemical (TVB-N, TMA-N, TMAO-N and pH)

TVB-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	133476.6	5	2669.5	**76.5177	2.3860
Within groups	18839.39	54	348.88		
Total	152316.0	59			

TMA-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	18323.72	5	3664.75	**140.01	2.3860
Within groups	1413.42	54	26.17		
Total	19737.14	59			

TMAO-N:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	16621.19	5	3324.24	**25.6570	2.3860
Within groups	6996.47	54	129.56		
Total	23617.66	59			

pH:

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	65.29	5	13.06	*1.3425	2.3860
Within groups	525.22	54	9.93		
Total	590.51	59			

APPENDIX - 12c Storage day versus Microbiology (TVC):

Source of variance	SS	df	MS	'F' stat.	'F' critical
Between groups	233.9	5	58.78	**6.8126	2.3860
Within groups	465.91	54	8.65		
Total	759.81	59			

SS= Sum of Square, MS= Mean of Square, df= degree of freedoms, stat.= statistical
** = Significant, * = Non significant