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Assessment of Total Volatile Basic Nitrogen (TVB-N) and Microbial Contents of Iced Marine Fish Species

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ABSTRACT

The total volatile basic nitrogen (TVB-N) and microbial contents of iced species from the Arabian Gulf Shaeri (*Lethrinus miniatus*) and Hamour (*Epinephelus* chlorostigma) were investigated as indices of spoilage. On day 3 and till the end of storage period Hamour had higher pH values than Shaeri. The TVBN values in Hamour had risen from 15.9 mg/100g at zero time to 72.76 mg/100g at 5 days and then decreased to 64.3 mg/100g at 7 days. The TVBN had risen in shaeri from 10.5 mg/100g at zero time to 74.22 mg/100g at 5 days , Two fish marine species reached their maximum TVBN content on the 5th day of storage and were close to the rejection limit (30 mg/100 g) by day 3 of storage. Bacteria grew rapidly between day 1 and 10. Hamour exceeded the maximum microbiological limit for fresh fish recommended by (ICMSF), log 7.0 CFU/g by day 7 of storage, while Shaeri did not reached that limit by day 7.day of storage. Initial total plate counts of marine fish species were 3.4 and 3.2 (log 10 CFU /g fish) for Hamour and Shaeri respectively. Hamour and Shaeri psychrotrophic counts increased progressively with storage period. Initial psychrotrophic counts were 3.6 and 2.9 (log 10 CFU /g fish) for Hamour and Shaeri, respectively; these counts increased with the increase of storage time to reach a maximum values of 5.9 and 6.3 (log 10 CFU /g fish) respectively at the end of storage. Shaeri had high coliform counts both initially and at the end of storage period than Hamour. proteolytic counts of all fish species increased with extended storage reaching their maximum count on the last day of storage period . Marine fish species had initially proteolytic counts of 2.3 and 1.4 log CFU/g fish reaching a maximum value of 5.6 and 5.1 at the end of the storage period for Hamour and Shaeri respectively. The study showed that in both cases, the total volatile basic nitrogen (TVB-N) and microbial contents had increased with increasing storage time.

KEYWORDS: *Marine fish, shelflife, postmortem changes, TVB-N and microbial counts*

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INRODUCTION

Fish are a great source of protein, vitamins, and minerals and omega-3 fatty acids, a key nutrient for brain development (Spencer et al., 1971, Jaclyn et al., 2010, Jinadasa 2014, Paul et al 2016). Postharvest losses caused by spoilage amount to about 10 to 12 million tonnes per year and in addition, it is estimated that 20 million tonnes of fish in a year are discarded at sea which is another form of post-harvest losses (FAO, 2010). Due to the role of microorganisms, enzymes, physical and chemical factors, fish is prone to spoilage during storage . Fish is a highly perishable and spoils quickly, vast variations in quality due to differences in species, environmental habitats, feeding habits and action of autolysis enzymes as well as hydrolytic enzymes of microorganisms on the fish muscle (Venugopal, 2002). Spoiled fish cannot meet the needs of people's nutrition, and also risk people's health. Therefore, it is an important index to determine the degree of fish freshness. which mainly includes the detection of sensory, physical, chemical, microbial and so on. These detections of fish freshness has important significance in public health. Dora and Hiremath (1987) reported that freshness has significant influence on the shelf-life of frozen stored fish . Loss of freshness followed by spoilage is a complex combination of microbiological, chemical, and physical processes (Pedrosa-Menabrito and Regenstnstein, 1988). Chemical methods such as measuring Total volatile basic nitrogen (TVB-N) appears as the common chemical indicators of marine fish spoilage and is important characteristic for the assessment of quality in seafood products (Amegovu et al., 2012, Wu and Bechtel, 2008).Total volatile basic-nitrogen (TVB-N) is responsible for the fish spoilage after their body defense mechanisms stop, which contribute to changes in edible and sensory qualities, including deterioration in color, texture, appearance, aroma and flavor. The consumption of meat and meat products mostly depends on color, appearance, flavor and taste (Risvik, 1994; Van Oeckel et al., 1999; Davoli and Braglia, 2007). The combined total amount of ammonia (NH_3), dim ethylamine (DMA) and trim ethylamine (TMA) in fish is called the total volatile base (TVB) nitrogen content of the fish and is commonly used as an estimate of spoilage and has been widely used as an index for freshness of fish (Wu and Bechtel, 2008).The increase in the amount of TVB parallel with the increase in TMA during spoilage. As the activity of spoilage bacteria increases after the death of a fish (Jinadasa 2014). Total Volatile Bases (TVB) are used as an alternative to measuring TMA content. Changes in TVB content during spoilage are very similar to those of TMA in the same species, except that the initial value of TVB is much higher (Pedrosa-Menabrito and Regenstein, 1988).It is known that loss of freshness and spoilage pattern in fish markedly varies from species to species. Once the fish dies, several postmortem changes take place, which are due to the breakdown of the cellular structure and biochemistry as well as to the growth of microorganisms that are either naturally associated with the fish, or associated to contamination during handling (Ehira &Uchiyama, 1987). However, the amount of Total volatile basic-nitrogen (TVB-N) produced is an indicator of the degree of spoilage. Due to limited shelf life of fresh fish, a variety of methods have been introduced in attempt to extend the shelf life and to keep good quality and fish safety; these methods included the use of vacuum-packaging , freezing , chilling and icing. Modified atmosphere (MA) packaging with elevated levels of carbon dioxide extends product shelf life by extending the lag phase of aerobic spoilage bacteria (Statham, 1984; Farber, 1991, Reddy et al., 1994).

MATERIALS:

Marine fish species

1. Longface emperor (Lethrinus miniatus) (Shaeri)
2. Hexagonal spotted grouper(Epinephelus chlorostigma) (Hamour)

Marine water species. Longface emperor (Lethrinus miniatus) (Shaeri) and Hexagonal spotted grouper (Epinephelus chlorostigma) (Hamour) with a weight of about (300 – 400 g) were purchased from a local fish market. The fish were caught from the Arabian Gulf and immediately dispatched in plastic container filled with ice. Upon arrival at the laboratory, fishes were divided into five treatment groups and stored in crushed ice in five fish ice boxes (70 × 40 × 35 cm each). Longface emperor (Shaeri) and Hexagonal spotted grouper (Hamour) were stored in box A, B, C, S, and H respectively. The ratio of fish to cruched ice was 1:1 (w/w) and storage temperature was about (3-4^{O}C) over the storage period. Whenever, necessary boxes were dranined of melted ice water and more crushed ice was added to the boxes to maintain the temperature at around 3-4 ^{o}C.

METHODS OF ANALYSIS:

Marine fish species was assessed initially and after 3, 5, 7, 10 and 13 days of cold storage. Assessments include chemical analysis of (TVB-N) and microbiological analysis of total count, coliform, psychrotrophic and proteolytic bacteria and pH measurement.

1. pH measurement

The pH was determined by adding 5 gm ground fish meat to 20 ml distill water. The mixture was well homogenized and the pH was measured using a digital pH meter (Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA).

2. Total volatile basic nitrogen (TVBN) Determination

Homogenates (10%) of fish meat were prepared by blending 10 gm of sample with 90 ml distilled water for 2 min using a sorvall omnimixer (5000 rpm).To 10 ml homogenate, an equal volume of 10% Trichloro acetic acid (TCA) was added. After 15 min the slurry was filtered through whatman No. 1 filter paper. One ml of TCA filterate was used to determine TVBN according to the method of Farber and Fero (1956).

3. Microbiological Analysis

Samples (10 gm) from each treatment at the appropriate storage period (0, 3, 7 and 10 days) were diluted with 90 ml 0.1% peptone water (Difco Labs, Inc. Detroit, MI) and blended for 1 min in Stomacher (Wseward, Labs, London, U.K). Appropriate serial dilution (10^{-3} to 10^{-5}) of the homogenate were placed into petri plates in duplicate. The colony forming units (CFU) were determined using the standard plate count agar (SPC , Difco). Plates were incubated as follows:

1. Total plate counts: 37^{o}C, 48 hrs.
2. Psychrotrophic counts: 7^{o}C, 10 days.
3. Proteolytic counts: SPC + 1.0% Skimmed milk and incubated at 37^{o}C and 48 hrs.
4. Coliforms the violet red bile agar (VRB) was used. The incubation conditions were 37^{o}C, 48 hrs.

Statistical Analysis

The data collected were subjected to analysis of variance and whenever appropriate the mean separation procedure of Duncan was employed (Steel and Torrie, 1980). The SAS program (SAS, 1988) was used to perform the GLM analysis.

RESULTS AND DISCUSSION

1. pH values:

Generally, Shaeri pH values showed increased slightly with increased in the storage period (day 3 to day 7) may have resulted from the production of both alkaline (pseudomonas) and acidic (lactic acid bacteria). Haard, (1992) reported that, those fish have a relatively high content of amino acids and peptides that probably serve to buffer changes in pH. However, hamour pH values decreased slightly through day 3, then tended to increase. On day 3 and till the end of storage period Hamour had higher pH values than Shaeri. Simeonidou *et al.,* (1998) and Church (1998) indicated that postmortem pH can vary from 6.0 to 7.1 depending on season, species and other factors like handling conditions. On the other hand several investigators (Pacheo–Aguilar *et al.,* (2000) , Barret *et al.,* (1965) reported pH values below 6.0 for several fish species e.g halibut, tuna, sardine and mackerel. In the current study, pH of Hamour varied from 6.9 to 7.4 showing an increment of 0.5 unit. Riaz and Quadri (1985) indicated that a pH increment of more than 0.2 unit means that the sample had deteriorated. Based on this parameter Hamour had an acceptable quality for 3 days on ice (Fig. 1).

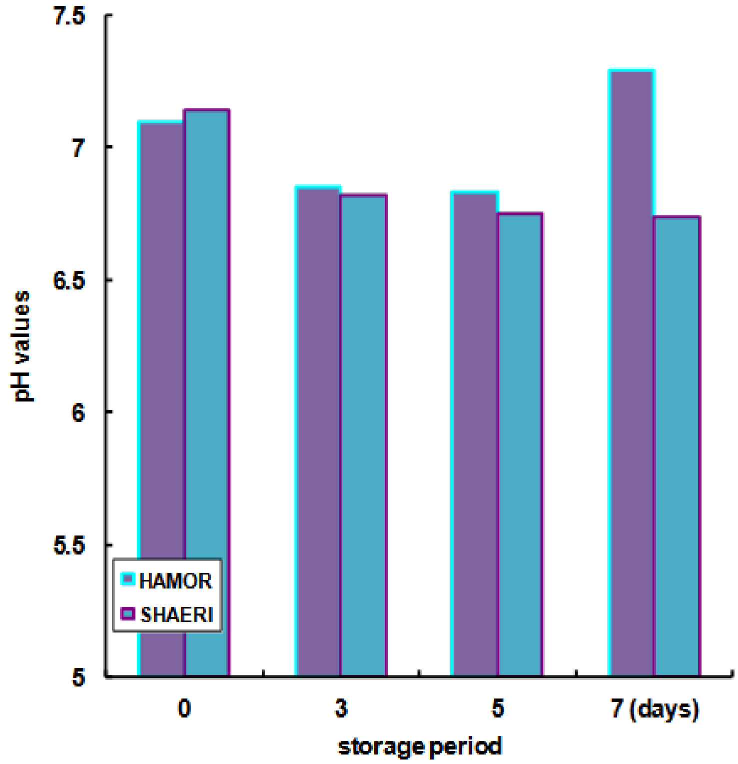

Fig.1: Changes in the pH values of marine fishes with storage period

2. Total volatile basic nitrogen (TVBN) Determination

TVBN is commonly used to evaluate fish muscle spoilage. For marine fish initially and throughout day 3, the changes in TVBN values were slow, then the changes were increased fast in TVBN values. The TVBN values particularly in Hamour had risen from 15.9 mg/100g at zero time to 72.76 mg/100g at 5 days and then decreased to 64.3 mg/100g at 7 days. A similar trend of TVBN values was also observed in shaeri during the storage period. TVBN had risen in shaeri from 10.5 mg/100g at zero time to 74.22 mg/100g at 5 days , However after 7days TVBN values decreased to 66.8 mg/100g . Two fish marine species reached their maximum TVBN content on the 5th day of storage and were close to the rejection limit (30 mg/100 g) by day 3 of storage. For several fish species, TVBN values were reported to increase curvilinearly or linearly with time (Perez-villareal and Pozo 1990; Gododlu et al., 1998). In this study TVBN of marine fishes species (Hamour and Shaeri) had, on the average, a wide range (13.3 to 73.49 mg/100g muscle) during the storage period. Such a result contradict with Mazaro-Manazo et al., (2000) who reported that TVBN content of Black Skipjack ranged from 25.5 to 34.3 mg N /100g muscle but agree with Gododlu et al., (1998) who reported a range of 13.2 to 64.8 mg/100g muscle during a storage period of 10 days. Suchitra and Sarojnalini (2012) reported that the gradual increase at the storage room temperature due to the elevation of temperature and subsequent microbiological and biochemical changes in the fish muscle. the continuous production of volatile bases due to the breakdown of proteins by action of microbes (Babu et al., 2005), and this is responsible for the generation of typical flavour and aroma of the final product (Majumdar et al., 2005) and also shows that higher liberation of TVBN were correlated with bacterial activity (Vanderzant et al 1973) (table 1).

Table.1: Changes in total volatile basic nitrogen (TVBN) values of marine fish with storage period. (mg/100g)

St. days	Hamour	Shaeri
0	15.9^{a}_{1}	10.5^{c}_{1}
3	28.5^{c}_{2}	26.6^{d}_{2}
5	72.76^{c}_{3}	74.22^{d}_{3}
7	64.3^{d}_{4}	66.8^{e}_{4}
10	-	-

Means in the same row bearing different superscripts letters different (p < .o5)
-means in the same column bearing different numericals are different (p < .o5).

3. Microbiological Analysis

Generally, Bacteria grew rapidly between day 1 and 10. Gram and Huss (2000) indicated that the bacterial numbers on the surface of fish increases with the increase in storage period. Hamour exceeded the maximum microbiological limit for fresh fish recommended by the International Commission on Microbiological Specification for foods (ICMSF), log 7.0 CFU/g by day 7 of storage. Initial total plate counts of marine fish species were 3.4 and 3.2 ($\log_{10}$ CFU /g fish) for Hamour and Shaeri respectively.At storage period tested Hamour had higher bacterial counts than Shaeri. While Hamour exceeded the maximum microbiological limit set by ICMSF (1978) by the 7th day of storage, Shaeri did not reached that limit even on the 10th day of storage. Reddy *et al.,* (1994) postulated that spoilage characteristic in fish fillet resulted when bacterial counts reached log 7.5 CFU/g fish, or higher (Fig. 2). Irrespective of the fish type Hamour or Shaeri psychrotrophic counts increased progressively with storage period. Initial psychrotrophics count of marine fish species had very comparable psychrotrophic counts. However, Hamour had slightly increased over Shaeri particularly in the first 7 days of storage while Shaeri had slightly increased over Hamour on the 10th day of storage. Initial psychrotrophic counts were 3.6 and 2.9 (log 10 CFU /g fish) for Hamour and Shaeri, respectively; these counts increased with the increase of storage time to reach a maximum values of 5.9 and 6.3 (log 10 CFU /g fish) respectively at the end of storage (Fig. 3). Obviously total coliform counts fluctuated during the storage period, fishes attained their maximum count at different times of storage. Shaeri had high coliform counts both initially and at the end of storage period than Hamour (Fig. 4). Generally proteolytic counts of all fish species increased with extended storage reaching their maximum count on the last day of storage period . Marine fish species had initially proteolytic counts of 2.3 and 1.4 log CFU/g fish reaching a maximum value of 5.6 and 5.1 at the end of the storage period for Hamour and Shaeri respectively (Fig. 5). A study conducted by Durairaj and Krishnamurthi (1986) showed that After 10 h at ambient temperature *L. rohita,* was completely spoild, while *C. mrigala* became unacceptable within 11 h. TVBN and total bacterial counts showed that *L. rohita* (0.5 kg and above) and *C. mrigala*(0.5 kg and above) were acceptable upto 7 to 8 days of storage in special ice in popular container(Bamboo basket lined with palmyrah mat) at ambient temperature.Influence of size of fish on shelf life in ice showed that *L. rohita* fishes below 500g were acceptable upto 6 days of storage in ice in popular container at ambient temperature while those above 1000g size were acceptable upto 8 days. Provision of alkathene lining to the popular container extended the storage life of *L. rohita* from 6 days to 8 days. Pre-chilled and iced *L. rohita* had a longer shelf life (9 days) than merely iced sample (7 days).

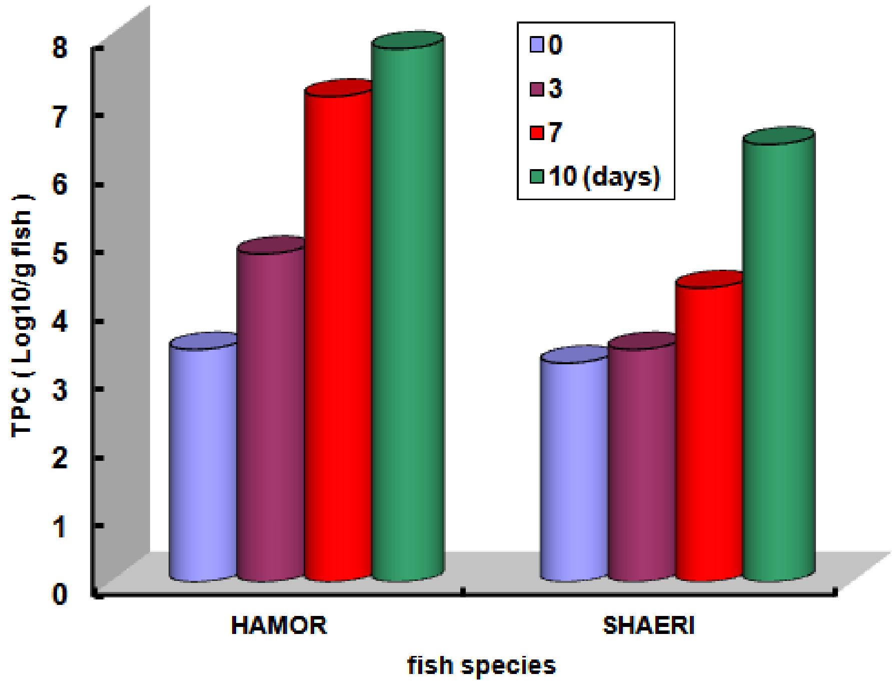

Fig.2: Changes in the TPC count of marine fish species with storage period.

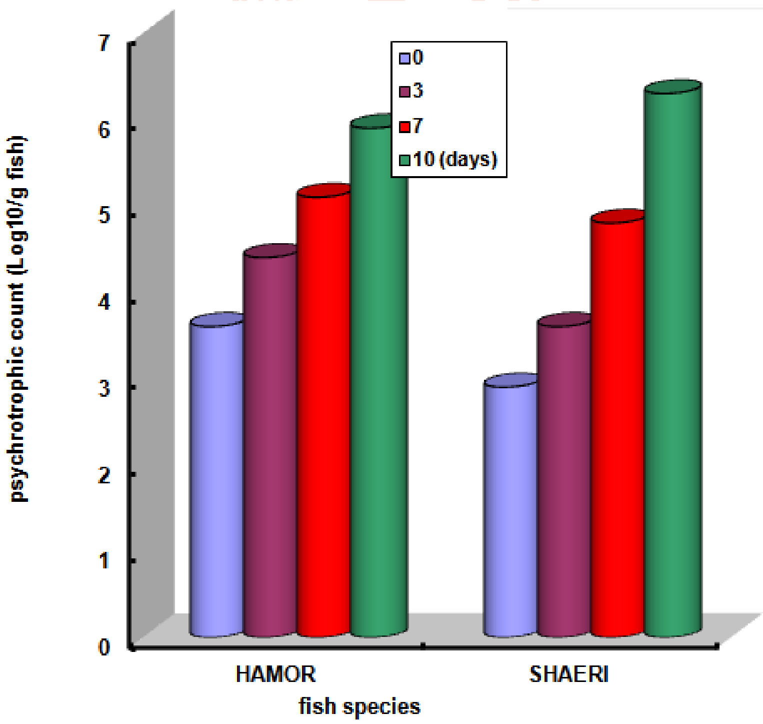

Fig.3: Changes in the psychrotrophic count of marine fish species with storage period.

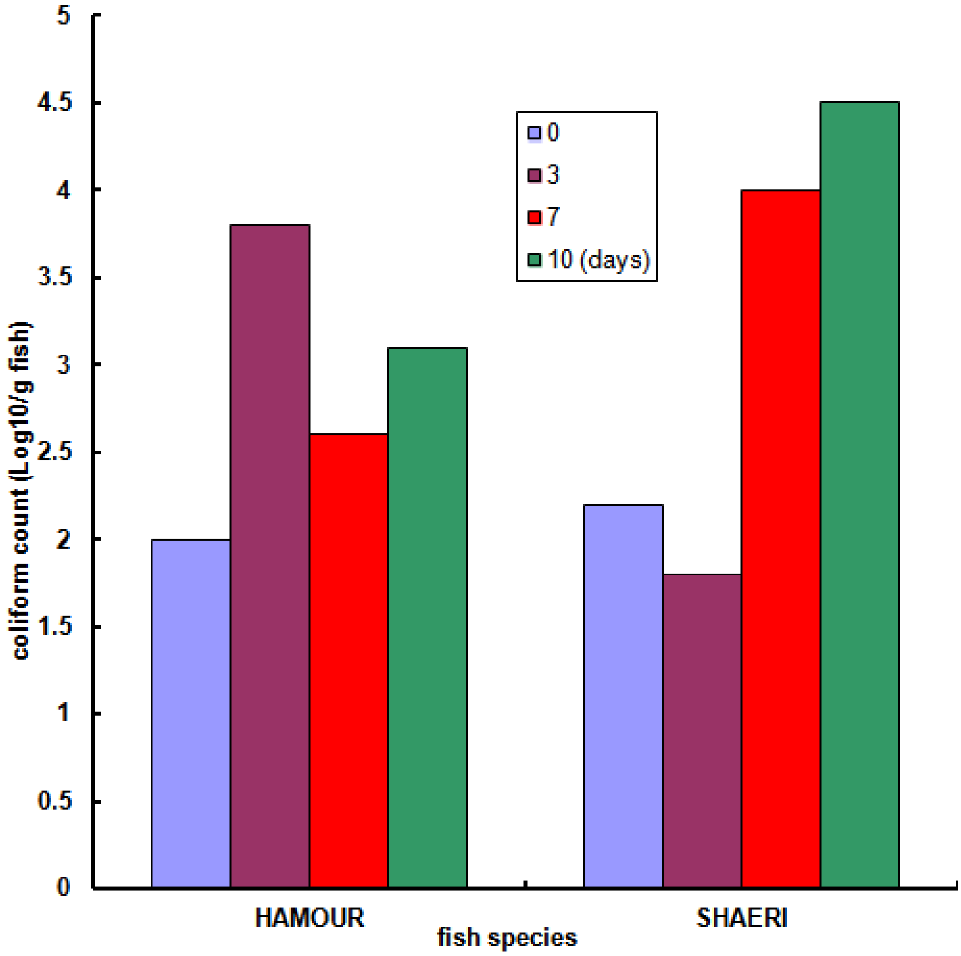

Fig.4: Changes in the coliform count of marine fish species with storage period.

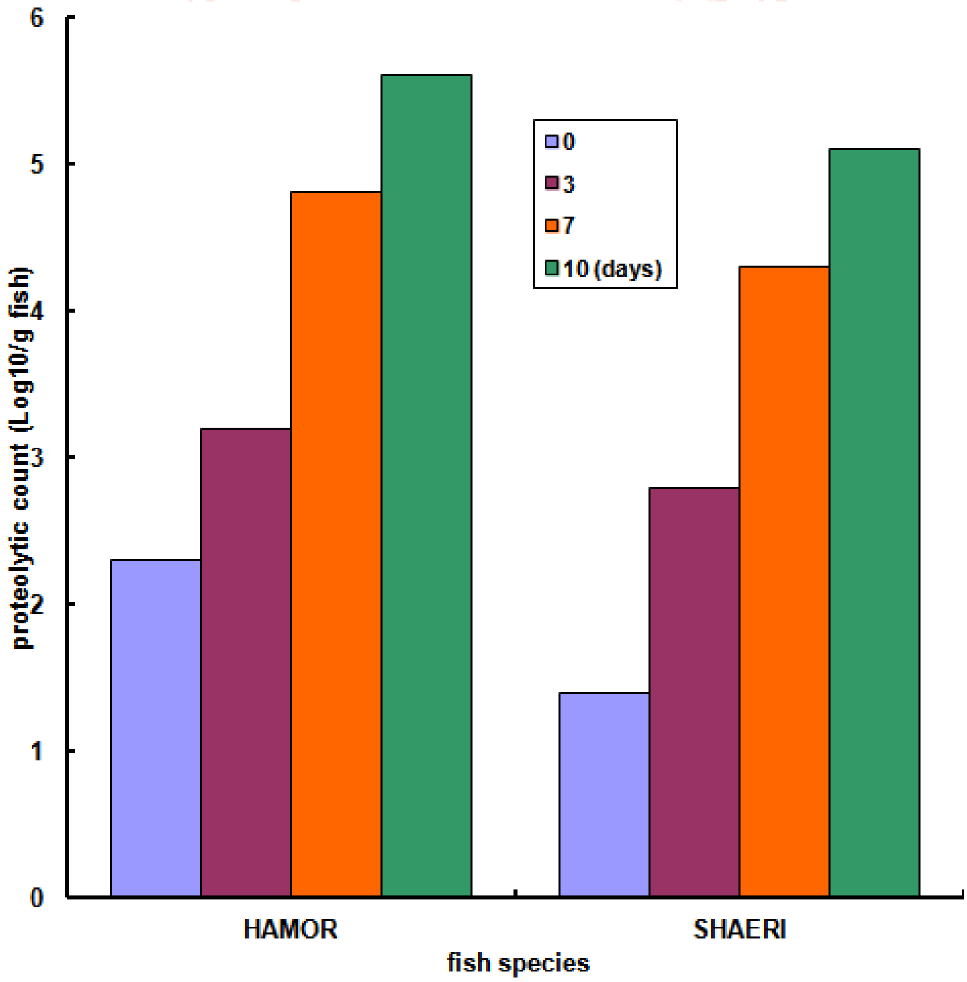

Fig.5: Changes in the proteolytic count of marine fish species with storage period

CONCLUSIONS:

Potentially a number of shelf life indices namely (TVBN, pH, and microbial tests such as TPC, Psychrotrophic, Coliform and Proteolytic counts) could be used to monitor changes in the quality of marine fish species with postmortem time. However, based on TVBN, marine fish species under investigation reached their maximum TVBN content on the 5th day of storage and were close to the rejection limit (30 mg/100 g) by day 3 of storage. This give an indication that the TVBN formation increased gradually with increase in storage period and varied with its concentration during storage period . Additionally, Bacteria grew rapidly throughout storage time.Microbial counts were assessed the applicability of the changes in some of the chemical as well as physical shelf life indices that could supplement sensory evaluation tests in predicting quality deterioration of marine fish species and ultimately determine their shelf life. Thus the TVBN and microbial counts were potentially risk in setting up food safety for consumption.

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