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Determination of Quality of Iced Freshwater Species Based on Total Volatile Base Nitrogen (TVB-N) and Microbial Contents Test

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ABSTRACT

The following two types of freshwater fish species {tilapia hybrid (Oreochromis niloticus x Oreochromis aureus) and the Common Carp (Cyprinus Carpio) were purchased from aquaculture farm and assessed for quality changes during 13 days postmortem of ice storage. The total volatile basic nitrogen (TVB-N) and microbial contents of iced freshwater fish species were assessed as indices of spoilage t 0, 3, 5, 7, 10 and 13 days postmortem. all fish species under investigation had pH value higher than 6.8. Tilapia fish had higher TVBN values than carp fish. Tilapia and carp reached their maximum TVBN content on the 7th and none of them reached the rejection limit (30 mg/100 g) during the storage period. In this study TVBN of freshwater fish species ranged from 18.67 to 20.07 and from 19.13 to 23.80 mg/100g muscle during the storage period for carp and tilapia respectively. The total plate counts for microbial growth of freshwater fish species were similar for all fish species under investigation. Bacteria grew rapidly between day 1 and 10. However, Tilapi had the lowest initial TPC and the highest TPC at the end of the storage period (10 days). Bacteria grew progressively on freshwater fish species to reach a high count of 6.5, 5.7 and 6 (log 10 CFU /g fish) for tilapia and carp respectively. None of freshwater fish species reached the maximum microbiological limit set by ICMSF (1978). psychrotrophic counts increased rapidly and tended to reach their maximum counts at the end of the storage period, and were 2.6 and 3.3 (log 10 CFU /g fish) for tilapia, and carp respectively. Total coliform counts were 1.3 and 2.3 log CFU/g fish and increased with extended storage to reach their maximum values of 4.1and 3.5 log CFU/g fish for tilapia and carp respectively..Proteolytic counts of all fish species increased with extended storage reaching their maximum count on the last day of storage period. The average proteolytic counts of freshwater species were 1.9 and 3.0 log CFU/g reaching a maximum values of 3.9 and 4.8 log CFU/g at the end of storage period for tilapia and carp respectively.

KEYWORDS: *Freshwater fish, Tilapia, Carp, Shelflife, postmortem changes, TVB-N and Microbial counts.*

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INRODUCTION

World food production of aquaculture has played an important role in the national economy and food sector, due to their contents of protein, vitamins, and minerals and omega-3 fatty acids. Currently capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish in 2010 of which about 128 million tonnes was utilized as food for people (Jinadasa, 2014). aquaculture has expanded from being almost negligible to fully comparable with capture production in terms of feeding people in the world (Seher Dirican 2017). Aquaculture has sustained a global growth, continues to grow, and is expected to increasingly fill the shortfall in aquatic food products resulting from static or declining capture fisheries and population increase well into the year 2025. (De Silva, et al 2001). 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, hydrolytic enzymes of microorganisms on the fish muscle (Venugopal, 2002), as well as physical and chemical activities However, these series of changes can be responsible for the fish spoilage after their body defense mechanisms stop, which lead to low qualities and deterioration in color, texture, appearance, aroma and flavor. Spoiled fish can not 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 on public health and the shelf-life of frozen stored fish.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). Changes in texture, smell, taste, or appearance cause fish to be rejected. 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).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 (NH3), dimethylamine (DMA) and trimethylamine (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 total volatile bases (TVB) is a group of biogenic amines formed in nonfermented food products during storage (Horsfall et al, 2006). 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). 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, Spoilage microorganisms include aerobic psychrotrophic Gram-negative bacteria, yeasts, molds, heterofermentative lactobacilli, and spore-forming bacteria. Psychrotrophic bacteria can produce large amounts of extracellular hydrolytic enzymes, and the extent of recontamination of food products with these bacteria is a major determinant of their shelf life.causing off-odors and flavors, and to visible changes in color or texture. (Rawat 2015). 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). A variety of methods have been used 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). Ice is capable of delivering a large amount of refrigeration in a short time and it maintain the temperature of fish close to the chilling temperature. (Ayers, 1980) reported that over 60 % of the fresh fish marketed is distributed in ice.

MATERIALS:

Freshwater fish species

1. Hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*)
2. Common Carp (*Cyprinus carpio*)

Freashwater species (hybrid Tilapia and common Carp) with a weight of about (300 – 400g) were caught early in the morning from a local fish farm. The fish were transported live in oxygenated water in plastic bags. 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). Hybrid Tilapia and common Carp 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 (3-4OC) 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 oC 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:

Methods of Analysis:

Freshwater species were 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:

The ultimate pH of muscle is normally about 6.2-6.6 in most fish. Initially all fish species under investigation had pH value higher than 6.8. The pH of freshwater species decreased steadily with postmortem time (Pacheo–Aguilar et al 2000), Several fish species e.g halibut, tuna, sardine and mackerel have pH values below 6.0 (Barret et al 1965). Riaz and Quadri (1985) indicated that a pH increment of more than 0.2 unit means that the sample had deteriorated (Fig.1).

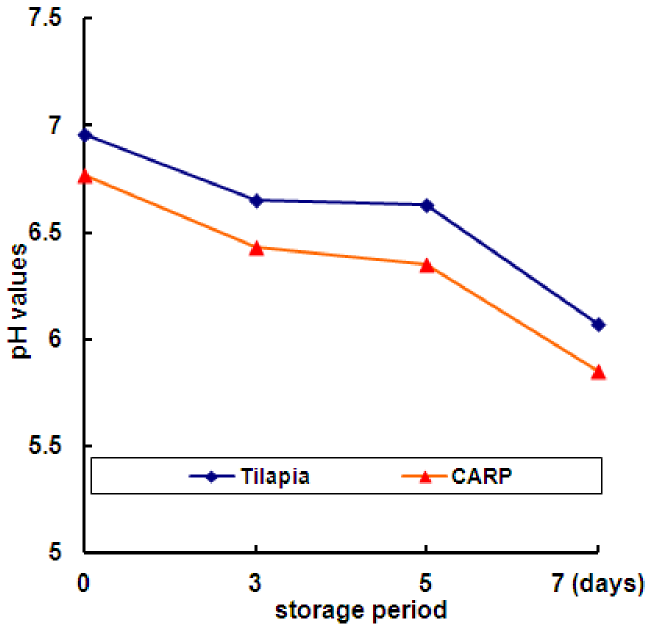

Fig.1: Changes in the pH values of freshwater fish species with storage period

2. Total volatile basic nitrogen (TVBN) Determination

TVBN is commonly used to evaluate fish muscle spoilage. For several fish species, TVBN values were reported to increase curvilinearly or linearly with time (Perez-villareal and Pozo 1990; Gokodlu et al., 1998). In this study freshwater fish species had high TVBN values (P < .05). Initially, tilapia fish had higher TVBN values than carp fish. The TVBN content of tilapia had steadily risen from initial value of 18.67mg/100g to 21.93 mg/100g at 7 days before decreasing to 20.07 mg/100g at 10 days. But, TVBN of the carp was almost constant through day 7 of storage, while on the 10th day of storage, its TVBN content was significantly (P < .05) lower than that at day 0 of storage. Generally, freshwater fish species reached their maximum TVBN content on the 7th. Yet none of the freshwater fish species reached the rejection limit (30 mg/100 g) during the storage period. In this study TVBN of freshwater fish species ranged from 18.67 to 20.07 and from 19.13 to 23.80 mg/100g muscle during the storage period for carp and tilapia respectively. None of the freshwater fish species carp and tilapia reached the maximum TVBN reported by Maia et al (1983). Also, initial values reported in the current study for carp and tilapia were substantially lower than those reported by Maia et al (1983). It was generally concluded that, on the average, freshwater species had a narrower range of TVBN during the storage period. 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 freshwater fish species with storage period. (mg/100g)

St. days	Carp	Tilapia
0	18.67^{a}_{1}	19.13^{b}_{1}
3	16.25^{a}_{1}	20.53^{b}_{2}
5	16.58^{a}_{1}	20.82^{b}_{2}
7	16.72^{a}_{1}	21.93^{b}_{3}
10	20.07^{a}_{2}	23.80^{b}_{2}

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

The bacterial numbers on the surface of fish increases with the increase in storage period (Gram and Huss 2000). The total plate counts for microbial growth of freshwater fish species were similar for all fish species under investigation in this study, bacteria grew rapidly between day 1 and 10. Gram and Huss (2000) indicated that the bacterial numbers in the surface of Tilapia increases with the increase in storage period. Apparently initial plate count of freshwater fish species were 3.1and 3.6 ($\log_{10}$ CFU /g fish) for tilapia, and carp respectively. Tilapia had the lowest initial TPC. Yet, it had the highest TPC at the end of the storage period (10 days). Reddy *et al.,* (1994) reported an initial count of 4.4 CFU/g of Tilapia. Bacteria grew progressively on freshwater fish species to reach a high count of 6.5, 5.7 and 6 ($\log_{10}$ CFU /g fish) for tilapia and carp respectively. Apparently none of freshwater fish species reached the maximum microbiological limit set by ICMSF (1978) for fresh fish. Reddy *et al.,* (1994) postulated that spoilage characteristic, in tilapia fillet generally resulted when bacterial counts reached log 7.5 CFU/g fish, or higher. However their fillet, appeared spoilage before the 10 day of storage, based on a strong off- odor and soft texture and the presence of thick slime on the fillet surface. (Fig.2). Irrespective of the freshwater fish species psychrotrophic counts increased progressively with storage period.The carp showing the highest count at day 3 and 10 of storage. Generally, psychrotrophics counts tended to reach their maximum counts at the end of the storage period. Initial psychrotrophics count of freshwater fish species were 2.6 and 3.3 (log $_{10}$ CFU /g fish) for tilapia, and carp respectively. Psychrotrophic bacteria grew progressively to reach a high count of 5.3 and 7.5 (log $_{10}$ CFU /g fish) after 10 days of storage for tilapia and carp respectively. On the 7th day of storage carp had substantially high count than both tilapia (Fig.3). Total coliform counts fluctuated during the storage period, fishes attained their maximum count at different times of storage. Irrespective of the sex, initial average total coliform counts of fresh tilapia fillets were less than log 1.5 CFU/g fish. A values which was way less than that reported by Reddy *et al.,* (1994) for fresh tilapia (log 2.6 CFU/g).Within freshwater fish species, average initial counts were 1.3 and 2.3 log CFU/g fish and increased with extended storage to reach their maximum values of 4.1and 3.5 log CFU/g fish for tilapia and carp respectively.. The highest initial and maximum coliform counts were observed to be for the carp and Tilapia respectively (Fig.4). Generally proteolytic counts of all fish species increased with extended storage reaching their maximum count on the last day of storage period . Initially, average proteolytic counts of freshwater fish species were significantly high (P < .o5), and proteolytic counts of all fish species increased with extended storage reaching their maximum count on the last day of storage period tested.Initially, average proteolytic counts of freshwater species were 1.9 and 3.0 log CFU/g reaching a maximum values of 3.9 and 4.8 log CFU/g at the end of storage period for tilapia and carp respectively (Fig.5). A study conducted by Durairaj and Krishnamurthi (1986) showed that After 10 h at ambient temperature *L. rohita, ,* and *T. mossambica* were 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 while *T. mossambica* (30 - 40 g.) was acceptable upto 6 days, 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) . Also a count of TVBN values and total bacteria have not raised to the expected limits.

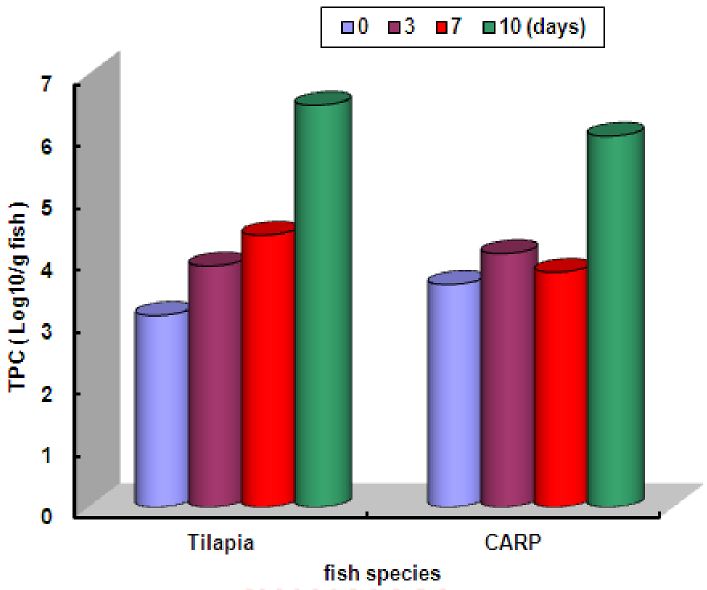

Fig.2: Changes in the TPC count of freshwater fish species with storage period.

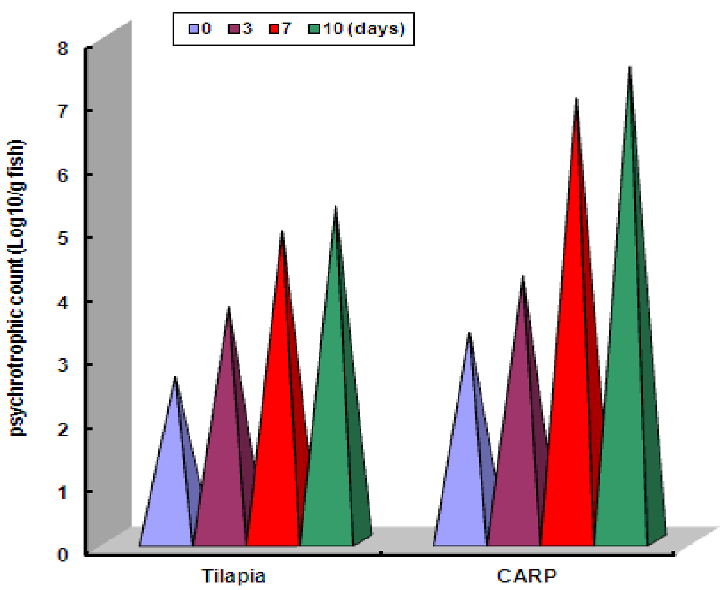

Fig.3: Changes in the psychrotrophic count of freshwater fish species with storage period.

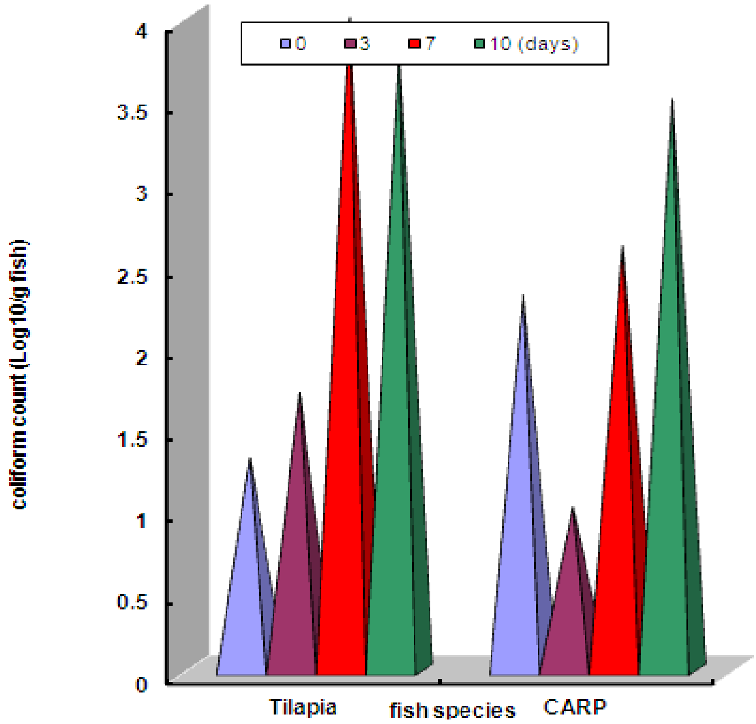

Fig.4: Changes in the coliform count of freshwater fish species with storage period.

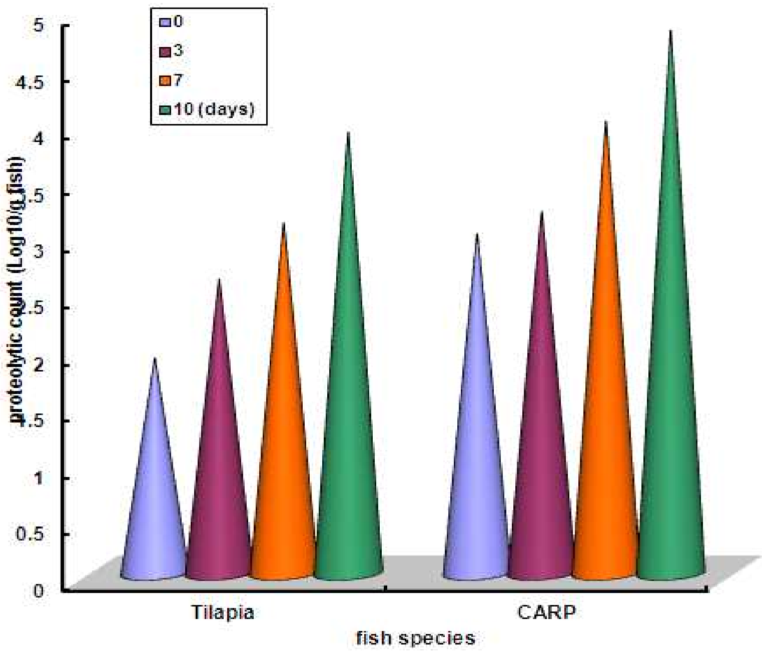

Fig.5: Changes in the proteolytic count of freshwater fish species with storage period

CONCLUSIONS:

Fish meat that contains protein, fat, water, vitamins and minerals can provide a good environment for the growth of microorganisms, mostly bacteria. So chill, freeze or ice and store immediately after capture which will help to extend the shelf life, increase the safety and maintain the quality of fish meat. Depending on the quality of the fish and the storage condition; the shelf life can be as long as many weeks or as short as few days. There is a direct correlation between the temperature of fresh fish and their shelf life; fish should be stored at temperatures near the freezing point of water (0 ºC) for maximum shelf life and at this point microorganisms, enzymes, and chemical activities will be minimized.

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REFERENCES:

[1] Amegovu AK, Sserunjogi ML, Ogwok P, Makokha V (2012). Nucleotited degradation products, total volatile basic nitrogen, sensory and microbiological quality of nile pearch (Latesniloticus) fillets under chilled storage. Journal of microbiology, biotechnology and food sciences 2, 653-666.

[2] Ayers JW (1980). Farm raised catfish. Processor's Report. Nat. Marine Fisheries Service. Little Rock, AR.

[3] Davoli R, Braglia S (2007). Molecular approaches in pig breeding to improve meat quality. Brief. Funct. Genom. Proteom. 6:313-321.

[4] De Silva SS (2001). A global perspective of aquaculture in the new millennium. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery & J.R. Arthur,

eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp. 431-459. NACA, Bangkok and FAO, Rome.

[5] Babu USJ, Rao BM, Khasim DI, Nair KGR (2005) Biochemical and Microbiological quality of formic acid silage and Lactobacillus fermented silage. Fish Technol. 42 (2):163-170.

[6] Barret I, Brinner L, Brown WD, Dolev A, Kwon TW, Little, A, Olcott, HS., Schaefer MB, Schrader P (1965). Changes in tuna quality, and associated biochemical changes, during handling and storage aboard fishing vessels. Food Tech 12, 108-117.

[7] Dirican S (2017) Future Aquaculture Trends of Sivas in the Central Anatolia of Turkey. International Journal of Innovative Studies in Aquatic Biology and Fisheries (IJISABF). 3:(2), PP 1-4.

[8] Durairaj S, Krishnamurthi S (1986). The Preservation of Some Indian Freshwater Fish. Fishery Technology. 23, 116-119.

[9] Ehira S, Uchiyama H (1987). Determination of fish freshness using the K value and comments on some other biochemical changes in relation to freshness. In D. E. Kramer & J. Liston (Eds.), Seafood quality determination (pp. 185–193). Amsterdam: Elsevier Science.

[10] Farber JM (1991). Microbiological aspects of modified atmosphere packaging technology; a review. J. Food Prot. 54, 58 .

[11] Gokodlu N, ozden O, Erkan N (1998). Physical, chemical and sensory analysis of freshly harvested Sardines (Sardina pilchardus) stored at 4 ºc. J. Aquat. Food prod. Technol. 7, 5-15.

[12] Gram L, Huss HH (2000). Fresh and process fish and shelffish in the microbiological safety and quality of food. Vol. 1. (ed. Lund, B. M., Baird-Parker, T. C. and Gould, G. W.) Aspen Publishers, Inc. Maryland, USA.

[13] Horsfall M, Kinigoma BS, Ayebaemi IS (2006). Evaluation of the levels of total volatile bases and trimethyleamine formed in fish stored at low temperature. Bulletin of the Chemical Society of Ethiopia, 20: (1).

[14] Jinadasa BKKK (2014). Determination of Quality of Marine Fishes Based on Total Volatile Base Nitrogen test (TVB-N). Nature and Science, 12: (5).

[15] Maia EL, Rodrigues-Amaya DB, Moraes MA (1983). Sensory and chemical evaluation of the keeping quality of the Brazilian fresh water fish Prochilodus scrofa in ice storage. J. Food Sci. 48 (4), 1075-1077.

[16] Majumdar RK, Subrata B, Anandan R (2005). Biochemical and Microbiological characteristics of salt fermented Hilsa (Tenualosa ilisha). Fish Technol. 42(1):67-70.

[17] Pacheco-Aguilar R, Lugo-Sanchez ME, Robles-Burgueno MR (2000). Postmortem biochemical and functional characteristic of Monterey sardine muscle stored at 0 ºC. J. Food. Sci. 65(1), 40-47.

[18] Perez-Villarreal B, Pozo R (1990). Chemical composition and ice spoilage of Albacore (Thunus alalunga). J. Food Sci. 55, 678-682

[19] Rawat S (2015). Food Spoilage: Microorganisms and their prevention. Asian Journal of Plant Science and Research, 5(4):47-56.

[20] Reddy NR, Schreiber CL, Bbuzard KS, Skinner G E, Armstrong, DJ (1994). Shelf life of Fresh Tilapia Fillets Packaged in High Barrier Film with Modified Atmospheres. Journal of Food Science, 59(2), 260-264.

[21] Rias F, Quadri RB (1985). Quality changes in lobster (Panulirus poliphagus) muscle during storage in ice. J. Agric. Food chem. 33, 117-122.

[22] Statham JA (1984). Modified atmosphere storage of fisheries products; the state of the art. Food Technol. 36, 233.

[23] Van Oeckel MJ, Warnants N, Boucque ChV (1999). Measurement and prediction of pork colour. Meat Sci. 52:347-354

[24] Wu T.H, Bechtel PJ (2008). Salmon by-product storage and oil extraction. Food Chemistry, 111(4): Pages 868-871.

[25] Risvik E (1994). Sensory properties and preferences. Meat Sci. 36:67-77.

[26] SAS (1988). SAS Users Guide, Release 6-03 Edition. SAS Institute Inc. Gary, NC, USA..

[27] Suchitra T, Sarojnalini C (2012). Effect of temperature on biochemical and Microbiological qualities of "Ngari". Nature Sci. 10(2):32-40.

[28] Vanderzant C, Mathys AW, Cobb BF (1973). Microbiological, chemical and organoleptic characteristics of frozen breaded raw shrimp. J. Milk Food Technol. 36:253-261.

[29] Venugopal V (2002). Biosensors in fish production and quality control. Biosensors and bioelectronics, 17, 147-157.