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Microbiological and Chemical Changes of Nile Tilapia (*Oreochromis niloticus* L.) Fillet during Ice Storage: Effect of Age and Sex

Alemu Lema Abelti
Zeway Fisheries Resources Research Center, P.O. Box 229, Ethiopia, Tel.: +251913189100

Abstract: Fish is one of the most highly perishable food products. The aim of this study was to investigate microbiological and chemical changes of Nile tilapia fillet during ice storage. Nile tilapia samples were collected from Lake Zeway, Ethiopia. The fillet was stored at 0°C for 30 days on ice. Total plate count, total and fecal coliform, total volatile base nitrogen and pH was measured at the interval of four days. Regardless of age and sex, Total plate count varied between 4 to 11.74 log cfu/g. Total coliform counts fluctuated between <3 to >1100 MPN/g. However fecal coliform changed between 23 to <3 MPN/g. Total volatile base- Nitrogen has increased from 7.5 to 42.0 mg N/100 g. Similarly, pH has increased from 6.1 to 7.6 during the ice storage period. The eventual increase observed in mean total plate count could be due to multiplication of organisms favored at the storage condition. The increased total coliforms may be attributed to fillet quality and temperature fluctuations. The increment in TVB-N during ice storage is associated with activity of micro-organisms during later stages of deterioration. The increase in pH values is due to the formation of basic decomposition products. Finally, Post mortem change of Nile tilapia fillet was noticed and bacterial metabolites like Total volatile base-nitrogen, Total plate count and pH were invariably increased throughout the storage time indicating that they are good parameters to predict shelf life.

Keywords: Chemical, microbiological, Nile Tilapia, pH, TPC, TVB-N

INTRODUCTION

Fish is one of the most highly perishable food products. During handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product. Most of the methods that have been used to estimate the quality of fresh fish measure or evaluate parameters that change, disappear or formed during deterioration of fish. These methods may be divided into several groups such as microbiological and chemical methods (Huss, 1995). Some of microbiological methods used to assess fish freshness are total plate count, total coliform and fecal coliform. Total plate count is good indicator of the sensory quality or expected shelf life of the product (Olafsdottir *et al*., 2006; Koutsoumanis and Nychas, 2000). The chemical method used as freshness indices is Total volatile basic amine. It is a general term which includes the measurement of trimethylamine (produced by spoilage bacteria), dimethylamine (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites) and other volatile basic nitrogenous compounds associated with seafood spoilage. TVB-N is good indicators for assessment of the freshness measurement of quality in such as ray fish (Ocaño-Higuera *et al*., 2011), Cazon fish (Ocaño-Higuera *et al*., 2009), industrial fish for meal and silage (Olafsdottir *et al*., 1997), Anchovies (Pons *et al*., 2006), Pacific saury (Sallam *et al*., 2007), Galda and Balda (Ali *et al*., 2010), Haddock and cod (Olafsdottir *et al*., 2006), Yellow grouper (Li *et al*., 2011), African cat fish (Adoga *et al*., 2010), Nile perch (Okeyo *et al*., 2009) and Nile Tilapia (Liu *et al*., 2010).

In freshly caught fish TVB-N content is generally superior to 10 mg/100 g and does not exceed 15 mg/100 g except for pelagic fish, 16-18 mg/100 g for sardine, 18-20 mg/100 g for mackerel, about 30 mg/100 g for albacore tuna and 6.5 mg/100 g for Nile Tilapia (Liu *et al*., 2010). The TVB-N content increases slightly during the first days of storage, this slight increase may reflect the amines production by autolytic processes (Ocaño-Higuera *et al*., 2009). Other chemical methods which can indicate spoilage in ice stored fish is pH. The pH of muscle tissue of live fish is close to neutrality (Huss, 1995). During the later post-mortem changes, pH is more or less constant or slightly increased due to the formation of basic compounds (Huss, 1995). Even though the changes in pH are generally rather small, they have great technological importance. Keeping these points in view, the present studies were conducted to investigate microbiological and chemical changes of Nile tilapia fillet during ice storage.

MATERIALS AND METHODS

Sample collection: Fresh fish was purchased from local fishermen at Bochessa, Korokonch and

Menefesha of Lake Zeway, Ethiopia. Live fish was transported to Zeway Fisheries Resources Research Center laboratory layering with flaked ice using ice box. Sex was identified by examining genital papilla and length was measured to the nearest 0.1 cm and converted into age using Von Bertalanffy growth function. A composite sample from the same age and sex was taken and refrigerated at 0°C in Styrofoam box layered with flake of ice and generally stored for thirty days. Every fourth day, microbial analysis (total plate count, total coliforms and feacal coliforms), spoilage indicator chemical (Total Volatile Base-Nitrogen) and pH was measured.

Sample homogenization: To enumerate CFU, 10 g of fish fillet was homogenized using 90 mL Maximum Recovery Diluents (MRD) sterile saline solution in stomacher bag and then ten-fold serial dilution was prepared.

Total Plate Count (TPC): Using sterile pipette, 1 mL of the initial inoculum was transferred into 9 mL of MRD. The procedure was repeated for as many serial dilutions as required (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}). The dilution was mixed using a vortex mixer for 10 sec. (0.1 mL) of the initial inoculum and each required dilution was aseptically spread plated on plate count agar using L-shaped glass rods into a labeled Petridish in duplicate. The petridish was inverted and incubated in an incubator at 35°C for 24 h. Finally the number of colonies were counted and multiplied by dilution factor to calculate the total colonies forming units per gram of sample.

Total coliforms count: From the homogenate, 1 mL of serially diluted (1:10, 1:100 and 1:1000) sample was transferred into 9 mL sterilized Lauryl Sulphate Tryptose (LST) broth in triplicate (3 test tubes). For each dilution, the tubes were incubated at 35°C for 48 ±2 h to evaluate gas formation (AOAC, 1998). Lauryl Sulphate Tryptose (LST) broth was used as a pre-enrichment media. After primary incubation, one (0.3 mm) loopful of positive tubes (gas formation by the action of the coliform bacteria in fermenting lactose medium tubes) was transferred to Brilliant Green Lactose Bile (BGLB) broth, further incubated at 35°C for 48±2 h for total coliforms count. Inverted Durham Fermentation Tube was added into test tubes before the addition of BGLB broth to allow easy identification of gas production. Then the number of tubes with positive gas production was counted. MPN (Most Probable Number) of Coliform bacteria per gram sample was calculated from MPN table based on the number of tubes of BGLB broth producing gas at the end of incubation period.

Feacal coliforms count: One milliliter of serially diluted (1:10, 1:100, 1:1000) sample homogenate was transferred into 9 mL sterilized Lauryl Sulphate Tryptose (LST) broth in triplicate (3 test tubes) form each dilution and the tubes will be incubated at 35°C for 48±2 h for gas formation (AOAC, 1998). The assumption is that feacal coliforms ferment lactose and produce acid and gas. After primary incubation, one loopful of positive tubes (gas formation by the action of the coliform bacteria in fermenting lactose medium tubes) was transferred to EC broth and incubated at 44.5°C for 48±2 h. Then the number of tubes with positive gas production was counted. MPN (Most Probable Number) of feacal Coliform bacteria per gram sample was calculated from MPN table based on the number of tubes of EC broth producing gas at the end of incubation period.

Measurement of pH: Five gram of fish sample was homogenized in 50 mL distilled water in the ratio 1:10 (w/v) using laboratory warring blender and the pH was measured using a digital pH meter by inserting the electrodes into the homogenates. The pH meter was calibrated using pH 4 and 7 buffer.

Determination of Total Volatile Basic-Nitrogen (TVB-N): Fish extracts for determination of Total Volatile Bases Nitrogen (TVB-N) was prepared by homogenizing 10 g of fish sample with 90 mL of 7.5% (w/v) aqueous Trichloroacetic Acid (TCA) solution in a laboratory homogenizer for 1 min at high speed. The homogenate was centrifuged at 3000 rpm for 5 min and the supernatant liquid was then filtered through Whatman No. 1 filter paper. TVB-N was measured by steam-distillation of the TCA-fish extract, using the method of Malle and Tao (1987). Twenty-five millilitres of the filtrate was added to a Kjeldahl-type distillation tube, followed by 5 mL of 10% (w/v) aqueous NaOH solution. Steam-distillation was performed using a vertical steam distillation unit and the distillate was received into a beaker containing 15 mL of 4% (w/v) aqueous boric acid and 0.04 mL of methyl red and bromocresol green indicator solution up to a final volume of 50 mL. The titration was allowed to run against aqueous 0.05 M sulfuric acid solution. The TVB-N content was calculated by the following formula:

$$\text{TVB-N} = \frac{14\frac{g}{mol} \text{X a X b X } 300}{25 \text{ mL}} \text{ (mg N per 100 g)}$$

where,
a = mL of sulfuric acid
b = Normality of sulfuric acid

Statistical analysis: Number of colonies from total plate count data was transformed into log10 CFU/g. For total coliforms and feacal coliforms, the numbers of positive test tubes were counted and most probable number of coliforms was computed from MPN table. Descriptive statistics was used to summarize the mean of TVB-N and pH during ice storage for thirty days.

Table 1: Log mean count (CFU/g) of total plate count of bacteria

Parameters		Storage time in days						
Sex	Age	0	6	10	14	18	22	26
Male	4	6.33	6.44	6.55	6.00	8.77	10.17	11.74
	5	6.33	6.69	7.94	8.32	9.75	11.28	11.36
Female	4	4.30	6.00	5.84	6.46	8.28	11.14	11.71
	5	4.00	5.49	6.07	7.94	8.01	11.46	11.65

Table 2: Most Probable Number (MPN) of total coliforms in Nile Tilapia fillet stored at 0°C

Parameters		Storage time in days						
Sex	Age	0	6	10	14	18	22	26
Male	4	39	7.3	<3	75	>1100	>1100	1100
	5	43	29	3.6	15	35	53	>1100
Female	4	9.4	16	15	35	>1100	>1100	>1100
	5	9.1	3.6	<3	160	35	>1100	>1100

Table 3: Most Probable Number (MPN) of faecal coliforms of Nile Tilapia fillet stored at 0°C

Parameters		Storage time in days						
Sex	Age	0	6	10	14	18	22	26
Male	4	23	3.0	<3	<3	<3	9.3	<3
	5	23	3.6	<3	7.2	<3	<3	<3
Female	4	6.2	3.6	7.3	<3	<3	6.1	<3
	5	3.6	3.6	<3	<3	<3	9.1	<3

Table 4: Total Volatile Base Nitrogen (TVB-N) in mg N/100 g of Nile Tilapia fillet stored at 0°C

Parameters		Storage time in days						
Sex	Age	0	6	10	14	18	22	26
Male	4	8.4±1.60	19.3±0.84	25.2±1.68	27.7±0.84	34.4±0.84	37.8±0.84	41.1±0.84
	5	9.2±0.84	21.0±0.84	25.2±1.68	31.0±0.84	36.1±0.84	37.8±0.84	39.4±0.84
Female	4	7.5±0.84	18.4±0.00	24.3±0.80	32.7±0.84	32.7±0.84	36.1±0.84	39.4±0.84
	5	7.5±0.84	17.6±0.84	25.2±1.68	31.0±0.84	31.0±0.84	42.0±1.68	34.4±0.84

Table 5: Change in pH value of Nile Tilapia (*Oreochromis niloticus*) fillet stored at 0°C

Parameters		Storage time in days						
Sex	Age	0	6	10	14	18	22	26
Male	4	6.1±0.01	6.8±0.02	6.5±0.00	6.6±0.02	6.8±0.02	7.0±0.01	7.3±0.01
	5	6.2±0.03	6.6±0.01	6.7±0.02	7.0±0.02	7.1±0.01	7.0±0.03	7.3±0.01
Female	4	6.3±0.02	6.7±0.00	6.9±0.02	7.0±0.02	7.1±0.00	7.2±0.04	7.4±0.05
	5	6.2±0.01	6.70±0.0	6.9±0.00	6.72±0.01	6.99±0.01	7.0±0.01	7.3±0.02

RESULTS

Total plate count: Log mean total plate bacterial count showed increasing trend from storage day zero to day 26 at levels main effects (Table 1). For 4 years male fish the log mean bacterial count ranged from 6.33 CFU.g on day zero to 11.74 CFU.g on day 26. Similarly, an increase of log mean bacterial count from 6.33 to 11.36 CFU.g was observed for five years male fish. Also, 4 and 5 years female fish log mean TPC ranged from 4.3 to 11.71 and 4 to 11.65, respectively on 0 to 26 days of storage.

Total coliforms and feacal coliforms: Total coliforms counts varied from 39 to 1100 MPN/g in four year male, 43 to >1100 MPN/g in 5 year male, 9.4 to >1100 MPN/g in 4 year female and 9.1 to >1100 MPN/g in 5 year female fish, respectively. From Table 2 it can be observed that, the initial total coliforms were higher in 4 and 5 year male fish (39 and 43 MPN/g) as compared to 4 and 5 year female fish (9.4 and 9.1 MPN/g). From Table 3 Feacal coliforms kept constant except for the first day starting from zero day of storage until the end of storage day irrespective of age and sex.

Total volatile base-nitrogen: The TVB-N contents of Nile Tilapia fillet stored on ice for 30 days was increased from 8.4 to 41.1 mg N/100 g in 4 year male fish, 9.2 to 39.4 mg N/100 g in 5 year male fish, 7.5 to 39.4 mg N/100 g in 4 year female fish and 7.5 to 34.4 mg N/100 g in 5 year female fish (Table 4).

Change of pH value: A change in pH value over the period of iced storage was shown in Table 5. pH value was increased from 6.1 to 7.3 in 4 year male fish, 6.2 to 7.3 five year female, 6.3 to 7.4 in 4 year female and 6.2 to 7.3 in 5 year female fish.

DISCUSSION

Total plate count: The initial bacterial load was between 4 to 6.3 log CFU/g on 0 day of storage. These

levels increased to exceed 7 log CFU/g of total plate counts and reached 11 log CFU/g at the end of storage days. Similarly, Hernández *et al*. (2009) had reported the total aerobic mesophilic bacteria load of 11.2 log CFU/g for meagre stored on ice for 18th day of storage. Yeasmin *et al*. (2010) had reported an increase of bacterial load from 7.5×10^3 to 6.2×10^9 CFU/g from 16 to 24 days of storage. Variation in the initial bacterial load could be ascribed to bacterial initial contamination level of fish skin because fresh caught fish might vary depending on the microbial load of the waters in which they live (Huss, 1988). The eventual increase observed in mean total plate count could be due to multiplication of organisms favored at the storage condition (Ibrahim and El-Sherif, 2008). To be considered safe, however, total plate counts of fish should never exceed 7 log CFU/g wet weight (ICMSF, 1986).

Total coliforms and fecal coliforms: The higher density in male fish fillet may be due to secondary contamination during handling and storage (Mandal *et al*., 2009). The high incidence of total coliforms at the end of storage day corroborates the findings of Arannilewa *et al*. (2005) which observed an increasing of total coliforms count with a prolonged storage of fish fillet. The increased total coliforms may be attributed to fillet quality, temperature fluctuations, time taken during the processing and time taken to transport fish (Mhango *et al*., 2010). The limit value for the total coliform bacteria is 160-210 MPN/g (2.20-2.32 log MPN/g) (Anon, 1996; Jay *et al*., 2005). Hence the fish fillet stored on ice at the end of storage day was not better quality. Feacal coliforms have more or less kept constant starting from zero day of storage until the end of storage day irrespective of age and sex. Feacal coliforms by themselves are not dangerous (pathogenic), they are often used as indicators to assess food safety (Jay *et al*., 2005). If feacal coliform counts are high (over 200 colonies/mL of sample), there is a greater chance that enteric pathogenic organisms are also present (APHA, 1999). Faecal coliforms such as *Escherichia coli* usually originate from faeces of warm blooded animals. Faecal coliform in fish demonstrates the level of pollution of their environment because coliforns are not the normal flora of bacteria in fish. From the present study, feacal coliform values were lower than cut off values indicating that lesser chance of pathogenic organisms can exists.

Total volatile base-nitrogen: TVB-N results of present study agree with the result of Ali *et al*. (2010) who studied the post mortem variation in TVB-N of Galda and Bagda. The initial lower level of TVB-N observed could be due to the lower levels of endogenous ammonia due to reduced microbial activity during the first weeks of storage of the fish on ice (Okeyo *et al*., 2009). The increment in total volatile base nitrogen in fish muscles during ice storage is generally associated with activity of micro-organisms during later stages of deterioration (Huss, 1988). This increase may be attributed essentially to ammonia produced from bacterial catabolism of nitrogen-containing compounds (Kyrana *et al*., 1997; Ocaño-Higuera *et al*., 2009; Okeyo *et al*., 2009; Liu *et al*., 2010). The study conducted on TVB-N and TMA levels during ice storage of European hake shown that TVB-N increased sharply during ice storage and the steeper increments of TVB-N observed between two ages of hake, may reflect a different rate of spoilage between the two fish age classes (Orban *et al*., 2011). Total volatile nitrogen has been widely used as an index for freshness of fish (Stansby, 1954; Huss, 1995). For several fish species, TVB-N values were reported to increase curvilinearly or linearly with the time. A level of 30 mg N/100 g of fish Muscle TVB-N has been considered as the upper limit above which some fishing products are considered spoiled and unfit for human consumption as Specified by European commission guidelines (Commission Decision 95/149/EC, 1995; Huss, 1995).

Changes in pH: Variations among the initial pH values may be due to diet, level of activity or stress during catch as well as type of muscle (Ocaño-Higuera *et al*., 2009). The initial pH value in 4 year male fish was lower than female fish may be due to physiological conditions or degree of ante-mortem activity or stress (Huss, 1995). In unstressed fish glycogen in the muscle would metabolized to lactic acid and account for low pH (Kyrana *et al*., 1997). The low pH value in male fish may have contributed to increased shelf life (Kyrana *et al*., 1997). The lower pH of fish flesh, the slower the bacterial growth and vice versa (Okeyo *et al*., 2009). Increase of pH during ice storage has frequently reported by different researchers (Arannilewa *et al*., 2005; Sallam *et al*., 2007; Hernández *et al*., 2009; Ocaño-Higuera *et al*., 2009; Liu *et al*., 2010). The increase in pH values after day 6 in present study might be attributed to the formation of basic decomposition products, such as ammonia and trimethylamine. The increase may also be due to an increase in total volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes and this corroborates with the increase in TVB-N (Kyrana *et al*., 1997; Okeyo *et al*., 2009). Such increase in the pH indicates the bacterial growth, loss of quality and possible spoilage (Sallam *et al*., 2007). pH can act as indicators of the fish freshness as it start with low reading at the early stage of storage and increased when the fish had been stored for certain period of time. So, by checking the pH of the fish fillet after a certain period of storage can determine the state of its freshness (Abbas *et al*., 2008). pH is important intrinsic factor related to fish flesh and influence freshness because of its influence on bacterial growth (Gram and Huss, 1996).

CONCLUSION

From the present study it can be concluded that, there is variation between age and sex of Nile Tilapia in regard to microbiological load and chemical during ice storage. Post mortem change of Nile tilapia fillet was noticed and bacterial metabolites like Total volatile base-nitrogen, Total plate count and pH were invariably increased throughout the storage time indicating that they are good parameters to predict shelf life.

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