Ethiop. J. Biol. Sci., 9(2): 183-193, 2010
 ISSN: 1819-8678

SHORT COMMUNICATION

PHYSICO-SENSORY AND MICROBIAL EVALUATION OF ICE STORED NILE TILAPIA FILLETS (*OREOCHROMIS NILOTICUS* L.) FROM LAKE HAWASSA

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ABSTRACT: Fillets from the Nile tilapia (*Oreochromis niloticus L.*) were obtained from Lake Hawassa, and were exposed to ice storage in five different packages designated as package I (muscle to ice ratio of 1:1 (w/w)), package II (muscle to ice ratio of 1:2), package III (a combination of muscle, ice and water of 1:0.2:0.5 (w/w/w)), package IV (muscle to water ratio of 1:1) and package V (stored in a refrigerator at 4^0C). Clean fresh fillets contained 5.06 log (c.f.u/g). Fillets stored in ice crystals in the ratio of 1:2 (package II) got spoiled after 24 hours, when the total bacterial count reached 5.31 log (c.f.u/g), while at the ratio of 1:1 (package I), the count reached 5.65 log (c.f.u/g). The fillets from these two packages were organoleptically acceptable for consumption up to the 24^{th} hour of storage and those stored in water at the 1:1 ratio (package IV) were acceptable up to the 15^{th} hour. At rejection, fish exhibited a strong fishy, sulfidy, putrid and ammoniacal odor. The respective counts of total lactics and psychrophiles initially were 1.70 and 4.27 log (c.f.u/g). However, after 24 hours of storage, they reached 2.89 and 5.32 log (c.f.u/g) in package II and 3.68 and 5.46 log (c.f.u/g) in package I, respectively. Our study revealed that the shelf-life of tilapia stored in ice was improved by about 12 hours.

Key words/phrases: Fillets, Ice storage, Lake Hawassa, *Oreochromis niloticus*.

INTRODUCTION

Tilapias are becoming increasingly prominent in fresh water aquaculture and are among the most studied groups of fish in African waters (Demeke Admassu, 1996). As sea foods, including tilapia, are highly perishable due to their high water activity (a_W), neutral pH and availability of autolytic enzymes, they should be kept and handled at low temperature to reduce the microbial growth (Sasi *et al*., 2000). This is particularly important as deterioration of fish mainly occurs as a result of bacteriological activity which leads to loss in quality and nutritive value (Liston, 1980;

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LeChevallier et al, 1980, Ashie *et al*., 1996; Olafsdottir *et al*., 1997). Several fish constituents, mainly non-protein nitrogen compounds can be decomposed by bacteria and this leads to the development of off-odors associated with spoilage.

The range of bacterial genera isolated in fish is related to the aquatic habitat of the fish and varies with factors like bacterial load and salinity of the water. Thus, bacteria recovered from the skin and gills may be transient rather than resident on the fish surfaces (Marian, 1990). Experiments on the spoilage of fresh tilapia flesh at low temperatures indicted that *Pseudomaonas, Serratia, Streptococcus, Escherichia* and *Bacillus* were the agents of spoilage (Keke *et al*., 2005).

One of the primary methods of maintaining freshness of fish is iced storage (Chapman L, 1990; Sasi *et al*., 2000; Benjakul L *et al*., 2002; Jeyasekartan G *et al*., 2004a). Ice exhibits a large cooling capacity, is a safe food-grade substance, portable, cheap and the raw material for its production is widely available. In addition, the reduction of the storage temperature to about 0^0C reduces the multiplication of pathogenic and spoilage microorganisms. Furthermore, temperature reduction inhibits rate of enzymatic reactions which are linked to early post mortem changes by extending the rigor mortis period. At present, it is widely used as a coolant for the shipping of fresh sea foods. According to Lima dos Santos *et al*., (1981) the quantity of crushed ice required for chilling fresh fish is substantial (1:1, fish to ice ratio) and sometimes higher in tropical areas.

Mogessie Ashenafi *et al*. (1995) reported that fishing at Lake Hawassa is very unhygienic and a 3-5 h exposure to temperatures between 20-25^0C permits spoilage bacteria to multiply. In a series of experiments, Huss *et al*., (1974) revealed the importance of hygiene during onboard handling of fish. They assessed the quality and storage life of completely aseptically treated fish, fish iced in clean plastic boxes with clean ice and fish treated badly (i.e. iced in old dirty wooden boxes). A considerable difference was found in the bacterial contamination of the three batches.

The microbial load of fresh tilapia fillet from Lake Hawassa was $9.5X10^6$ c.f.u/cm^2 (Mogessie Ashenafi, 1989). The loss of fish as a result of bacterial spoilage at the lake was not fully assessed. Furthermore, there are few or no reports regarding the postharvest handling of fish and effect of ice storage on the bacteriological quality of tilapia fillets from Lake Hawassa. Here we report, the shelf-life and changes in the sensory and bacteriological quality of fillets from tilapia (*O. niloticus*) preserved in ice.

MATERIALS AND METHODS

Sampling: Tilapia fillets were obtained from a fish landing center at Lake Hawassa, which is situated within 2 km of the Food Science and Post Harvest Technology Laboratory at the University of Hawassa, Ethiopia. Fresh samples were brought to the laboratory about 3 hours after capture and thoroughly washed with potable water. The samples were divided into five packages (designated as package I, II, III, IV and V). Packages I-IV were placed in polythene bags, sealed airtight with cellophane tape and stored at room temperature (27-30^{0}C) in thermocold boxes. Package V was stored in a refrigerator at 4^{0}C. The constituents/treatments of the packages are shown in Table 1. During the experiment, re-icing was not done. Triplicate analysis was conducted for bacteriological, sensory and physical quality.

Table 1. Treatments used during the storage trials of tilapia fillets from Lake Hawassa.

Package	Treatment	Remark
I	fillet to ice ratio of 1:1 (w/w)	for 500 gm of fillet, 500 gm of ice crystals was used
II	fillet to ice ratio of 1:2 (w/w)	for 500 gm of fillet, 1000 gm of ice crystals was used
III	fillet to ice and water ratio of 1:0.2:0.5 (w/w/w)	for 500 gm of fillet, 100 gm of ice crystals and 250 gm of water was used
IV	fillet to cold water ratio of 1:1 (w/w)	for 500 gm of fillet, 500 gm of water was used
V	500 gm of fillet	stored in the refrigerator

Physical and sensory evaluation: Temperature and pH were measured using an Ultra freezer temperature probe (Consort Model T 852, Belgium) and an ORION pH meter, respectively. The temperature of the fillets in package V was not measured as it was assumed to be constant. Sensory quality and acceptability of fillets were assessed by a panel of five trained individuals working in the area of Fisheries and Food Science Laboratory at the University of Hawassa. Odour, texture and general appearance were observed and ranked according to a ten point scale, the scores being given in a decreasing order (10-9 for excellent, 8-7 for good, 6-5 for fair and acceptable, 4-3 for poor and 2-1 for very poor). The overall sensory quality of the fillets was calculated from the mean of the scores given by the panel (Lawless and Heymann, 1999).

Bacteriological analysis: The bacteriological analysis included total bacterial load, psychrophiles and total lactics. Twenty five gram of muscle was cut into small pieces using a sterile knife and homogenized using a Stomacher Lab Blender in 225 ml of sterile physiological saline (0.85%), after which serial decimal dilutions were made using the same diluent (Evancho *et al*., 2001). Appropriate dilutions were spread plated in triplicate onto plate count agar (PC, Hi-Media laboratories, Mumbai, India) for the enumeration of total bacterial load and psychrophiles. PC plates for total bacterial load were incubated at 37^0C for 24 h, whereas for psychrophilic counts, incubation was carried out under a refrigerated condition (4^0C) for 7 days. Total lactics were enumerated using *Lactobacillus* MRS Agar (OXOID) and the pour plate technique, followed by incubation at room temperature for 48 h.

RESULTS AND DISCUSSION

In recent years many types of analyses have been developed which can be used to evaluate fish freshness and to detect spoilage. These include physical, chemical, electrochemical, sensory and microbiological methods. The most common method for the determination of the bacteriological quality of seafood is total viable count. Although, total viable count is taken as an indicator of the hygiene status of the product, it is seldom a good indicator of the sensory quality or expected shelf-life of the seafood products (Huss *et al*., 1974).

Physical and sensory evaluation: Fig. 1a shows the temperature profile of tilapia fillets stored at different conditions. Immediately after packaging, the temperature of packages I and II was 24^0C while that of III and IV was 25^0C. The room temperature at that time was 27^0C. The lowest temperature recorded for packages I and II (-1 and -5^0C, respectively) was at the 3^{rd} hour, and this seems a good result for storages using ice crystals. Although, the temperature in all the cases gradually increased throughout the storage period, sub-zero temperature was maintained up to the 10^{th} hr for package II.

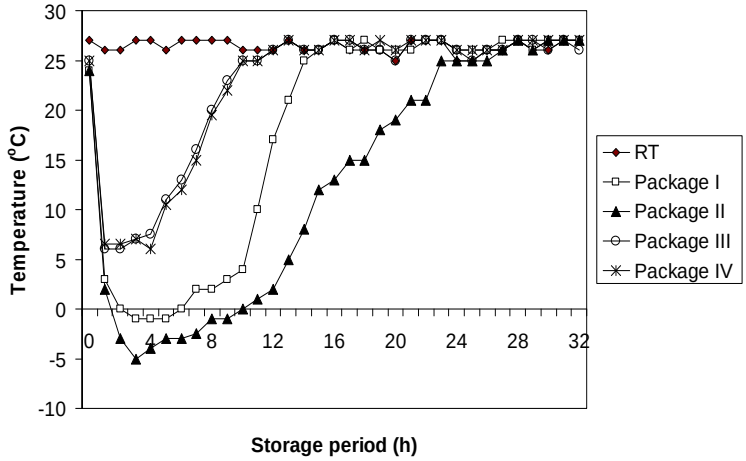
Temperature, processing and atmospheric conditions during storage are the most important factors that affect the rate of fish spoilage. Microbiological and enzymatic activities are highly influenced by temperature and from 0^0C to 25^0C microbiological activity is relatively more important than enzymatic activity. The optimal temperature for the growth of pathogenic bacteria in fish is about 37^0C though they can also multiply at temperatures between 20^0C and 50^0C (Jimmy, 1999).

The pH value of the fresh fillets was 6.54 and in all cases it generally decreased during the storage period, but remained above 6.00 (Fig. 1b). This might be attributed to acid production by the multiplying microorganisms. The post mortem reduction in the pH of fish muscle has an effect on the physical properties of the muscle. A drop in pH results a reduction on the net surface charge of the muscle proteins. As a result, the proteins partially denature and lose some of their water-holding capacity.

During cooking, muscle tissue in the state of rigor mortis loses moisture and is unsuitable for further processing which involves heating, since heat denaturation enhances the water loss. Loss of water has a detrimental effect on the texture of fish muscle and there is an inverse relationship between muscle toughness and pH. An unacceptable level of toughness (and water-loss on cooking) occurs at lower pH levels (Love, 1975).

The first sensory changes of fish during storage are concerned with color, texture, odor and appearance. Fresh tilapia fillets in the present study showed a slightly grayish color, firm texture and a pleasant odor with a sensory score of 9.4 (Fig. 1c). Packages I and II were organoleptically acceptable up to the 24^{th} hour. After this time, the muscles exhibited a soft texture, a fading color and ammoniacal odor with a sensory score of 4.2. Package IV exhibited a spoilage odor at the 12^{th} hour at a sensory score of 3.8, while package III was rejected by the panelists at around the 18^{th} hr, when the sensory score reached 4.1. The refrigerated fillets (package V) were rejected after twelve days of storage. In an earlier report by Mogessie Ashenafi *et al*. (1995) spoilage of tilapia from Lake Hawassa, which was stored at 4^0C occurred after 11 days when the aerobic mesophillic count reached 10^7 c.f.u/g. In the present study, fillets at the time of rejection generally exhibited a whitish color, soft muscle and a spoilage odor of strong fishy, sulfidy, putrid and ammoniacal. It was also confirmed that deterioration and spoilage of tilapia fillets from Lake Hawassa is temperature-dependent.

a



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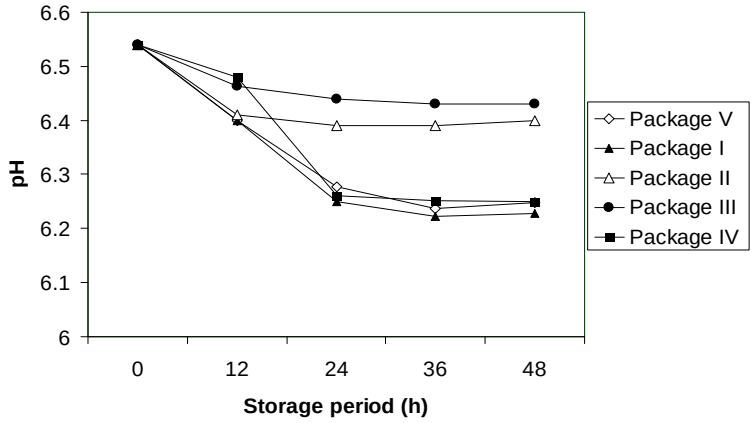

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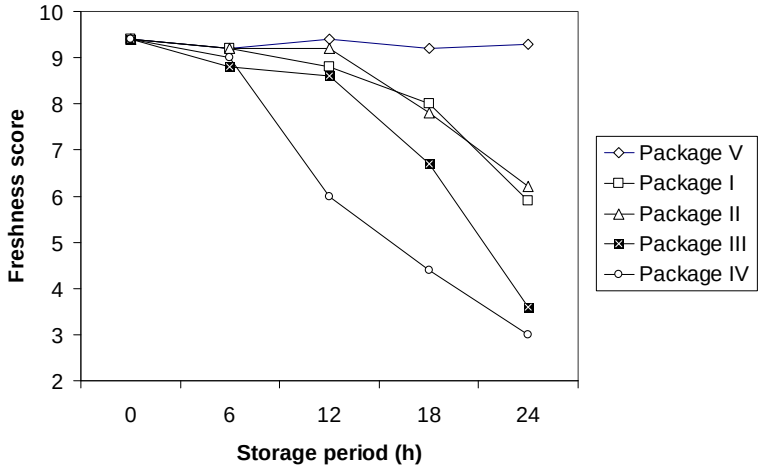


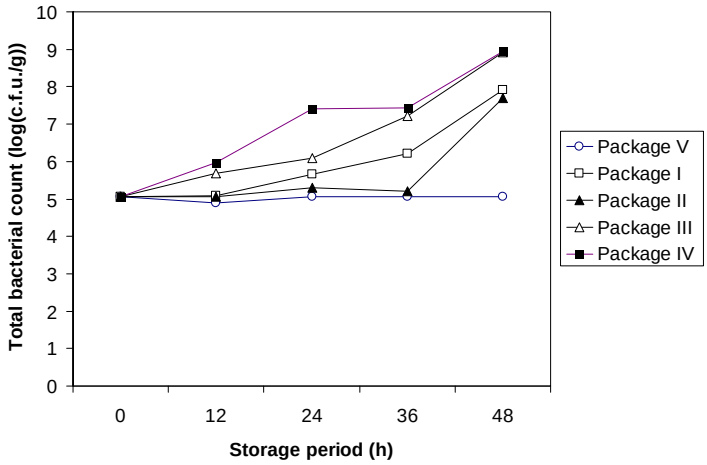
Fig.1. Changes in temperature profile (a), pH (b) and sensory quality (c) of tilapia fillets (*O. niloticus*) from Lake Hawassa stored in different conditions

Bacteriological analysis: Figs. 2a, b, c show the total bacterial load, total lactics and psychrophilic counts of fresh tilapia fillets stored at different conditions. Initially the value of the total bacterial count was 5.06 log (c.f.u/g). After 24 hrs of storage it reached 5.65 log (c.f.u/g) and 5.31 log (c.f.u/g) for packages I and II, respectively, and this level correlates well with the rejection time of the two packages by the panelists. Mogessie Ashenafi *et al*. (1995) reported that the initial aerobic mesophilic count of tilapia fillet from Lake Hawassa was $8.0x10^3$ to $2.5x10^4$ c.f.u/g. The low initial bacterial count in our study also agrees with the findings of Shewan, (1977) who reported that only a limited number of bacteria invade fish flesh during ice storage. Many bacteria are unable to grow at temperatures below 10^0C, and even cold tolerant bacteria grow slowly and sometimes with extended lag phases when temperatures approach 0^0C. There was a gradual and sharp increase in the bacterial population of packages III and IV throughout the storage period and reached 6.08 and 7.41 log (c.f.u/g) after 24 hours of storage, respectively, while it was still 5.07 log (c.f.u/g) in package V (refrigerated fillet at 4^0C).

Fresh tilapia fillets exhibited an initial total lactics count of 1.70 log (c.f.u/g). The population increased by nearly a log in packages I and II and reached 3.68 and 2.89 log (c.f.u/g), respectively, after 24 hours of storage. In packages III and IV the counts, respectively, were 3.68 and 3.69 log (c.f.u/g) after 24 hours, while in package V the level recorded after 12 hours of storage (1.82 log (c.f.u/g)) was maintained until the 48^{th} hour. Although lactic acid bacteria are not considered as belonging to aquatic environments, *Carenobacterium*, *Vagococcus*, *Lactobacillus*, *Enterococcus* and *Lactococcus* have been found in freshwater fish and their surrounding environment (Austin and Austin, 1992) and *Lactobacillus sp*. have been associated with fish diseases (Cone, 1982). Thus, assessment of lactics as fish pathogens is vital.

The initial psycrophilic count in the present study was 4.27 log (c.f.u/g). After 12 hours of incubation it reached 4.92, 4.88, 4.89, 4.88 and 4.89 log (c.f.u/g) for packages I-V, respectively, (without any significant difference) and this level was nearly maintained up to the 24^{th} hour of storage. In packages I and II, the level of psychrophiles reached 5.46 and 5.32 log, (c.f.u/g) after 24 hours of storage. After this time, however, the increase in the total psychrophilic count was sharp, except for package V (refrigerated fillet).

a



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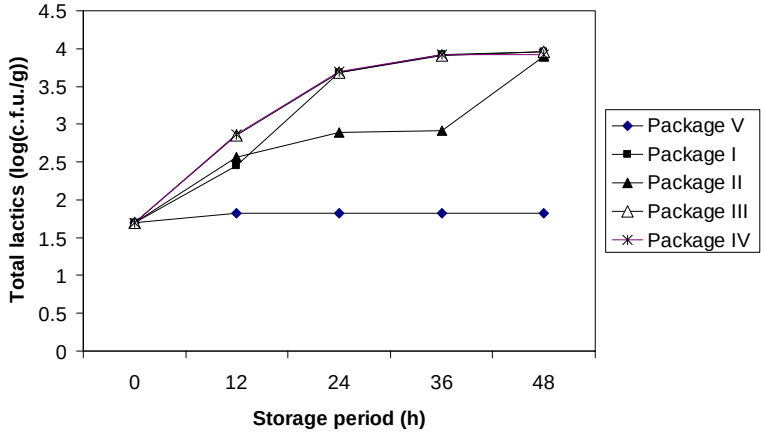

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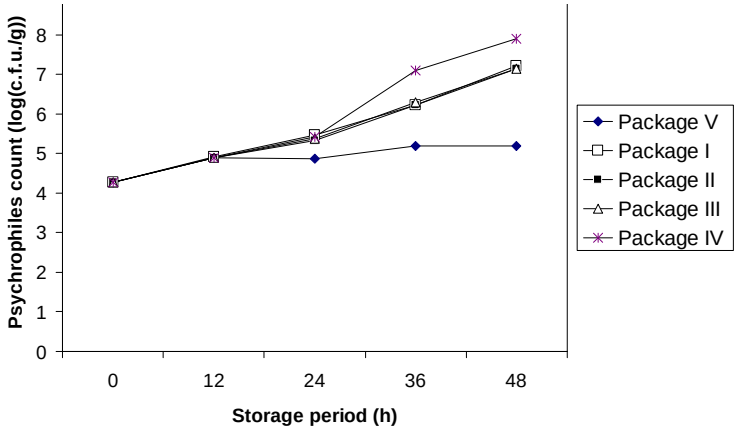

Fig. 2. Changes in total bacterial count (a), total lactic (b) and total psychrophiles of tilapia fillets (*O. niloticus*) from Lake Hawassa stored at different conditions.

A factor mentioned as influence on the number and types of bacteria is the method by which fish are harvested (Nickelson and Vanderzant, 1976). In addition, the great diversity of fish would also give rise to diversity among the bacteriological populations normally associated with the various fish species. While species differences may be important, micro-flora of fish is more directly related to environmental factors. It was further indicated that warm-water fish seem to harbour a more mesophilic, gram-positive microflora (*Micrococci, Bacilli, Coryneforms*), while cold-water fish carry a predominantly gram-negative population *(Moraxella, Acinetobacter, Pseudomonas, Flavobacterium,* and *Vibrio)* (Shewan, 1977). Furthermore, spoilage patterns during iced storage are usually quite similar, and are caused by *Alteromonas putrefaciens* and *Pseudomonas* sp. (Barile *et al*., 1985a, b). The dominant microorganisms of well cleaned fresh *Oreochromis niloticus* from Lake Hawassa were reported to be dominated by *Acinetobacter* and *Micrococcus* (Mogessie Ashenafi *et al*, 1995). Eshetu Eshetu Yimer (2000) identified *Shigella*, *E. coli*, *Citrobacter*, *Klebsiella*, *Oxytoca* and *Yersinia enterocolitica* from apparently healthy fish from Lake Ziway.

CONCLUSION

This research was able to detect that bacterial contamination of tilapia at the fish landing site of Lake Hawassa is considerable. The shelf-life can be improved by storing the product into ice and water and thereby inhibiting the microbial growth. More efforts should be made to design methods that can extend the shelf-life of tilapia at the lake.

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