

Survey of contaminant bacteria on *Oreochromis niloticus* and *lates niloticus* at Elmourda fish market

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Research Paper

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ABSTRACT

This study tend to determine the microbial load on fresh and chilled fish and to identify some contaminant bacteria. The fish were divided into two groups (one was put in a container and mixed with ice ratio 1:1, layer of ice and layer of fish for 48 hours. The second group was treated as fresh. Samples were taken from three regions of fish muscles; these are caudal, middle and anterior region. Finally, the whole samples were analyzed. The result obtained revealed that the bacterial count in fresh fish in *Oreochromis niloticus* and *lates niloticus* is $4.69 \times 10^5 \pm 1.35 \times 10^5$ and $3.69 \times 10^5 \pm 0.89 \times 10^5$ and in chilled fish of *Oreochromis*

niloticus and *lates niloticus* is $5.65 \times 10^5 \pm 1.88 \times 10^5$ and $3.81 \times 10^5 \pm 1.22 \times 10^5$ cfu/g respectively. The result analyzed show that there was highly significant difference in total bacterial count ($P < 0.05$) between chilled *Oreochromis niloticus* and *Lates niloticus* while there was no significant difference between fresh *Oreochromis niloticus* and *lates niloticus*. In addition, the result indicates that *Staphylococcus* and *E.coli* were isolated as contaminant bacteria, while *Salmonella* was not isolated from both fresh and chilled *Oreochromis niloticus* and *lates niloticus*.

Key words: chilled, *Oreochromis niloticus*, *lates niloticus*, *Staphylococcus E.coli* and *Salmonella*.

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INTRODUCTION

Fish is a major source of protein and its harvesting, handling, processing and distribution provide live hood for millions of people (ALJufaili and Ofara, 2006). It is the most important animal protein in food available in the tropics, and it represents about 14% of all animal protein on a global basis (Abolagba and Omelle, 2008). Fish is regarded a healthier meat option due to the high content of long chain polyunsaturated fatty acids which associated with improving health and preventing disease of old age. Capture fisheries and aquaculture supplied the world with about 142 million tons of fish in 2008 of this, 115 million tons was used as human food (FAO, 2010).

The intensive farming of tilapia (*Oreochromis niloticus*) is rapidly expanding and tilapias (including all species) are the second most widely farmed fish in the world with annual production exceeding 2 million tons in 2005 (FAO, 2007). Moreover, the *O.niloticus* has many outstanding advantages such as easy to culture, high growth rate, easy breeding, high fibrils protein, good taste, white

cotton meat like sea bass fish, high nutrition and having more Omega-3 than other wild fresh water fishes and wild estuarine fishes (Aquatic Animal Research Centre Choroen Pokphand, 1999).

Nile perch (*Lates niloticus*) that is native to Ethiopia country introduced in the Lake Victoria during late 1950S and other Lakes in Africa. The species is of great commercial importance as a food fish. The Nile perch is also popular with sport anglers, as it attacks artificial fishing lures and rose in aquaculture. The yield of fillets from a completely un gutted fish is about 30%. The remainder is head, skin, guts, bones and fins plus meat attached to the filleting frame. The frames used to be smoke-dried for local consumption, while heads and skins used as fuel under frying pans to collect oil from the guts. Now, the companies process the filleting waste to fishmeal. However, the swim bladder dried and sold to traders for export to Southeast Asia where they used as food. Nile perch meat has a high content of omega-3 fatty acids (Werimo, 1998).

The microbial flora associated with freshly harvested fish is principally a function of the environment in which the fish caught and not of the fish species; hence, the indigenous microbial populations of fish can vary significantly (Shewan, 1977).

Fish because of their soft tissues and aquatic environment are extremely susceptible to microbial contamination. Many of them potential spoilers, present in the surface slime, on the gills and intestines of live fish, although the flesh itself is normally sterile, Bacterial growth and invasion on the fish are prevented by the body's natural defense system during life, but after death the defense system breaks down and the bacteria multiply and invade the flesh. Microbial actions play a large part in the spoilage of fish (Eyo, 2001). This study tend to determine the microbial load on fresh and chilled fish and to identify some contaminant bacteria.

MATERIALS AND METHOD

Fourty fish samples (20 fresh, 20 chilled) of *Oreochromis niloticus* and *lates niloticus* were purchased from Elmourada fish market, Omdurman, Sudan during April to June 2012. Then iced in thermostatic containers and transported to the Sudan University of Science and Technology, College of Veterinary Medicine and Animal Production. The fish were divided into two groups one was put in a container and mixed with ice ratio 1:1, layer of ice and layer of fish for 48 hours and the second group was treated as fresh. Samples were taken from three regions of fish muscles; these are caudal, middle and anterior region. Finally, the whole samples were analyzed.

Bacteriological Examination

Plate count agar

The plate count agar was prepared by dissolving 32 g of nutrient agar in 400ml distilled water then, it was sterilized by autoclaving at 15 pound per square inch pressure (121c⁰) for 15 minutes. The samples were homogenized in sterile mortar and put in sterile tubes.

Preparation of serial dilutions

Separated sterile pipettes were used, decimal dilution of 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ and others were prepared, and samples were homogenized by transferring 1ml of previous dilution to 9ml of diluents.

Total viable count (TVC)

The test was done according to Guinn *et al.*, (1999). Immediately sample dilutions and agar medium were

mixed thoroughly and uniformly by alternate rotation and back and forth motion of plates on flat level surface. The poured agar let to solidify; the solidify Petri dishes were inverted and incubated promptly for 48±2hrs at 37c⁰. Fifty to three hundreds colonies were counted. The total colony count per milliliter was calculated by multiplication of the number of colonies counted by dilution level.

Bacterial identification

First isolation

First, the sample was inoculated in nutrient agar and Macconkey agar and incubated at 37c⁰ for 24 hours. Then Supplemented plates were prepared for subculture so as to get pure isolates depending on the appearance of bacterial growth on the surface of the media and color of the colonies.

Biochemical test: All biochemical tests were done according to methods described by Barrow and Feltham (1993).

Catalase test: One drop of hydrogen peroxide was placed on microscopic slide using sterile glass rod. Small part of isolated colony was taken and emulsified in the hydrogen peroxide drop. The productions of gas bubbles were considered a positive reaction.

Indole test: Sterile tube was used and inoculated with isolate then covacs solution was added and incubated at 37c⁰. If red ring occurred in the surface of the tube it considered a positive reaction.

Sugars reaction: Sugar media enriched with serum and/or yeast extract in test tubes contained inverted Durham's tubes were inoculated with the isolate under test. The inoculated liquid sugar media were incubated at 37c⁰ for 24hours and examined for production of acid or gas. The production acid was indicated by change of Andrade's indicator into pink rosette in the upper part of Durham's tube indicated production of gas.

Statistical analysis: The Obtained results were analyzed statistically by T test described by Pomez (1984).

RESULTS

The result obtained revealed that the bacterial count in fresh fish in *Oreochromis niloticus* and *lates niloticus* is 4.69×10⁵±1.35×10⁵ and 3.69×10⁵±0.89×10⁵ and in chilled fish of *Oreochromis niloticus* and *lates niloticus* is 5.65×10⁵±1.88×10⁵ and 3.81×10⁵ ±1.22×10⁵cfu/g respectively. The result analyzed show that there was highly significant difference in total bacterial count (P < 0.05)

Table 1. Microbial load for fresh and chilled *Oreochromis niloticus* and *Lates niloticus*.

Fish type	Microbial load of fresh Mean± Std. Deviation	Microbial load of chilled Mean± Std. Deviation
<i>Oreochromis niloticus</i>	4.69×10 ⁵ ±1.35×10 ⁵ c.f.u/g	5.65×10 ⁵ ±1.88×10 ⁵ c.f.u/g
<i>Lates niloticus</i>	3.69×10 ⁵ ±0.86×10 ⁵ c.f.u/g	3.81×10 ⁵ ±1.22×10 ⁵ c.f.u/g
Significant	No significant	*

*= Significant at (P>0.05).

Table 2. Tests of contaminant bacteria of fresh and chilled fish (*Oreochromis niloticus* and *lates niloticus*).

Organism Treatment	Staphylococcus	Salmonella	E.coli
Chilled fish	+ ve	- ve	+ ve
Fresh fish	+ ve	- ve	+ ve

between chilled *Oreochromis niloticus* and *Lates niloticus* while there was no significant difference between fresh *Oreochromis niloticus* and *lates niloticus* (Table 1).

In addition, the result indicates that *Staphylococcus* and *E.coli* were isolated as contaminant bacteria, while *salmonella* was not isolated from both fresh and chilled tilapia and *lates niloticus* (Table 2).

DISCUSSION

Microbiological tests is to enumerate and characterize the micro-organisms most important in fish and fishery products by looking at the factors that affect their growth and survival and where they are mostly likely found in the processing plant. In this study, the total number of bacterial count for fresh and chilled *Oreochromis niloticus* for 2-4 days was 4.6×10⁵ ± 1.3×10⁵ and 5.6 ×10⁵±1.88×10⁵ cfu/g of fish meat respectively, and this number was in the accepted limit mentioned by SSMO (Sudanese Standards and Metrology Organization, SDS357) which was 5× 10⁵ to 5× 10⁶ cfu/g for fresh fish products. In addition, this number was in the normal range stated by Liston (1980) which was 10² to 10⁷ cfu/g of fish meat. It is considered as normal range of fresh as mentioned by Hoffman (1971) who stated that the bacterial count of 10² to 10⁷ cfu /g of skin surface is normal range.

This is accepted limit compared to Anon (1991) who said that the total mesospheric aerobic bacterial counts over 10⁶.cfu/g was regarded as accepted limit for sea foods. In addition, this result is in agreement with Musa and Ahmed (2011) found that the bacterial count of fresh and chilled *Oreochromis niloticus* was 8.4×10⁵ and 3.2×10⁵ cfu/g of fish meat respectively.

In case of *Lates niloticus* the total viable count of bacteria in fresh and chilled fish was 8.9×10⁵ ± 3.69×10⁵ cfu/g and 9.3×10⁵± 1.6×10⁵ cfu/g of fish meat respectively. This result is differ from the finding of Amegovu et al., (2012) who stated that the total bacterial

count of Nile perch catch by different methods of catch stored for 7 days was 3.9 x 10⁴ . In addition, this result agrees with Kapute et al., (2013) who found that the total bacterial count reach 2.1×10⁵ for the gill of Nile perch. This result was considered as accepted number of bacteria compared to that mentioned by Anon (1991) who said that the acceptability limit is 10⁶ cfu/g for mesophilic aerobic bacteria. These results agree with finding of Chou (1993) who reported that the total aerobic plate count of unwashed and washed *lates niloticus* from mince were 5×10⁶ cfu/g and 10⁷ cfu/g respectively. Also, result in agreement with Liston (1980) which was 10²-10⁷ cfu/g of fish meat. Olafsdottir (1997), reported that the total viable count of fish products is 10⁷ to 10⁸ cfu/g at the point of sensory rejection.

In addition, the result revealed that there was highly significant difference in total bacterial count (P < 0.05) between chilled *Oreochromis niloticus* and *Lates niloticus* while there was no significant difference between fresh *Oreochromis niloticus* and *lates niloticus*. In addition, Shewan (1977) reported that the bacterial flora on freshly caught fish depends on environment rather than fish species, and this reflects the wide range of bacterial count. Also fish spoil at very different rates, and differences in surface properties of fish have been proposed to explain this. Skins of fish have very different textures. Thus, *Oreochromis niloticus* may have a very fragile integument spoil rapidly compared to *lates niloticus* that has a very robust dermis and epidermis. Furthermore, the latter group has a very thick slime layer, which includes several antibacterial components, such as antibodies, complement and bacteriolytic enzymes. This finding may coincides to some extent with result of (Hielmland and Christie, 1983) who claimed that Although, very wide variations occur, tropical fish species often have prolonged shelf lives when stored in ice when comparisons are made, data on fatty fish like herring and mackerel should probably be omitted as spoilage is mainly due to oxidation.

In addition, the result indicates that *Staphylococcus* and

E.coli were isolated as contaminant bacteria, while *salmonella* was not isolated from both fresh and chilled *Oreochromis niloticus* and *lates niloticus*. Pathogenic bacteria associated with fish and fishery product can be categorized into two general groups: (1) bacteria (indigenous bacteria) that belong to the natural microflora of fish (*Clostridium botulinum*, pathogenic *Vibrio* spp., *Aeromonas hydrophila*); (2) enteric bacteria (no indigenous bacteria) that are present due to fecal contamination (*Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Staphylococcus aureus*) (FDA, 2011).

In polluted waters, high numbers of *Enterobacteriaceae* may be found. In clean temperate waters, these organisms disappear rapidly, but it has been shown that *Escherichia coli* and *Salmonella* can survive for very long periods in tropical waters and once introduced may almost become indigenous to the environment (Fujioka et al., 1988). Humans are common asymptomatic carriers of enterotoxigenic *S. aureus* in nose, throat, and skin. Thus, food handlers can be an important source of food contamination (Gtz, 2002), and this enhances the recurrence of food contamination. Different in the taxonomic composition of microflora of fish are due to regulation by local ecological and physiological condition.

Each area including amount and type of different available nutrients, pH and nature of adhesion factors for each bacterial groups in the epithelial cells. Control of enteropathogenic *E.coli* and other food borne pathogens such as *Salmonella* and *Staphylococcus aureus* could be achieved. Precaution should include adequate cooking and avoidance of recontamination of cooked meat by contaminated equipment, water or infected food handlers.

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