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Evaluation of Microbes under Stored Condition at 4°C in Freshwater Fish *Oreochromis mossambicus* Collected from Local Fish Market Ukkadam, Coimbatore

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Abstract: ***The present study was carried out to determine the abundance of pathogenic microorganisms in the frozen fish sample (Oreochromis mossambicus) collected from Ukkadam local market, Coimbatore district. The sample was kept under 4°C frozen temperature for 7 days (frozen period). The serial diluted fish muscle samples (10^{-5}) in nutrient agar medium after 24-36 hrs were quantified for microbial pathogens using microbial counting chamber. At the end of the experiment the selected fish muscle samples during the storage period was found to be contaminated with pathogenic bacteria ranging from 3.74×10^5 to 16.90×10^5 cfu/g. The total viable count of selected sample was gradually noted to be increased during the study period. Such quantity of pathogens in the selected experimental fish sample may claim a serious public health risk.***
Key words- Fresh water, Oreochromis mossambicus, Fish quality, microbial content

I. INTRODUCTION

Aquatic ecosystem in general ordinarily supports a variety of waste which may be assimilated in bio-systems without causing serious changes in abiotic and biotic characteristics of water. And now-a-days, contaminants reach levels in excess of the assimilative capacity of receiving waters. Contaminants of varied nature exist in surface waters which include multiple chemical compounds and different products of industrial and agricultural revolution. There are many pathways by which pollutants leave their sites of application and distribute throughout the environment and enter the aquatic ecosystem. The major route of pollutants into aquatic ecosystems is through rainfall, runoff and atmospheric deposition. Thus, the aquatic environment is continuously at the mercy of man's negative impact on it and there is drastic decline in quantity as well as quality of the fish inhabiting it. Since, condition of the fish is strongly influenced by environmental conditions it can be used as an index to assess the status of the aquatic ecosystem in which fish live. The world's demand for an aquatic source of foods is on the rise not only because of its growing population, but also because of a preference for healthier foods for human beings (Abimorad and Carneiro,2007).The quest for fish as a food source especially by the local populace is unending, either as a result of its relative affordability and/or because of the nutrients derived from it.

Fish is known to be highly nutritious and excellent source of animal proteins which is consumed by a larger percentage of populace of India because of its availability and palatability. Fish is also, a vitamin and mineral rich food for young and old consumers (Moghaddam *et al.*,2007; Koffi-Nevry *et al.*, 2011). Fish meat contains significantly low lipids and high water compared to that of other white or red meats (Nestel, 2000). The nutritional and medicinal values of fish products depend on their proteins, lipids, minerals and vitamins. Fish and fishery products also contain vitamins and minerals which play essential role in human health. As the proximate composition of fish species is different among the fish species, measurements of some proximate profiles such as protein, lipids and moisture contents are often necessary to ensure that they meet the requirements of food regulations and commercial specifications (Tawfik, 2009).This knowledge of the biochemical composition of fishes is essential to estimate their energy value and to plan the most appropriate industrial and commercial processing (Tsegay *et al.*, 2016).

In fact, tropical countries like India are not suitable for conservation methods and fish consumption is a problem since it is a highly perishable product. In urban areas, like Coimbatore city freezing is the most used method but the cold supply chain often not guaranteed. As we know quality control and marketing of fish and fish products are like the two sides of a coin. One cannot do without the other. So, quality, i.e. the wholesomeness or the state of excellence of a particular product in terms of its appearance, shape, colour, taste and competitiveness in price to the buyer should fulfill the customer's requirements. The living habitats of fish are completely different from its post-harvest environments and its quality depends on many factors, such as intrinsic composition, degree of spoilage, damage, deterioration during harvesting, cleaning, washing, handling, preservation,

processing, storage, transportation, distribution and marketing. A prolonged shelf-life and better quality can be maintained even under natural conditions in the cold countries than in tropical ones. But still fish and fishery products can undergo undesirable changes during frozen storage condition and duration. Microbial contamination by bacteria and viruses can also lead to the decrease in the safety consumption level of the seafood as well as the increase in chemicals contamination (Fleming *et al*., 2006).

Microbial and biochemical reactions cause public health risk which could arise from specific activities along the harvesting, production and processing supply chain. A study conducted by (Sudheesh *et al*., 2013) on the hygiene status too supported this statement. Food-borne or waterborne microbial pathogens are leading causes of illness and death in less developed countries, killing an estimated 1.9 million people annually at the global level. Lack of basic knowledge about proper food hygiene, preservation technique and sanitation among local vendors adds to the risk associated with food poisoning and disease outbreaks Rane 2011. Present day consumer preference for fresh food leads to the use of frozen storage and cold chain from the farm to table. Freezing and frozen storage have been largely used to retain the freshness of the fish which can be reflected by assessing the sensory and nutritional properties (Khidhir *et al*.,2013). During frozen storage fish muscle may, however, lead to denaturation due to formation and accretion of ice crystals and aggregation of especially myo-fibrillar proteins. These changes result in altered functional properties, changed textural attributes and reduced water holding capacity and juiciness.

Objective: So, the aim of the present study was carried out to determine effect of frozen storage (4°C) period -7days on microbial impact on fish species *Tilapia mossambicus* collected from Ukkadam market, Coimbatore.

II. MATERIALS AND METHODS

Coimbatore city also known as Kovai is the second largest city in the Indian state of Tamil Nadu. It is situated in the Western corner of Tamil Nadu and is surrounded by the Western Ghats on all sides. It is located on the bank of Noyyal river. It is one of the fastest growing cities in India and a major industrial hub in South India. It is often referred to as the "Manchester of South India" due to its textile industries. Coimbatore is also referred to as the Pump City/Industrial city. This city lies between 11°00′58″N 76°58′16″E11.0161°N 76.971°E covering an area of 642.12 km^2.

A. Sample Collection

Ukkadam is a neighbourhood of the city of Coimbatore in the Indian state of Tamil Nadu. It falls between the 10°59′28″N 76°57′40″E coordinates. Fish samples were collected for the present study from the Fish landing center Plate 1(A) and Fish market Plate 1(B), Ukkadam with the help of skilled local fishermen.

Fig - 1(A) Fish landing center-Ukkadam

Fig -1(B) Fish Market-Ukkadam

B. Laboratory Procedures

Immediately photographs were taken prior to preservation. Collected fish samples were neatly packed in polythene zap bags. Neatly labeled the time of collection with date and brought to the laboratory for further taxonomic identification.

C. Identification of Fish Species

The collected fish was identified to species level using standard taxonomic viz. Fishes of India, FAO identification sheets, ITIS (Integrated Taxonomic Information System) standard report (http://www.itis.gov), and other reference books using standard keys of Jayaram (1999), Qureshi and Qureshi (1983), Talwar and Jhingran (1991) and Day Francis (1878). Fish Base website

was also referred for various aspects of fish fauna (www.fishbase.org).

Fig -2 *Tilapia mossambicus*

D. Wet lab analysis

1) Microbiological Analysis: Samples of fish muscle from *Tilapia mossambicus* were aseptically obtained by dissection and then prepared for plating following Andrews and Hammack (2001). All the dissecting apparatuses, such as scalpel, forceps, scissors, knives, mortar, pestle and glassware, were sterilized with 100% ethanol and kept in hot air oven at 180ºC for 6 h prior to dissection.

E. Statistical Analysis

The experimental data collected were subjected to statistical analysis. Data was analyzed using one-way analysis of variance (ANOVA) and the significant differences between means were determined. Differences were considered to be significant when $p < 0.05$. Data were analysed using SPSS package (Version 11).

III. RESULT AND DISCUSSION

A. Microbial Analysis

The microbial parameters of ice stored fish are presented in Table.1 and Plate- 1A-1D. In the present study the total plate count at value of 5 log has been recorded for mostly consumed species and *O. mossambicus*. The total plate count of the meat of the *O. mossambicus* species during different periods of ice storage showed an increasing trend and registered a log value of 3.74×105 cfu/g (0 Day- Fresh fish) to 16.90×105 cfu/g (7th day stored fish). With further increase of the ice storage the TPC values increased due to possible proliferation of psycrophiles.

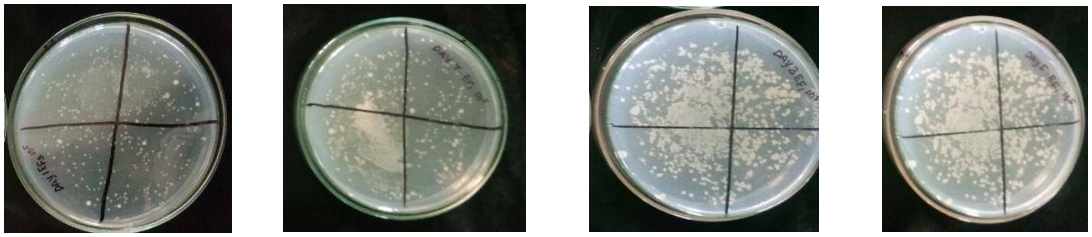
The flesh of the live healthy fish is sterile and microorganism present on the outer surface but after post mortem, the flesh is colonized by a wide variety of microorganism with broad temperature range due to the poikilothermic nature of fish (Huss *et al.,* 1997). The bacterial count (log cfug-1 meat) was gradually increased ($p < 0.05$). Increase in spoilage with the reduction of quality in the major substrates caused rapid deterioration of carps. The growth of all these bacteria increased significantly since the temperature of the storage condition favoured their growth.

Table.1 Changes in total plate count (TPC) in meat of *Oreochromis mossambicus* during frozen storage(4°C) period

Frozen storage period (Days)	**Total plate count (Cfu/g of meat)**
0	3.74×105
1	7.05×105
3	9.75×105
5	13.50×105
7	16.90×105

Plate.1A-1D Showing the number of microbial colonies (cfu/ml/gm) in 10^5concentrate of raw fish muscle of *Oreochromis mossambicus* collected from Ukkadam whole sale fish market

Day-1 **Day-3** **Day-5** **Day-7**



IV. CONCLUSION

Based on the results obtained from this study, it is recommended that microbial count can be used as an indicator of quality deterioration of selected fishes. From day three the count of microbes increased gradually. Therefore, in order to upgrade the quality of fish to meet consumers the expectation the enhancement of biochemical nature of the stored fish is necessary. So, existing freshwater fish quality can be improved via proper fish processing practice, application of modern fishing equipment, fish catching methods, catch handling on board, amount of ice used to chill the catch, sanitation of harvesting vessels and other processing tools/equipment and appropriate packaging material and storage facility so that we can meet the future protein demand and prevent malnutrition diseases among people all over the world.

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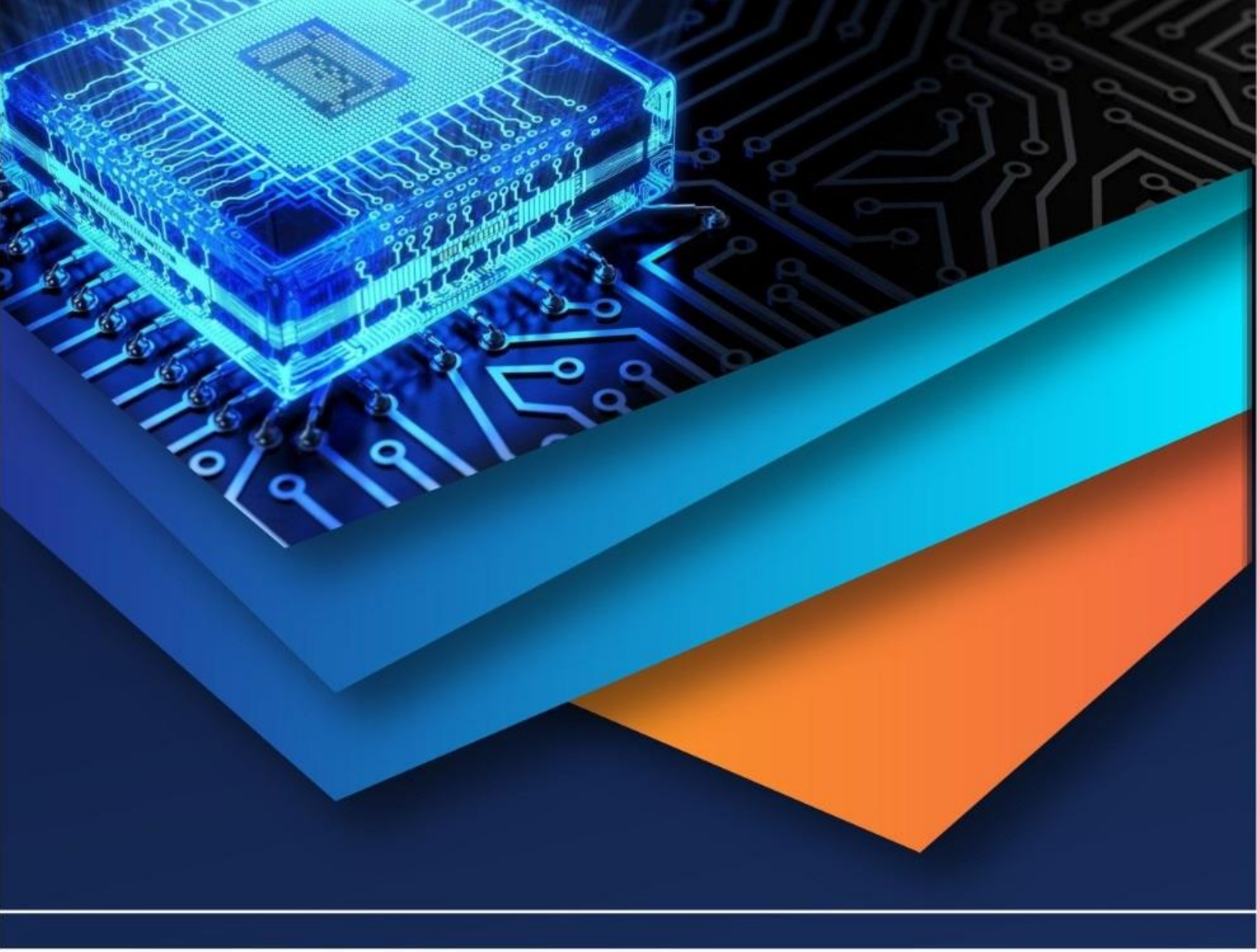

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