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Short Communication

# Effects of Storage Temperature on the Microbiological Quality of Fish Meat from Two Different Managemental Systems

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## ABSTRACT

Food spoilage is a complex process in which food deteriorates to that extent at which it becomes unfit for human consumption. Food spoilage due to microorganisms is a serious issue which causes approximately 25% food loss globally Present study revealed the effects of storage temperature associated with bacterial spoilage of fish meat. A total 24 samples were procured during three different seasons from local retail shops (n=12) and supermarket (n=12) in order to observe the bacteria which are associated with meat spoilage. Total viable count of aerobic psychrophilic, mesophilic and thermophilic bacteria was performed through selective enrichment and culturing of samples to determine the diversity of bacteria. *Staphylococcus aureus* (42%), *Enterococcus faecium* (54%), *Salmonella* sp. (38%), *Bacillus* sp. (71%), and *Moraxella* sp. (63%) were isolated from the samples collected from two different managemental systems. Statistical analysis revealed that temperature and seasonal variations alongside storage conditions affect not only the shelf-life of fish meat but also increase the spoilage microbiota population, hence, altering the quality profile of meat. Therefore, we can conclude that storage temperature is a crucial factor in determining the quality of fish meat.

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**Authors' Contribution**
**RHD, FA and AAS conceived and designed the study. RHD executed the experiments and KI analyzed the data. RHD and AK prepared the manuscript. MM and MIR contributed in preparation of manuscript and analyzing the data.**

**Key words**
**Food spoilage, Fish meat, Temperature, Psychrophiles, Mesophiles, Thermophiles**

Food spoilage is a world-wide concern which results into approximately 25% food loss due to microbial contamination owing improper handling and onsite storage (Eyo, 2014). Spoilage is the deterioration of food caused by pathogenic organisms and making it off flavor and off odor (e.g., when it is sour, rotten or moldy (Doulgeraki *et al*., 2012). Food spoilage can be due to chemical, enzymatic or microbial activities. Due to this spoilage, a considerable amount of fish is lost that leads to economic losses annually. Several parameters affect the growth and metabolism of these organisms such as storage temperature, types of preservation methods, atmospheric pressure, salt concentration, etc. (Bekaert *et al*., 2015). Most importantly, temperature variations during storage cause lipid oxidation and protein degradation that results in the compositional changes in fish meat leading to its spoilage. Typical bacterial count of $10^7$-$10^8$ CFU/g is normally found on such spoiled fish (Fukui *et al*., 2012).

Microbiological contamination of fish has been reported to be dependent on water, fishing conditions, inappropriate processing, distribution and storage facilities (Mahboob *et al*., 2014). This microbial degradation of fish leads to change in sensory properties, which makes the fish unsuitable for human consumption. In order to reduce the economic losses due to spoilage, there is a need to identify spoilage causing bacteria and to establish effective management system, for handling and storage of fish (Jan *et al*., 2014). Advancement in the development of preservation techniques has led to subsequent reduction in the loss of food items due to spoilage by understanding the growth and activity pattern of spoilage microorganisms (Zhou *et al*., 2010). Even though, chilling is the most efficient and extensively used technique of preservation as it keeps hold of the original characteristics of fish meat, but chilling alone does not guarantee the required value and freshness of fish meat (Claussen, 2011).

At some point during the production, transportation, vending and domestic storage of fish meat till its final serving as a meal, pathogens that are most commonly involved are *Salmonella* spp., *Staphylococcus aureus*, pathogenic *Escherichia coli, Listeria monocytogenes, Clostridium* spp., *Shigella* spp., and *Yersinia spp*. They

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are serious pathogens that accountable for a total of 42% foodborne outbreaks, which occurs across the globe (Yeni *et al*., 2014). Objectives: Therefore, the findings of this study investigated total viable bacterial count in fish meat collected from two different management systems i.e. local fish market and supermarket to determine the extent of bacterial contamination in fish meat. The effects of seasonal variation were also determined which in turned affected the storage duration of the fish meat. Culturing of pathogens that can be a source of contamination was also carried out.

*Materials and methods*

Samples ($n$=24) of freshwater carp *Labeo rohita* were procured from local market and supermarket of Lahore, Pakistan to study the effect of storage temperature and duration of storage on the microbiological profile affecting its shelf-life of fish meat. The temperature of the fish samples was recorded with the help of digital thermometer prior sampling to mimic the onsite storage temperature during transportation as well as storage up to 8 h. Samples were collected around 6 a.m. afterwards, transported as whole un-gutted fish in sterile (UV-irradiated) zip-lock bags to University Diagnostic Laboratory (Bacteriology section), University of Veterinary and Animal Sciences (UVAS), Lahore in ice packed insulated boxes.

Samples were collected from both managemental systems in spring, summer and winter season to observe the effects of seasonal variations on the viable bacterial load. Each sampling (spring (March-April)/summer (August-September)/winter (November-December)) was comprised of 4 whole un-gutted fish from each system. The temperature range varied in the local market from 18°C, 27°C and 9°C in spring, summer and winter seasons, respectively, at the time of sample collection. While the supermarket maintained its temperatures up to 1°C owing the availability of fishery chillers.

To remove any external debris, the exterior of the fish was rinsed with sterile distilled water. Evisceration was carried out with sterile equipment to avoid contamination samples. Afterwards samples were further processed for bacterial isolation. A 25g portion of interior flesh content was taken aseptically and processed for bacteriological analysis.

Samples were homogenized in 225ml of sterile physiological saline NaCl 0.85% (w/v) and were serially diluted. Samples were inoculated on general purpose media and selective media to perform culturing and isolation of selective bacteria. After inoculation, the bacterial colonies isolated from samples were subjected to bacteriological characterization procedures. The presumed colonies were first determined on selective media, while further identification was carried out using morphological and biochemical methods. All media used were from Oxoid Ltd, UK. The plates were incubated aerobically at 4°C for 7 days, 37°C and 45°C for 24 h for mesophiles and psychrophiles, respectively.

Following homogenization, the samples for aerobic psychrophilic (AP), aerobic mesophilic (AM) and thermophilic count were serially diluted and cultured using pour agar-overlay method on all-purpose medium (Nutrient Agar, Oxoid, UK). Readings from total viable count (TVC) was determined after every two h up to eight h to determine the effect of storage duration on viable load. After spreading, the plates were incubated at 4°C for 7 days, 37°C and 45°C for 24 h for mesophiles and psychrophiles, respectively.

*Salmonella* spp. was determined according to the method of Andrews and Hammack (2014). Detection of *S. aureus* was performed following the method of Cappuccino and Sherman (2007). *Enterococcus faecium* was determined according to method of Cappuccino and Sherman (2007). *Moraxella* spp. were subjected to culturing on blood and chocolate agar, given the aerobic incubation for 24 h at 30-37°C with conventional biochemical tests like catalase test, oxidase test, urease and hydrogen sulfide activity was also carried out. Sugar fermentation (glucose, sucrose, maltose and lactose) was also performed.

The significance of differences between means ($P<$ 0.05) total viable count, seasonal variations and storage temperature were analyzed using one way analysis of variance (ANOVA).

*Results and discussion*

Microbiological quality of the samples was observed to be affected in all three seasons. The effects of temperature variations in different seasons on the total viable count were recorded up to 8 h of storage. Bacterial spoilage of fish meat was recorded in samples procured from two different managemental systems. Both systems were having different hygienic status, which in turns affects the bacterial load in the meat. Fish samples were analyzed for total viable count (TVC) for aerobic psychrophilic, mesophilic and thermophilic bacteria. The microbiological quality of samples varied between all three seasons regarding total counts of aerobic psychrophilic, mesophilic and thermophilic bacteria (Fig. 1). The poorest quality was observed during summer season, where 65% of the fish were found unacceptable for consumption. While for the spring and winter seasons it was recorded as 53% and 43%, respectively. Moreover, total bacterial counts were significantly high at $P < 0.05$ in fish samples procured during summer season.

Similar observations were reported by Popovic *et al*. (2010) showed that the microbiological quality of individual samples varied widely between different fish species and also between winter and summer seasons regarding total

counts of aerobic mesophilic and psychrophilic bacteria. The poorest quality was for fish samples, where 66.6 per cent of fresh and frozen fish were found unacceptable by Croatian standards.

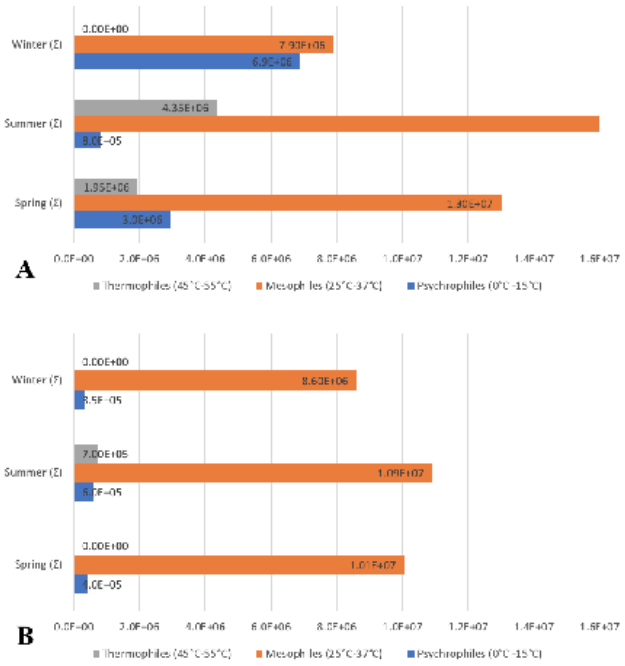


Fig. 1. Bacterial pathogens in fish meat samples procured from local market (A) and super market (B).

The *Salmonella* spp. isolates enriched in selenite cysteine broth showed brick red turbid appearance due to reduction of selenite. The colonies on Salmonella Shigella (S.S) agar displayed smooth translucent black colonies within due time of 48-72 h. Colonies on XLD were bright pink with black centers. IMViC test resulted positive for Indole, Methyl red and citrate utilization while negative for Voges Proskauer. Catalase was recorded positive while oxidase was negative for the isolates. Gram staining revealed typical gram negative pinkish red rods.

*S. aureus* was selectively enriched in BHI broth and raised medium to large slightly translucent or creamy colonies with clear zone of hemolysis was observed on 5% sheep blood agar. Golden brown pinhead and yellow colonies with fermentation of mannitol were seen on trypticase soy agar and mannitol salt agar, respectively. Purple stained grape like clusters were observed in microscopy after gram staining. The isolates were catalase and coagulase positive.

Samples processed for *E. faecium* were enriched in trypticase soy broth with 6.5% NaCl for purification of enterococci from streptococci. The isolates gave esculin hydrolysis in the presence of bile salts showed blackening on bile esculin azide agar which does not permit the growth of gram negative bacteria. The isolates were further tested for the arabinose fermentation on cephalexin aztreonam arabinose agar to distinguish between *E. faecium* and *E. faecalis*. Gram positive (purple-stained) diplococci were observed microscopically which turned out to be catalase negative and oxidase positive.

Active spoilers involved *B. cereus* which was enriched in BHI broth (1% maltose and 10IU polymyxin B/ml). Followed by streaking on MYP agar the plates were observed for typical colonies that appeared as dry, flat and white colonies surrounded by wide opaque zone. Conventional biochemical tests resulted in a positive catalase and citrate test while recorded negative for oxidase test and gelatin hydrolysis. Gram staining revealed typical gram positive rods stained purple.

Given the aerobic incubation for 24 h, *Moraxella* spp. revealed grey-white, waxy and crumbled appearance with no hemolysis on blood agar. Gram staining showed gram negative short rods and coccobacilli. Conventional biochemical tests which resulted in positive catalase and oxidase test. No hydrogen sulfide and urease production was recorded. Ability to ferment glucose, sucrose, maltose and lactose was not recorded.

*In vitro* assay during present work revealed that the samples were primarily contaminated by bacterial pathogens like *Salmonella* spp., *S. aureus* that are capable of causing gastrointestinal illnesses. Others included the not so classic food-borne organism *E. faecium* (but is associated with foodborne outbreaks) as well as active spoilers like *Bacillus cereus* and *Moraxella* spp. as at chilled temperatures. Incidence of isolated bacterial species in the samples was found to be *Salmonella* spp. (38%), *S. aureus* (42%), *E. faecium* (54%), *Bacillus cereus* (71%) and *Moraxella* spp. (63%). The results are in general agreement with those previously reported by Popovic *et al*. (2010) proposed that unacceptable Enterobacteriaceae levels were obtained in 40% of the freshwater fish summer samples (Fig. 2).

The conditions at local market for extended time of storage and selling of fishes are not ideal and may prompt to deterioration suggesting that fish should not be stored beyond six hours at ambient temperature. Therefore, hygienic conditions and use of clean water during processing of fish should be practiced. Post-harvest, freshly caught fish should to be appropriately stored at low temperatures to control growth of bacteria.

A similar study by Eze *et al*. (2011) suggested that aquaculture items can harbor pathogens, which are part of the natural microbiota of the surroundings and bioburden of the fish samples was determined by using agar plate method. *S. aureus*, *E. coli* and *Lactobacillus plantarum*, in particular, were the most commonly occurring pathogens linked to fish. The incidence of *S. aureus* in

fish was credited to the contamination due to non-hygienic handling by workers. This recommends that fish with this pathogen post-harvest must have been infected through management.

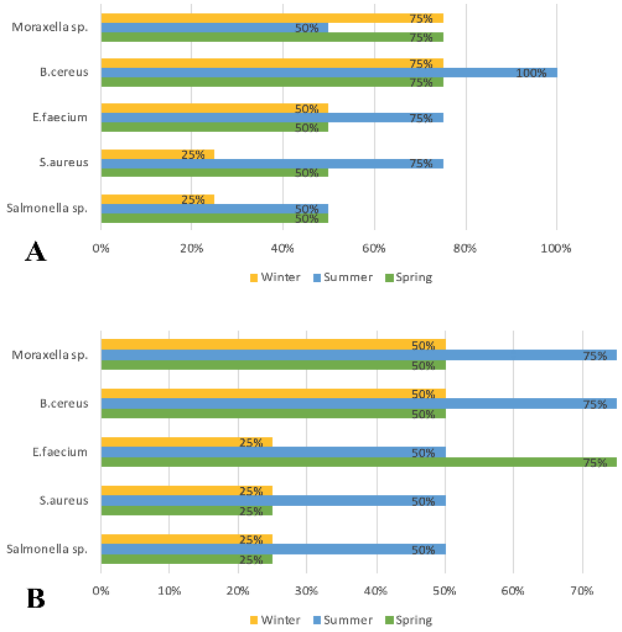


Fig. 2. Bacterial pathogens recovered from local fish market samples (A); super market samples (B) (All Seasons).

Falcao *et al*. (2002) have suggested that the ice which is used to refrigerate seafood might be contaminated with pathogenic microorganisms and can be a source of infection for humans, as they retrieved the presence of high numbers of coliforms, heterotrophic indicator microorganisms and pathogenic strains from ice that was used for chilling fish and other seafood items. Therefore, some of the contamination detected in the current study could be due to the ice used for chilling purposes.

*Conclusion*

The current study focuses on aspects of fish spoilage due to bacteria in particular. Temperature variations during three different seasons and storage conditions not only affected the storage life but also the microbiota population that causes spoilage leading to the change in composition and the quality profile. Hence, we can conclude that maintaining storage temperature is crucial feature in keeping the quality of fish meat.

*Statement of conflict of interest*

The authors have declared no conflict of interests.

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