Winter School on Product Development and Seafood Safety organized by CIFT Cochin 11 Nov. - 10 Dec. 2003,351-355.

Microbial Aspects of Fresh and Chilled fish

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The fishing activity was a monopoly of the marine sector till recently. With dynamic developments taking place in aquaculture, there is a shift in fish productivity from marine to aquaculture sector as evidenced by latest data. This necessitates thorough insight into the matters related to spoilage pattern, effect of handling and processing conditions on the bacteria and the safety for human consumption of fish from culture environments.

The major determinant both in the quality of seafood and its safety is the presence of various types of bacteria in the raw material. Fish even when it is alive, may carry bacteria on the skin surface, gills or intestine. Depending on the route of entry into fish body, microorganisms can be grouped into three classes as

- Bacteria naturally present in the aquatic environment
- Bacteria derived by way of pollution of aquatic environments
- Bacteria acquired by the seafood from terrestrial environment through contamination.

The body of fish acquires bacteria from the surrounding water or sediment and they are naturally present in fish in its very fresh condition. They are called the commensal or indigenous flora. Most of the members of this group may be harmless. But a few species can act as the spoilage organisms. The action of spoilers leads to undesirable changes, as also pathogens and make the fish unacceptable to the consumer. The second group is indicators of pollution for good manufacturing practices and these are considered as index of quality. They may be introduced to aquatic system and fish, mainly by sewage contamination, but they can also contaminate fish during later stages of handling and processing. The source of contamination in later stages can arise from food handlers or processing surfaces. There is a permitted limit for such organisms eg. *E.coli* 20/g. Some of the index organisms like *E.coli* and *Staphylococcus aureus* also act as food borne pathogens.

The pathogenic bacteria are either naturally present in the environment or they can enter as contaminants. Examples of naturally occurring pathogens are *Vibrio parahaemolyticus* and *Clstridium botulinum*. Ceratin other pathogens like salmonella and *Vibrio cholerae* are considered as contaminants from fecal sources. They are expected to be absent (zero tolerance) in fish as they get into the fish as contaminants. It is still a debated matter whether we should impose zero tolerance for indigenous pathogens as they are naturally present in the fish and hence total eradication of such pathogens is a distant possibility.

The quality of the fish is affected by a number of intrinsic and extrinsic factors; but the microbiology, to a great extent, plays a leading role in deciding the final quality. The finding that microbial flora of fish is a reflection of the environment from where it is caught emphasizes the role of environment in the quality of fish.

The total number as well as species wise distribution of various bacteria may vary from fish to fish depending on the intrinsic or extrinsic factors .The

intrinsic factors are those that are inherent with the sample such as type of fish species, age, geographical location etc., hence they cannot be controlled. The quantitative and qualitative distribution of the bacteria in freshly caught fish from the on board of vessel or the landing center fairly depicts the natural bacterial population in the sample.

In the case of freshly caught fish from marine, brackish water or freshwater areas the total plate count were in the range of 10^2 to 10^6/g with majority of cases lying in the range10^3 to 10^5/g. Very high counts of the order 10^7 - 10^8/g were noted from intestines of fish and shrimp. The bacterial count was maximum in the intestines and minimum on the skin surface. The high bacterial counts in the intestine emphasis the need to remove the gut portion to prevent cross contamination. As per ICMSF specification, bacterial count above 10^6/g has been considered as unacceptable in quality, while BIS and FDA standards have set a limit of 10^5/g as the maximum limit.

The bacterial types that dominated in freshly caught fish and prawn from marine ,brackish water or fresh water areas have been studied. Fresh water fish like Rohu carried higher percentage of Gram positives belonging to the family Micrococcaciae and Bacillaceae, which together comprised 50% of the total. Gram negatives also were present and they were Pseudomonas and Enterobacteriaceae. In brackish water fish, Gram positives like micrococci formed only 25% or less and gram negatives Pseudomonas, Vibrios, Acinetobacter and Moraxella prevailed. In marine environment, a conspicuous abundance of gram negatives like is observed. It may be stated that there was not much variation in bacterial diversity among habitats, but their percentage occurrence varied considerably. The shellfish carried bacteria that were almost similar to the finfish. But some of the bacterial genera like Moraxella and Acinetobacter were noted as prominent members on shellfish.

The pollution of the environment causes loss of quality of fish and this is estimated by the counts of bacterial pollutants. Important members of this group are total coliforms, fecal colliforms and *E.coli, Staphylococcus aureus*, fecal streptococci and *Clostridium perfringenes*. These counts were higher in farmed fish and shellfish compared to those from the wild. The aquaculture practices and operations such as pond fertilization with organic manure and animal excreta and supplementary feeding with pelleted feed, livestock and fish wastes impose a high probability of contamination on the farmed species. Hence a greater incidence of fecal bacteria in marine fish/shrimp can be considered as a result of post-harvest contamination whereas it is an inherent problem in the farmed fish/shrimp.

The pathogenic bacteria that are present in the fish can be either inherent or as a result of contamination during later stages of handling and processing. There is report of the isolation of salmonella and *V.cholerae* both from wild catch and cultured varieties. *Clostridium botulinum, V.parahaemolyticus* and *V.vulnificus* have been isolated from marine and brackishwater fish/shellfish. Emerging pathogens like *Bacillus cereus, Aeromonas species,Plessiomonas shigelloides* also have been detected. The isolation rate was low or random in most of the wild system and no general conclusion can be drawn regarding any specific association between a particular pathogen and environment But it was observed that there was a greater detection rate of pathogens in the farm environments, farmed fish and shrimp compared to that from the wild. This can be attributed to the farm practices including feed and manure.

When left at ambient temperature, which is usually 28±2°C, tropical fishes get spoiled within 6 to 12 hours, depending on the intrinsic and extrinsic factors. Bacteria most often involved in the spoilage of fish are part of the natural flora of fish. Initially their number may be small.They compete with other flora and becomes dominant at the time of

spoilage.The predominant kind of bacteria causing spoilage of fish vary with temperature of storage of fish and the processing treatments the fish has undergone. At ambient temperature spoilage (28±2°C), many bacterial species are found to cause spoilage. These are Vibrios, Pseudomonas, Aeromonas ,Moraxella and others. Bacteria belonging to genera Escherichia, Proteus, Serratia are also sometimes reported as spoilers of fish at high temperatures. At chill temperature fewer species viz Shewanella putrefaciens and Pseudomonas species (*P.putida, P.fluorescens*) are most likely to dominate followed by Moraxella and Flavobacterium. Under anaerobic conditions, Clostridium species can cause spoilage. Fish processed under various conditions have different spoilage flora. Thus salted fish are spoiled by salt tolerant (halo tolerant) or halophilic bacteria depending on the salt content in the fish. Molds are the chief spoilage organisms of smoked fish or pickled fish. Mold growth occurs at reduced water activity.Canned fish is spoiled by anaerobic spore-forming bacteria like *Clostridium botulinum* and *Bacillus stereothermophilus*. Spore formers can withstand the canning temperature and survive. Frozen fish has no spoilage problem when the product is in the frozen state, but the product may spoil during delayed thawing

In order to prevent such spoilage, many methods are in practice. Chilling, freezing, drying and use of chemicals are some of the usual methods. The basic principle involved in these methods of preservation of fish is to control the growth and activities of the microorganisms

Microbiology of chilling

The temperature has been widely exploited to control growth and activities of microorganisms. Both high as well as low temperatures have been exploited for fish preservation. Preservation by use of low temperature is considered more appealing than high temperature as it keep the product in natural state; but extended shelf life is not guaranteed by this method. Chilling is the cheapest and the most widely accepted short-term preservation method for fish.

Ice as the source of bacteria

Ice that is used for chilling fish is found to carry bacteria. Bacteria get entry into ice from crushing equipment, cooling tank, containers, fish holds, surface etc. But the count is never higher than 10^3. There is difference between factory ice and trawler ice. Factory ice is reported to carry greater numbers of Corynefomes, Flavobacterium, while trawler ice is dominated by Pseudomonas, Flavobacterium.

Changes during ice storage

The bacterial flora of fish held in ice will differ from flora of a newly caught fish.Flora changes depend on,

- Temperature of fish
- Icing efficiency
- Insulation efficiency
- External temperature
- Packing density

Microbiology of chilled of fish

Chilling is the most prevalent method of preserving fish. By this method, the temperature of the fish is lowered to near 0.1°C in about 2-3 hours. This lowering of temperature arrest almost all enzymatic changes and destroys about 50-60% of the mesophilic bacteria. The activity and growth of all other bacteria, which are cold loving (psychrophilic) and cold tolerant (psychrotrophic), may be slowed down. As a result, the spoilage of fish held in ice is delayed to a considerable length of time. During iced storage of fish, there is an initial drop of bacterial count due to the death of the cold sensitive mesophiles. The initial surviving cold

tolerant bacteria, however get adapted to growth in low temperature. Consequently, there is a gradual increase in population, which takes about 6 to 8 days to reach a count of one million per gram or above. By that time, the fish must have reached the stage of incipient spoilage.

During storage under the best condition (ideal) where temperature never rises above 0°C, bacterial count increases after 1-2 days and reaches maximum of 10^7-10^8/g in 9-10 days. The pattern of quantitative changes taking place in marine, brackish water or freshwater fish is comparable, but the time required for bringing out these changes may vary.

Storage of fish in ice leads to a selection of certain genera and elimination of certain others. The bacterial genera that survive at lower temperature alone can remain in the iced fish. Thus most of the mesophiles that dominate freshly caught fish are destroyed by the lethal action of chilling temperatures. Typical ice-storage studies have shown that the psychrotrophic count increases with storage period while mesophilic count gets reduced. Thus mesophiles like vibrios get reduced and psychrotrophic species like Pseudomonas and Alteromonas increase to enormous numbers. In some cases, when fish is densely packed, anaerobic conditions occur particularly towards the side of the container leading to a specialized flora and leading to the so-called stinker fish.

A preponderance of a particular genus at a given time in the bacterial population is indicative of its strong involvement with spoilage phenomenon. According to certain researchers, only a small fraction of bacteria of a particular group can cause spoilage, the reminders probably exist as free riders and are involved in some synergism with weak spoilers. Hence even though 80% of the flora of mackerel at the time of spoilage is made of Pseudomonas, only a fraction of them will be responsible for actual spoilage changes. Major observations noted during ice storage are

1. Decline of Gram positives
2. Emergence of a single dominant genus (Pseudomonas) as terminal flora
3. Pseudomonas group emerged as the terminal flora at the time when freshwater fish like rohu became unacceptable. But there was no spoilage odour/foul smell, which usually observed in the case of marine fish. This shows role of bacteria in the spoilage of cultured fresh water fish is limited.

In the case of shellfish like prawn, peeled prawn keeps better than headless which in turn has better keeping than whole prawn. A possible explanation for this behaviour is that cephalothorax and the exoskeleton, which is the source of surface and gut spoilage, have been removed in the case of peeled prawn. Surface spoilage seems to be more significant than gut spoilage since peeled prawn keep better than headless prawn. The quantitative changes in bacteria are much same as for fish.

On the basis of organoleptic scoring and TPC, which are good tools for quality evaluation, the shelf life in ice of different fish varieties was estimated. The results indicate that although initial flora from different fishery environs varies, spoilage flora is comparable. Marine fish showed the lowest shelf life of the order of 6-8 days. While brackish water fish had 12-14 days storage life in ice, freshwater fish could be stored up to 18 days or more. An interesting observation was that even when the TPC reached 10 million and the fish became totally unacceptable organoleptically, the spoiling smell observed in the case of marine fish was totally absent for fresh water fish. On the other hand, in marine fish like sardine and mackerel spoilage became apparent in 6-8 days with foul smell and slimy appearance and this corresponded to a TPC of 1 million/g. This finding indicate that role of bacteria in the spoilage is rather limited in the case of freshwater fish.

Psychrotrophic/Psychrophilic bacteria in iced fish and their role in fish spoilage

A major difference between temperate and tropical fish is the greater preponderance of the psychrophilic bacteria in the former group. The occurrence of psychrophilic bacteria in tropical fish is very low. About <5% of the heterotrophical bacterial population is found to be psychrotrophic in nature while in cold water fish like Flat head, flounder and Alaska pollak, it is 12 to 39 %. This small fraction of psychrotrophic bacteria comprises mostly pseudomonas and alteromonas. In some cases, when fish is densely packed, anaerobic conditions occur particularly towards the side of the container leading to a specialized flora leading to the so-called stinker fish. Alteromonas produce spoilage problems in fish in tropical countries. They are naturally present in the freshly caught fish and their number is greater in fresh fish compared to fishery products. One of the reason for the longer shelf life of tropical fish in comparison with temperate fish is the lower incidence of these psychrotrophs in the former.

Control measures for enhancing the effectiveness of chilling

1. As far as possible reduces the initial contamination before chilling. Prevention of contamination before chilling requires a systematic study of the various processing techniques in relation to source of contamination and the route of contamination. The spread of contamination can be controlled by effective time-temperature manipulations. Further contamination can be prevented by their elimination by process such as washing, gutting etc.
2. Ensure effective chilling process. The optimum temperature of most bacteria lies in the range of 20-30° C but may be viable upto 5° C. Failure to cool rapidly at the growth range may result in multiplication of bacteria
3. Prevent subsequent contamination by strict sanitation procedures.
4. Avoid fluctuations in temperature as this may lead to growth and toxin production by some bacteria.