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Microbial Safety of Fish and Fishery Products

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Fish is a highly perishable commodity. Spoilage of fish begins as soon as the fish dies. In tropical conditions, fish spoils quite rapidly, within a few hours of landing, if not preserved properly. The spoilage rate of fish can be reduced by good handling practices and effective temperature control from the very beginning. In raw fish, spoilage takes place mainly due to three reasons viz., (1) enzymatic action, (2) chemical spoilage, and (3) microbial spoilage. Among these three the microbial spoilage is very important and described in detail.

Microbial spoilage

The major cause of fish spoilage is bacteria, particularly in the case of marine fishes. The body fluids and flesh of freshly caught fish are free from bacteria except when the fish has bacterial disease. The bacteria present on skin, adhering slime, gills and intestine is normally saprolytic. Once the fish is dead, the bacteria invade the fish tissues causing spoilage and production of undesirable compounds. The type of bacteria on fish is very much dependent on the microbial flora of the environment.

Two types of microorganisms are of concern

1. Saprophytic or spoilage bacteria
2. Pathogenic bacteria

Spoilage bacteria

The important classes of spoilage organisms found in tropical species are *Pseudomonas, Flavobacteria, Acinetobacter, Aeromonas* and *Moraxella.* The spoilage bacteria are characterized by their ability to produce hydrogen sulphide (H_2S), reduce trimethyl amine oxide (TMAO) to trimethyl amine (TMA) and convert urea to ammonia. Many volatile compounds are also produced by *Pseudomonas* group of bacteria. The quantitative measurement of these compounds indicates the degree of spoilage. In the freshly landed fish total bacterial counts are in the range of 10^3-10^6 cfu/g and when the counts rise above 10^7 cfu/g, fish flesh starts visibly to spoil.

Pathogenic bacteria

Pathogenic bacteria associated with seafood are of two types

a. Indigenous bacteria
b. Non-indigenous bacteria

Indigenous bacteria

They are widely distributed in the aquatic environment. These pathogens occur in minimal numbers, and are not a serious problem in fresh fish. However, their growth and multiplication in seafood is a serious problem and cause illness. The important examples of this class are *Clostridium botulinum Vibrio* and *Aeromonas.*

Non-indigenous bacteria

Non-indigenous bacteria enter in seafood through external contaminants. The main sources include polluted aquatic environment, sewage, excreta from animals, birds, human beings, workers handling the material as well as the surface and environment where the seafood is processed. The examples of this class of bacteria are *Salmonella, Shigella, Escherichia coli,* and *Staphylococcus aureus.*

Factors influencing growth of different classes of bacteria

Bacteria are single-celled (unicellular) microorganisms widely distributed in nature belonging to the kingdom Prokaryote. The most outstanding characteristic of the organisms belonging to this kingdom is that the genetic material is not bound by a membrane. Bacterial growth is markedly influenced by many factors such as temperature, pH, salinity, oxygen content etc.

(1) Temperature

Each bacterial species has temperature range in which it has optimum growth. Also, there is a minimum temperature below which the bacterial growth does not takes place and a maximum temperature above which bacteria cannot grow. The optimum temperature is the most favourable temperature for growth. Depending on their temperature preference bacteria are broadly divided into three groups.

Psychrophilic bacteria : They are cold loving bacteria. They grow usually between temperature ranges of 0-20^{o}C, the optimum being 15^{o}C. This group includes most of the bacteria causing spoilage of refrigerated or iced food items. The examples of this class are *Pseudomonas, Alteromonas,* and *Moraxella*

In actual practice, truly psychrophilic bacteria are not usually encountered. These cold loving bacteria usually have a growth temperature range of 0-35^{o}C and hence also called as psychrotrophic bacteria.

Mesophilic bacteria : Majority of the bacterial species belong to this group. They grow within the temperature range of 20-45^{o}C with an optimum of 30-37^{o}C. Most of the pathogens belong to this group. The examples of this class are *Salmonella, Vibrio,* and *Streptococcus.*

Thermophilic bacteria : Bacteria which grow best at higher temperatures come under this group. Their growth temperature range is 45-70^{o}C, the optimum being 55^{o}C. Bacteria belonging to this group are quite rare. Such bacteria are found in natural hot springs. Certain bacteria causing spoilage of canned foods belong to this group. The examples of this class are *Bacillus coagulans, B. stearothermophillu* etc.

(2) Oxygen / air

Depending upon the requirement of oxygen or air for growth, bacteria are divided into 4 groups viz., *aerobic bacteria (*Bacteria requiring the presence of free oxygen or air for growth are called

aerobes. e.g. *S. aureus),* anaerobic bacteria (bacteria which can grow only in the absence of oxygen or air. e.g. *Clostridium* sp), facultative anaerobic bacteria (bacteria growing in the presence and absence of free oxygen), and microaerophilic bacteria (bacteria grows in the presence of very little free oxygen, e.g. *Lactobacillus, Streptococcus*).

(3) Salinity and pH

Salinity and pH are the primary physicochemical parameters controlling bacterial growth and have a marked effect on the maximal proliferation of bacteria.

Majority of the bacterial flora of marine fishes are gram negative, non-spore forming rods/cocci whereas gram positive in freshwater fishes. Brackish water fishes have both gram negatives and gram positives bacteria. Bacterial count from skin, gills and intestine of fish varies with the geographical origin of fish. The body fluids and flesh of freshly caught fish are mostly free from bacteria but saprophytic bacteria are usually present on the skin, gills and intestine. Soon after death these bacteria invade the fish tissue and leads to the spoilage of fish. There are three main routes for this attack.

1. From the gills into the flesh through the vascular (circulatory system)
2. Through the skin by penetration
3. Through the peritoneal lining, from the intestinal lining

Invasion of bacteria through the first and second routes is faster. Entry through the peritoneal lining can take place only after perforation of stomach and intestinal walls, which normally takes longer time. When left in ambient temperature, which is usually $28\pm4^{o}C$ tropical fishes get spoiled within 6-12 hours, depending on their size. In order to prevent such spoilage, many methods are in practice. Drying, icing, freezing, canning and smoking are some of the preservation methods followed in fish processing industry.

(A) Drying : A minimum level of water should be present in the medium/ substrate allowing the bacteria to grow. Such available water in foods or other substances is described by the term water activity (aw). Below a minimum level of water activity, microorganisms cannot grow. Most of the fish spoilage bacteria do not grow below aw of 0.91. During the drying of fish, water is removed from the fish muscle to an aw of 0.9 or below so that bacterial action is completely prevented.

(B) Icing : Icing is the most prevalent method of preserving fish. Ideal icing involves packing crushed ice and fish in layers in insulated boxes, in the fish to ice ratio of 1:1 (w/w). By this, the temperature of the fish is lowered to near $0.1^{o}C$ in about 2-3 hours. Reduction in temperature arrest most of the enzymatic changes and kills 50-60% of mesophilic bacteria. It also reduce the activities and growth of all other bacteria, those are cold loving and cold tolerant. As a combined effect of all these 3 factors discussed earlier, the spoilage of fish will be delayed to a considerable duration in ice. During iced storage of fish, there is an initial drop of bacterial count due to the death of the bacteria; however, some group of bacteria may get adapted and survive at low temperature. Consequently, there will be gradual increase in bacterial count, which may takes 6-8 days to reach one million/gm or above and the fish may reach to the incipient spoilage stage. In tropical fishes, psychrotophs are the actual spoilers during iced storage than psychrophiles. These psychrotophs, whose population is very low in the fresh tropical fish, easily adapt to grow at the low temperature in ice and may

flourish very rapidly, leads to the spoilage of fish. Further, psychrotrophs have a shorter generation time compared to psychrophiles.

(C) Freezing : Freezing of fish is done at -40°C, and the frozen fish is stored at -18 °C to -20 °C. During freezing, 80-90% of the Gram negative bacteria die out, and the residual bacterial can not grow in the frozen storage. But, before cooking, the frozen fish has to be thawed. During the thawing process the residual bacteria, which are predominantly Gram positive, can cause spoilage of the thawed fish. Hence, frozen fish will have to be thawed within the shortest possible time.

(D) Salt curing and fermentation : There are essentially two types of products where the preservative action of salt is the predominant process, dry salted and wet salted or pickled fish products. Dry salting is used only for non-fatty fish. There are two types of spoilage of this product. One is growth of the extremely halophilic bacteria which causes a condition known as “pink”. These pink halophilic bacteria (*Halococcus, Halobacterium*) are strongly proteolytic and produce off-odours and flavours in the product. The other type of spoilage is moulding by a highly osmophilic type of fungus known as “dun” (*Sporendonema and Oospora*). Wet salting is used for fatty fish species such as herring and anchovy. The fishes are mixed with salt and kept in a closed container. Three types of spoilage are known for these products characterized by the presence of sour, sour/sweet and putrified off-odours and off-flavours. This type of spoilage is caused by growth of Gram negative, halophilic, obligate anaerobic rods. The second type of spoilage is characterized by the development of fruity off-odours and is caused by growth of osmotolerant yeast species. Third type of spoilage is caused by a Gram negative, halophilic, aerobic, non-motile, rod-shaped *Moraxella* like bacteria.

(E) Canning : The canning process is a sterilization technique that kills microorganisms already present on the fish, prevents further microbial contamination, and inactivates degradative enzymes. In this process fish are hermetically sealed in containers and then heated to high temperatures for a given amount of time. Canned fish can be stored for several years. However, sterilization does not kill all microorganisms, and bacterial growth and gas production may occur if the products are stored at very high temperatures. Fish is a low acid food and can be processed safely only at temperatures reached in a steam pressure canner. Failure to heat process fish at 240° F or higher may allow spores of the dangerous heat-resistant bacteria, *Clostridiurn botulinum,* to survive, germinate, and grow. The poison produced by botulinum bacteria cause botulism, a deadly food poisoning.

E) Smoking : Smoking or smoke curing of fish is a method of preservation effected by a combination of drying and deposition of smoke constituents. When fish is smoked it is subjected to four basic treatments viz., brining, drying, smoking and heat treatment. Formaldehyde, acids and phenols are the important constituents of smoke involved in smoke curing of fish. Among these, phenolic constituents are supposed to be the most effective in preserving fish.

Quality control and its maintenance are the most important criteria for all the processed and preserved seafood. Quality control in seafood means all the steps taken between harvesting and retail trade to protect the quality of the final product. Many advanced and developing countries have already adopted the Hazard Analysis Critical Control Point (HACCP) concept for the seafood processing. With a view to harmonizing the various quality management systems the International Standards Organisation has developed a set of standards called ISO 9000 series. The HACCP

concept can become a part of these standards. The HACCP concept proposed by the US FDA has been taken as a standard process control system for assuring food safety by international bodies. It has been identified as the global unified quality assurance system for producing safe and better quality fish products at a global level.

(A) The main elements of the HACCP system are:

1) Identify potential hazards AND TO Assess the risk of occurrence.
2) Determine the Critical Control Points (CCPs)
3) Establish criteria to be met to ensure that each CCP is under control.
4) Establish a monitoring system.
5) Establish corrective action when CCP is not under control.
6) Establish procedures for verification.
7) Establish documentation and record-keeping

(B) Application of HACCP system in fishery harbor

HACCP concept is elaborated to the fishery harbors and landing centers for ensuring seafood safety. The main elements of it are:

1) Landed fish should not be exposed to the sun and should be iced.
2) Inspect fish for appearance and odour and reject fish of unacceptable quality.
3) Periodically perform bacteriological tests on representative samples.
4) Follow a cleaning schedule for all work areas and surfaces, using water containing 5 to 10 ppm of free chlorine.
5) Remove all fish slime and blood by hosing down with chlorinated water. At the end of the day, rinse all surfaces with clean water having 5 ppm of chlorine.
6) Apply personal hygiene rules strictly to prevent contamination of fish. Smoking and spitting in work areas should not be permitted. Hands must be washed with bactericidal soap prior to handling fish and after a visit to the toilet.
7) Check that water supply and treatment systems are in order. Water and ice samples should be analysed as per testing schedule by ISO certified laboratories for levels of chemical and bacteriological contamination and potability certificates obtained.
8) The harbour should be free from litter and other wastes.
9) Check to ensure that all drainage systems are in good working order**.**
10) The harbour should be free of animals, rodents and pests.
11) Ensure that there are no bird nests in the fish handling area.
12) Check that wastes are being disposed of sanitarily.
13) Check cold storage equipment to ensure that the right temperature is being maintained.
14) Ensure that all precaution and warning signs are readable

C) The ISO-9000 series certification of the International Standards Organization

For seafood processing establishments, the most relevant standards of the ISO 9000 series are the ISO 9001 and 9002. The former is a quality system standard that lays down requirements for product development, production, delivery and after sales functions. The latter concerns only production and delivery. The ISO 9003 deals with quality system requirements for final inspection and testing.

Microbiological criteria (MC) and testing of quality of fish and fishery products

Traditionally, control of microorganisms in food was demonstrated by microbiological testing of samples at various stages of production and the final product. Results were compared with criteria developed to give some degree of assurance that the food was safe and of good quality. It is now fully recognized that this type of activity can never give an absolute assurance of product quality and safety. A much higher degree of assurance can be provided by a preventative approach based on the application of the Hazard Analysis Critical Control Point (HACCP) principles at all steps in the food supply and processing system. Three types of MC are generally recognized according to their use:

1) Standards
2) Guidelines
3) Specifications

These terms have been defined and redefined a number of times, but it is generally recognized that the term “standard” is a MC contained in a law or regulation with mandatory compliance. A microbiological “guideline” is a MC applied at any stage in food processing and aids in identifying situations requiring actions for food safety or quality reasons. A “specification” is a MC used for contractual purposes by food business as part of their own safety management system and should not be confused with legal requirements.

Microbiological standards to be met

Sampling plan and recommended microbiological limits for seafood (ICMSF 1986)

Product	Test	Case	Plan Class	no. of samples	no. of positive results	Limit per gram or per cm^2	
						Per g	Per cm^2
Fresh and frozen fish	APC	1	3	5	3	5×10^5	10^7
	E. Coli	4	3	5	3	11	500
Precooked breaded fish	APC	2	3	5	2	5×10^5	10^7
	E. Coli	5	3	5	2	11	500
Frozen raw crustaceans	APC	1	3	5	3	10^6	10^7
	E. Coli	4	3	5	3	11	500
Frozen cooked crustaceans	APC	2	3	5	2	5×10^5	10^7
	E. Coli	5	3	5	2	11	500
	S. aureus	8	2	5	0	10^3	-
Cooked, chilled, and frozen crabmeat	APC	2	3	5	2	10^5	10^6
	E. Coli	6	3	5	1	11	500
	S. aureus	9	2	5	0	10^3	-
Fresh and frozen bivalve molluscs	APC	3	2	5	0	5×10^5	-
	E. Coli	6	2	5	0	16	-

Standards and criteria of fish and fishery products under the food sanitation law, Japan.

Classification	Standards and criteria	Remarks
Fish paste products (fish sausage and ham)	-Coliform organism: negative/g	There are also processing & preservation standards
Boiled octopus	- Viable bacteria count: 1.0 x 10^5/g or less- Coliform organism: negative/0.01 g	Only frozen octopus. There are also processing & preservation standards*
Raw oyster for uncooked	- Viable bacteria count: 5.0 x 10^4/g or less- *E. coli* MPN/100 g: 230 or less	-

US FDA Regulatory requirements for pathogens

Parameters/indicators of microbial safety	Bacterial counts
Total viable count	Not to exceed 100,000 per gram
Salmonella	Not to be detected in 25g of meat
Vibrio cholerae	Not to be detected in 25g of meat
Listeria cooked only	Not to be detected in 25g of meat
E. Coli	Less than 10 per gram
S. aureus	Less than 1000 per gram
Faecal coliforms	None

As a relatively “high-risk” perishable food, fish and fishery products are subjected to a range of food safety requirements related to general hygiene and specific microbiological and chemical contaminants. Thus maintenance of microbial quality and ensuring the safety of fish and fishery products at all the levels of production to final consumption is the prime challenge for the fishery industry. Food production and processing sector can start on the journey towards world class quality by building a foundation using the quality tools: ISO 9000, HACCP and Good Manufacturing Practices (GMP).

Suggested Reading

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