Chapter 2

Post- Mortem Changes and Mechanism of Fish Spoilage

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Products with no proven quality find little space in the market. The term quality represents the consumer acceptability of the commodity. The eating quality of fish determines the acceptability of fish as food to the consumer. The quality of fish begins to deteriorate immediately after catch. The changes taking place in fish after death are collectively called "post-mortem changes" and they can be grouped as follows:

- Hyperaemia
- Rigor Mortis
- Autolysis
- Microbial putrefaction/decomposition
- Lipid oxidation/autoxidation
- Discoloration

Hyperaemia:

During the harvest, the fish passes through various levels of struggle due to asphyxia or unfavorable conditions. At this time, the mucus glands in the skin secrete a large quantity of mucus, forming a thick layer of slime on the skin's surface. This phenomenon is known as "Hyperaemia". The slime contains an antimicrobial substance lysozyme which protects the fish from the attack of spoilage bacteria. After death, the immunological properties are lost, and microbes easily proliferate on the glycoprotein content of the slime imparting an offensive smell and increasing the penetration further into the muscles and internal organs.

Rigor mortis:

After the capture till the point of consumption, the fish undergoes a large number of physicochemical changes, which can be classified into three stages:

1. *Re-rigor state:* It is the state of fish from death till the onset of rigor mortis. At this stage, the fish is extremely fresh and the muscles remain elastic. This state is characterized by a fall in ATP and creatinine phosphate and glycolysis.
2. *Rigor mortis:* It is the state of fish from the onset of rigor mortis till its disappearance. At this stage the fish muscles become stiff. The stiffening of muscle after death is called

"rigor mortis". It starts 1-7 h after death, reaches a peak between 5-22 h, and its total duration range from 31-120 h.

3. *Post-rigor state:* It is the state of fish after the disappearance of rigor mortis. During this stage, the meat tenderization takes place making the fish organoleptically acceptable.

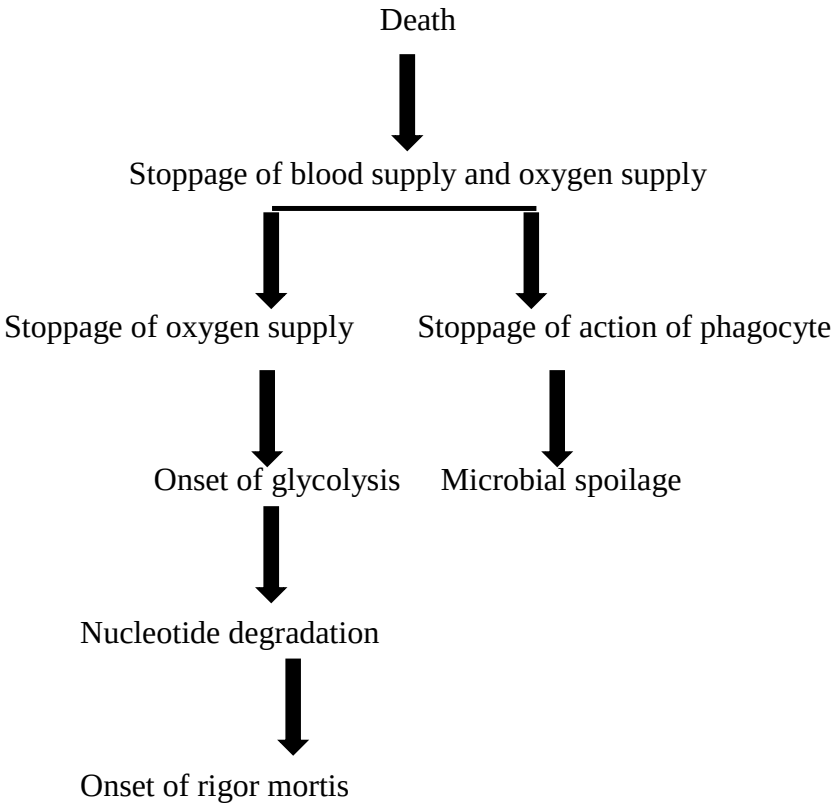
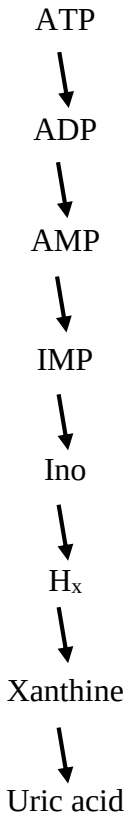


Fig1. Post-mortem changes of fish

Post-mortem Glycolysis: On cessation of oxygen supply to the muscles, the main carbohydrate source, glycogen broken down to lactic acid by anaerobic pathways -hydrolytic/amylolytic pathway & phosphorolytic pathway. As lactic acid accumulates in the system, the pH of the system falls from the initial physiological pH of 7.2-7.4 to the ultimate post-mortem pH of 6.0-6.2.

Nucleotide degradation: Nucleotide degradation is one of the earliest indices to assess freshness. It reflects both the action of autolytic enzymes and bacterial action. The nucleotide degradation products – Inosine Monophosphates (IMP), Hypoxanthine (H_x) or K value clearly reflect the quality loss in fish. After the death of the fish, Adenosine triphosphate (ATP) is degraded by endogenous enzymatic action and forms Adenosine diphosphate (ADP), Adenosine monophosphate (AMP), IMP, Inosine (Ino), and H_x successively. Hypoxanthine is

further degraded by xanthine oxidase to xanthine and uric acid. The degradation of ATP up to IMP is very fast, but the degradation of IMP is relatively slow. IMP imparts a pleasant, sweet taste and flavor (Umami taste, especially in crabs. Degradation of IMP to inosine and hypoxanthine results in a bitter taste and progressive loss of desirable flavor. The sequence of nucleotide catabolism in fish is given below:



Autolysis:

The process of breakdown of fish tissue after death by endogenous enzymes is known as "Autolysis". The breakdown of proteins, lipids and nucleic acids into their simpler units of significance during autolysis, results in softening of the tissue.

Microbial putrefaction/decomposition:

Autolysis alone will not spoil fish but helps the spoilage process by providing a nutrient-rich medium to the microbes. Microbes secrete their own enzymes to hydrolyze tissue components into simpler substances. Microbial putrefaction is the process of conversion of tissue components by microorganisms into off-odor and off-flavor substances to make the fish spoil.

Lipid oxidation/autoxidation:

Lipid oxidation is the limiting factor in fatty fish during storage, which results in rancidity (development of off-flavor and off-odor). The factors affecting the onset and development of rancidity are

1. Degree of unsaturation
2. Type and concentration of antioxidants
3. Pro-oxidants
4. Moisture content
5. Oxygen availability
6. Temperature
7. Degree of exposure to light

The major chemical indicators for the determination of the extent of oxidative rancidity are anisidine value (AV), peroxide value (PV), and thiobarbituric acid value (TBA). Peroxide value is also known as hydroperoxide value, used as a measure of the extent of oxidation in the early stages. It measures the primary products of lipid oxidation, which break down into secondary products of oxidation or reacts with protein. An increase in PV is most useful as an index of the earlier stages of lipid oxidation; as the oxidation proceeds the PV starts to fall. AV and TBA measure the secondary product of lipid oxidation. TBA measures the malonaldehyde produced during lipid oxidation. It can be assessed that if the PV value is 10-20 mg oxygen/kg or TBA is above 1-2 mg of malonaldehyde per kg of the sample, then the fish will in all probability smells and taste rancid. During prolonged storage of fish, PV, AV, and TBA values may increase reaching a peak and decline.

Spoilage of fish:

The process of quality deterioration or change in fish or fish product that renders it less acceptable, unacceptable, or unsafe for human consumption is known as spoilage. Spoilage can be

1. Microbial
2. Physical
3. Chemical

The most commonly used method for the quality evaluation of raw fish is sensory evaluation. Although the method is simple and rapid, the main disadvantage is the lack of objectivity. During spoilage, a number of chemical reactions are taking place in the fish muscle. Various

compounds are formed during these reactions, which are quantitatively determined, correlated with sensory characteristics, and used as spoilage indices.

Spoilage indices:

During spoilage, various compounds are produced in the fish muscle by autolytic enzymes, putrefactive microorganisms, or chemical reactions and gradually get accumulated in the flesh. Hence the quantitative determination of these compounds will provide a measure of the spoilage process. The spoilage indices for fish and shellfish are as follows:

1. Volatile bases
2. Nucleotides
3. Lipid oxidation products

Total Volatile Bases:

Volatile bases are produced by spoilage bacteria in fish. They are basic nitrogenous compounds such as ammonia, trimethylamine (TMA), Trimethylamine oxide (TMAO), and Dimethylamine *etc*., The most commonly used index of quality for the freshness of fish is the Total Volatile Base Nitrogen value (TVBN) along with Trimethylamine. Fish with a TVBN value of 20mg/100g is considered very fresh. The limit of acceptability of TVBN is 35-40 mg/100g beyond which the fish is considered as spoiled.

Trimethylamine (TMA):

Trimethylamine is the specific index used for assessing the freshness of marine fish. In most cases, the TMA concentration is extremely low, normally under 1mg N/100g. Studies indicate that in a few bivalves, the TMA content is about 20mg N/100g. In elasmobranchs and marine teleosts, the viscera, especially the spleen, liver, and kidney contain the most TMA and the muscle the least. The midgut gland has the highest level of TMA in squid.

TMA is derived from TMAO which is critical for osmoregulation in marine fish. Two types of enzymes are considered to be responsible for the reduction of TMAO to TMA and to DMA and formaldehyde (FA)- endogenous enzymes in fish, and exogenous enzymes produced by spoilage bacteria. The strains of bacteria capable of reducing TMAO to TMA have been found in most species of the Enterobacteriaceae including *Escherichia coli, Achromobacter, Micrococcus, Flavobacterium*, nonfluorescent *Pseudomonas, Clostridium, Alcaligenes,* and *Bacillus* spp. TMAO is reduced by bacterial enzymes to TMA while the endogenous enzymes reduce TMAO to DMA and then to FA. During frozen storage, the production of DMA is greater than that of TMA. Hence DMA can be used as an index of enzymatic deterioration during frozen storage and TMA as an index of pr-freezing quality. The

formation of DMA is accompanied by the equimolar formation of formaldehyde (FA), which can cause the denaturation of myofibrillar protein in fish flesh.

A level of 10-15 mg TMA-N/100g muscle is considered as the limit of acceptability. This level increases with storage time during iced storage hence TMA can be used as a good index of spoilage.

Ammonia:

Bacterial spoilage of fish generates small amounts of ammonia from the free amino acids. The ammonia content can be used as an indication of the extent of spoilage. A greater amount of ammonia is produced during the spoilage of elasmobranchs due to the high content of urea in their flesh. Shellfish can also produce a large amount of ammonia than marine fishes at the early stages of spoilage.

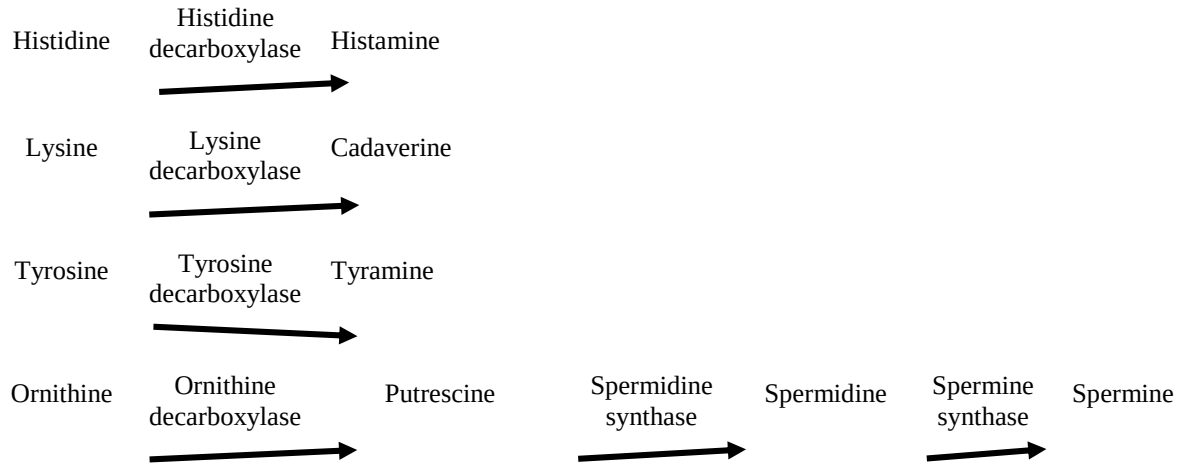
Biogenic amines:

Biogenic amines are non-volatile compounds, found at very low levels in fresh fish. Important biogenic amines are histamine, cadaverine, putrescine, tyramine, tryptamine, spermine and spermidine. Histamine is known to be the causative factor of scombroid poisoning/histamine poisoning in histamine-forming fishes such as mackerel, tuna, sardine, bonito, herring, anchovy *etc.,* Food Safety and Standards Authority of India has identified the following family of fishes as histamine forming fish species.

1. Carangidae – 30 species of fishes including jacks, scads, pompanos, queen fishes, kingfishes, and trevallies
2. Chanidae (Milkfish)
3. Clupeidae – 33 species of fishes including Sardine and Shad
4. Coryphaenidae (Mahi Mahi/Dolphin fish)
5. Engraulidae – 9 species of anchovy
6. Istiophoridae – 9 species of Marlin/Sailfish
7. Mugilidae (Mullet)
8. Pristigasteridae – 2 species of Ilisha/Pellona
9. Scombridae – 32 species of fishes including Mackerel, Tuna, Bonito, and Seer fish
10. Xiphiidae (Swordfish)

These fishes are found to be having high free histidine content which gets converted into histamine during spoilage. The biogenic amines formed during the spoilage of fish are found to be thermally stable and thus can be used as an indicator of poor quality of raw material in preserved/processed fishery products. Cadaverine and putrescine are found to be potentiators

of histamine. The direct precursors of histamine, cadaverine, and putrescine are histidine, lysine, and ornithine respectively. Putrescine is also an intermediate of a metabolic pathway that leads to the formation of spermidine and spermine.



Although biogenic amines have been associated with fish spoilage, the legal limit has been established for histamine only. As per Food Safety and Standards Regulation (FSSR, 2011), the maximum permissible level of histamine content in fish and fishery products is 200mg/Kg. Fishes with histamine content up to 20mg/kg are considered to be safe for consumption, 20-100mg/Kg is probably safe while ≥100mg/kg is toxic and unsafe for consumption.

Studies also indicated that cadaverine and putrescine can also be used as freshness indices for fish and shellfish respectively. Fish and fishery products containing cadaverine below 15mg/100g are considered as good for consumption, 15-20mg/100g indicates potential decomposition, and over 20mg/100g advanced decomposition. The quality Index (QI) and Biogenic Amine Index (Bai) are also used to indicate the freshness of fish.

$$QI = \frac{Histamine + Putrescine\ + Cadaverine}{[1 + (Spermidine + Spermine)]}$$

$$BAI = (Histamine + Putrescine + Cadaverine + Tyramine)$$

Indole: Indole is a spoilage indicator in shrimp and crab. Indole (2,3-benzopyrene) is a degradation product of tryptophan. Indole is highly volatile and soluble in different solvents such as hot water, alcohol, ether, and benzene. Shrimp with indole content <25mg/100g is organoleptically acceptable.

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