Fish Spoilage and Quality Assessment

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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 properly cooled. The spoilage rate of fish may 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. Enzymic action
2. Microbial action
3. Chemical action

Enzymes and bacteria do not cause any deteriorative changes in the living cell bacause of the natural defensive mechanism. In dead fish, enzymes become involved in autolytic changes and bacteria can invade the fish muscle and proliferate there. The fish gut is rich in proteolytic enzymes and in dead fish it digests the gut and belly region making the fish very soft. Bacteria that are present on the surface, gills and gut of the fish invade the dead fish, decompose the tissue and bring about undesirable changes. Off odours and off flavours, slime, gas production, discolouratioin and soft texture are the obvious signs of spoilage. Components involved in spoilage process are protein, lipids, carbohydrates, nucleotides and other non-protein nitrogen compounds. The rate of spoilage is temperature dependent and lowering the temperature will reduce the rate of spoilage.

Autolytic spoilage (Enzymatic)

Autolytic spoilage is responsible for early loss of quality of fresh fish. The first enzymatic change in fish muscle is the gradual hydrolysis of glycogen to lactic acid which is known as glycolysis.

1. Glycolysis

After death, the blood circulation stops and the cells are no longer supplied with oxygen and hence glycogen will not be converted into carbon dioxide and water unlike in the case of living cells. In the postmortem period, glycolysis proceeds via the anaerobic pathway where the end product is lactic acid. As lactic acid accumulates, the pH of the muscle falls. In fish, glycolysis will continue until the supply of glycogen is completely used up. In general, fish muscle contains a relatively low amount of glycogen compared with mammalian muscle, and the final postmortem pH is consequently higher. This makes the fish meat more susceptible to microbial attack. However, there is great variation in the glycogen contents of different species; for example tuna has levels comparable with those found in mammals. Rested fish contains more glycogen than exhausted fish and well fed fish more than starved fish. Within the fish, glycogen is more concentrated in the dark muscle than in the white muscle. In stressed fish, glycogen is rapidly depleted.

The lactic acid formed lowers the pH from 7.0 - 7.2 to around 6.2-6.5. In some species the final pH will be 5.8 - 5.6. The decline in pH is accompanied by the natural postmortem stiffening called *rigor mortis. Rigor mortis* begins 0 - 8 hrs. after death and lasts for 10 - 120 hrs. depending on exhaustion, temperature, handling (physical damage) and size of fish. The rigor is resolved after this stage.

2. Flavour changes in fish (Nucleotide degradation)

The most significant enzyme deteriorations are those that affect flavour. The nucleotide degradation in fish muscle produces many flavour bearing compounds. These compounds are formed by the splitting of ATP (adenosine triphosphate) by a series of dephosphorylation and deamination reactions.

ATP —> ADP —> AMP —> IMP —> Inosine —> Ribose —> Hypoxanthine.

Progressively it is hydrolysed to ADP (adenosine diphosphate), AMP (adenosine monophosphate) and to IMP (inosine monophosphate) and ammonia. At ambient temperature, ATP breakdown occurs very rapidly and IMP accumulates in the fish tissue. In fresh fish, the level of IMP is very

high and it imparts a desirable sweet, meaty and characteristic flavour to fish. As autolysis proceeds further, the level of IMP decreases and neutral tasting inosine or bitter tasting hypoxanthine accumulates in the tissue. As a result, fish becomes more insipid. Some of these compounds increase with time and have been used as indices of fish freshness.

3. Belly bursting

Enzymic spoilage causes belly bursting in fish, especially during a period of high food intake. These fishes will have a large content of digestive enzymes in the digestive tract. Such fish will degrade quickly and spoil easily soon after they are caught. In the dissolved gut components, bacteria proliferate and produce gases such as CO_2, and H_2. This gas production leads to belly bursting after short storage period. Keeping the fish live for some time will retard this.

4. Colour changes in fish

Colour is an important factor in seafood quality. Colour change in seafoods is caused by enzymic or non-enzymic action such as fat oxidation or by pigments. Colour change in seafood is an indication of spoilage. The important discolouration in seafoods caused by enzymes or fat oxidation is illustrated below :

a. Black / blue discolouration

Shrimp

The development of black spot in shrimp is due to the presence of an enzyme, polyphenol oxidase (PPO). The black spot (melanin) is formed by the oxidative reaction of tyrosinase on tyrosine. Different species of shrimp undergo postmortem discolouration to different extents. The pigment is formed on the internal shell surfaces, or in advanced stages, on the underlying shrimp meat. This makes the shrimp unattractive for marketing. Ths shrimp phenol oxidase differs from that found in mussel or lobster in that it is not activated by trypsin. Sulphite preservatives can be used to prevent black discolouration resulting from phenolase reaction. Dipping shrimp in 0.2 - 0.5% sodium bisulphite for one minute is usually adopted in industrial practice.

Lobster

Black spot occurrence during icing and frozen storage of lobster is a serious problem which has resulted in significant commerical losses. Tyrosinase activity was identified in the blood with lesser amounts in other tisses. Discolouration at the butt of the tail, black spot between the segments and other deteriorations are more pronounced in moribund losters. The tendency for blackening is also influenced by moulting cycle.

Crab

Phenolases have been implicated in blue-black colouration of crab. The enzyme is present in the blood. Various additives have been shown to prevent blue discolouration (eg : organic acids, sodium bisulphite and EDTA). The haemocyanin induced blackening is also prominent in crab and is non-enzymic.

b. Yellowing of fish flesh

Frozen storage of some fish may result in yellowing of flesh below the skin. Freezing or other processes disrupt chromatophores and release carotenoids and their migration to the subcutaneous fat layer causes yellowing. Yellowing associated with lipid oxidation and carbonyl-amine reaction is observed during frozen storage.

c. Brown discolouration

Brown or yellow discolouration is caused by the reaction of protein or amino acids with product of lipid oxidation. Brown discolouration is observed in a variety of processed products including white pomfret, sardine, jack mackerel, salted shark, marine eel etc. Discolouration due to protein-lipid browning is greater in fatty fish than lean fish.

Microbial spoilage

Fish spoilage is mainly due to the action of bacteria. Bacteria are present on the surface slime, skin, gills and intestine of fish. In dead fish, bacteria begin to invade the tissues causing spoilage and production of undesirable compounds. The type of bacteria on the fish is very much

dependent on the microbial flora of the environment. However, in the fish processing industry, two types of micro - organisms are of concern.

1. Saprophytic or spoilage type bacteria

These organisms are responsible for the spoilage of fish. The important class of spoilage organisms found in tropical species are *Pseudomonas, Flavobacteria, Acinetobacter, Aeromonas* and *Moraxella.* The spoilage bacteria are characterised by their ability to produce H_2S, reduce trimethylamine oxide (TMAO) to trimethylamine (TMA) and convert urea to ammonia. Many volatile sulphur compounds are also produced by *Pseudomonas.* A quantitative measurement of these compounds indicates the degree of spoilage. Fish flesh starts visibly to spoil when bacterial level rises above 10^7 organisms / g.

The flesh loses its culinary qualities like juiciness, firm texture etc. changing it into a product that becomes soft with loss of flavour, discolouration and off flavour. The major deteriorative changes brought about by microorganisms in fish are the following :

a. Formation of ammonia

Spoilage organisms convert many nitrogen compounds into off smelling volatile bases. Non-protein compounds present in fish are good substrate for spoilage organisms. The free amino acid pool in the muscle of fish is readily utilised by typical spoilage organisms by the process of deamination. This results in the formation of ammonia which is the primary compound produced during decomposition of fresh fish. Ammonia is the major component in the total volatile nitrogen (TVN) fraction which often is used as a quality indicator for fresh fish. Urea present in elasmobranchs like sharks and rays is degraded to ammonia by bacterial action. Thus, high level of ammonia in these species is an indication of spoilage.

b. Formation of TMA

Marine fish is characterised by the presence of an odourless compound called trimethylamine oxide (TMAO). Marine flat fish and teleosts contain

low levels of this compound (0.1 - 0.5%), while elasmobranchs (shark, rays etc.) and gadoids contain very high levels (1 - 1.5%). Spoilage bacteria convert this substance into foul smelling trimethylamine (TMA). TMA is produced in fish muscle slowly at first then at a greater speed in fish stored at ambient temperature, in ice or in refrigerated seawater. The fishy odour is produced when it reacts with fat.

c. Histamine formation

Microbial spoilage of fish produces the toxin, histamine in certain fishes. Histamine poisoning or scombroid fish poisoning is very frequent in many countries. Scombroid fishes and other dark muscle fishes contain high levels of free amino acid, histidine, in their muscle. During spoilage, histidine is converted into histamine by bacteria. Over 50 species including popular species such as tuna, bonito, mackerel, blue fish, dolphin fish (Mahi mahi), carangids, herring, sardine and anchovies have shown to be a potential threat of histamine poisoning. Histamine production increases with temperature and 37°C is the optimum temperature for microbial activity. *Morganella morganii, Klebsiella pneumoniae* and *Hafnia alvei* are the main spoilage organisms producing histamine. Low temperature storage, right from catch, reduces histamine production.

d. Indole production

Conversion of tryptophan to indole is another result of amino acid decomposition by bacteria. The FDA uses indole level along with sensory evaluation for measurement of shrimp decomposition.

e. Other compounds formed during bacterial spoilage

A number of other extractives are available in fish for bacterial action such as free amino acids, sugars, peptides, creatine, as well as lipid and proteins. Chemical examination of spoiling fish muscle has shown that organoleptically the most important constituents are the volatile sulphur compounds such as hydrogen sulphide (H_2S), dimethylsulphide $(CH_3)_2S$ and methylmercaptan (CH_3SH). Esters of lower fatty acids such as acetic, propionic, butyric and hexaenoic acids are also produced. Volatile sulphur

compounds influence the organoleptic characters, especially odours, in spoiling fish. The overall qualitative chemical picture of spoiling fish is summarised below :

Substrate	Compounds produced by bacterial action
Inosine	Hypoxanthine
Carbohydrate & Lactate	Acetic acid, CO_2 & H_2O
Methionine & cysteine	H_2S, CH_3SH and $(CH_3)_2S$
Tryptophan	Indole
Glycine Leucine & Serine	Esters of acetic, propionic, butyric and hexaenoic acids
Trimethylamine oxide	Trimethylamine
Urea	Ammonia
Lipids	Carbonyls
Proteins	Tyrosine, indole, skatole, putrescine, cadaverine.
Histidine	Histamine

Some of the spoilage bacteria are proteolytic and undoubtedly contribute to the ammoniacal odour by producing ammonia from the protein breakdown.

2. Pathogenic bacteria

The pathogenic bacteria associated with seafoods are of two types :

a. 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 can cause illness.

Eg. *Clostridium botulinum, Vibrio sp., Aeromonas sp.*

b. Non-indigenous bacteria

They occur in seafood as a result of contamination. The source 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.

Eg. *Salmonella* sp., *Shigella, E. coli* and *Staphylococcus aureus*

Chemical spoilage (oxidation of fish lipids)

Fish lipid is characterised by a high level of polyunsaturated fatty acids (PUFA) and hence undergoes oxidative changes. With fatty fish in particular, fat oxidation gives rise to problems such as rancid flavour and odour as well as discolouration. Lipid oxidation is by two processes (a) Auto oxidation - action of O_2 on the unsaturated fatty acids and (b) Lipid hydrolysis - an enzymatic hydrolysis with free fatty acids (FFA). Oxidative rancidity is of great concern in fatty fish storage. In pelagic species like sardine, mackerel and herring, rancidity has been detected during spoilage. At first, hydroperoxides are formed, which further degrade to form aldehydes and ketones with typical rancid flavour. The oxidation is initiated and accelerated by heat, light (UV-radiation), presence of several organic or inorganic compounds (eg. Cu and Fe), moisture content, large surface area, presence of air etc. Antioxidants such as α tocopherol, ascorbic acid, citric acid or carotenoids can inhibit oxidation.

Factors affecting spoilage

The rate at which spoilage occurs varies with species of fish, sanitary conditions, methods of handling and storage. The rate of spoilage is highly temperature dependent. Increasing the temperature from 0 to $5^{O}C$ doubles the spoilage rate of many species of fish. So, chilling the fish immediately after catch (rapid chilling), careful handling, gutting and maintaining good hygiene will retard the spoilage rate.

Methods of quality assessment

The degree of spoilage or quality can be assessed in four different ways, namely;

Organoleptic assessment

Chemical method

Instrumental method and

Microbiological method

Organoleptic assessment

Sensory method is used throughout the fish processing industry to judge the quality of the product. All visible signs of deterioration can be detected efficiently by sight, smell or touch. With some practice, the whole pattern of changes from very fresh to very spoiled fish can be differentiated easily and rapidly by sensory means. The quality can be assessed either by the dispassionate objective assessment or by the hedonic systems. Numerical score sheet can provide quantitative measurements.

1. Sensory assessment of fresh fish

Fish freshness is usually assessed by the general appearance, raw odour, colour of the gills, condition of the eyes and firmness of the flesh. Appearance of the eyes of bony fish is a good guide to the degree of spoilage. The shiny and brilliant skin in fresh fish becomes dull, with lack of lustre and faded appearance as spoilage progresses. The eyes are bright with black, clear pupil and convex protruded cornea in very fresh fish; the gills are bright blood red and have a fresh seaweedy smell. As the fish spoils the colour of the gills changes to pale red to bleached white often covered with thick mucus. The firm, springy and elastic flesh of very fresh fish becomes soft when spoiled.

a. Scoring and grading for fish freshness

Scoring is the most commonly used method for assessing freshness of chilled fish. The deterioration in fish quality is followed with the aid of a score sheet. Usually, numerical scores are given to

(i) Raw fish - appearance, odour and texture

(ii) Cooked fish - flavour, odour and texture

Freshness grades

Grades have the same meaning as scores. In the EEC scheme of grading fish (chilled fish) four grades are given (E, A, B and C) corresponding to the various stages of spoilage. E is the most fresh and C unfit for human consumption.

b. Quality assessment by taste panel study

Taste panel study is most satisfactory for quality assessment of fish. A hedonic scale or scoring method can be adopted here.

Hedonic Scales

Excellent	Good	Average	Poor	Bad
Like extremely	Like slightly	Neither like nor dislike	Dislike slightly	Dislike extremely

In scoring, a scale from 10 to 0 is used. Samples retaining the odour and flavour typical of the species are given scores above 6 depending on the intensity of sweetness or flavour. Scores of 5 and 4 indicate fish with slight off odour and off flavour. Scores below 4 can be used when strong unpleasant off odour and off flavour develop. The scoring pattern is given below :

Sample preparation

Fish to be tested must be steamed in a closed dish over boiling water for about 20 minutes (time appropriate to the thickness of the piece). It can also be prepared by cooking in a pouch over boiling water.

Scoring system for cooked fish

Fresh sweet flavours characteristic of species	-	10
Some loss of sweetness	-	9
Slight sweetness and loss of the flavour characteristic of the species	-	8
Neutral flavour, difinite loss of flavour but no 'off' flavour	-	7
Absolutely no flavour, as if chewing cotton wool	-	6
Trace of 'off' falvours, some sourness but no bitterness	-	5
Some 'off' flavour and some bitterness	-	4
Strong bitter flavours, rubber-like flavour, slight sulphide like flavour	-	3
Strong bitterness, but not nauseating	-	1
Strong 'off' falvours of sulphides, putrid, tasted with difficulty	-	0

Environment

Testing environment may influence the result. A special taste panel room is normally recommended. The environment shall have

- An odour free room
- Proper lighting / coloured light
- Screened, individual booths.

Chemical methods of quality assessment

In chemical assessment of quality, the various products of spoilage in fish muscle are quantitatively determined and correlated with sensory characteristics. These compounds are produced in fish muscle by autolytic enzymes, putrefactive micro-organisms or by chemical reactions like lipid oxidation. During spoilage, these compounds gradually accumulate in the flesh and hence their determination provides a measure of the progress of spoilage, The compounds found most useful as quality indices are

Volatile bases - Basic nitrogenous compounds such as ammonia, trimethylamine oxide (TMAO), trimethylamine (TMA), dimethylamine (DMA) etc.

Nucleotides - Degradation products from Adenosine triphosphate (ATP) eg:- inosine monophosphate (IMP), hypoxanthine (Hx) etc.

Lipid oxidation products - Peroxides, hydroperoxides, aldehydes etc.

a. Total volatile bases (TVB)

TVB refers to all the volatile basic compounds and comprises mainly TMA and ammonia. The TVB-N value is the most common index of quality used universally for deciding the state of freshness of fish (along with TMA). Some TVB is present even in very fresh fish (<20mg%). Volatile bases are produced by spoiling bacteria.

A level of 35-40mg TVB-N/100 g. of muscle is usually regarded as the limit of acceptability beyond which the fish can be regarded as too spoiled for most uses.

b. Trimethylamine (TMA)

TMA level in fish muscle has been used as a more specific index of bacterial spoilage. A level of 10 - 15mg TMA-N / 100 g. muscle is taken as the limit of acceptability. Its level increases with storage time at ambient temperature, in ice or in refrigerated seawater and hence forms a good index of spoilage.

Determination of TVB-N and TMA-N

TVB-N and TMA-N can be accurately determined by Conway microdiffusion method. Conventional steam distillation method can be adopted for the determination of both TVB-N and TMA-N. Here, the TCA extract of the muscle is made alkaline with NaOH in a steam distillation apparatus and the evolving bases are absorbed in standard acid and determined by titration.

c. Histamine as an index of spoilage :

In fishes like mackerel, tuna, bonito, herring, sardine etc. the production of toxic amine (histamine) is an indication of spoilage.

Histamine along with other amines (eg. Cadaverine) cause high toxicity. Dark fleshed fish have high histidine content and spoilage organisms convert it into histamine. USFDA enforces a maximum level of 50mg / 100g of histamine in fish tissue.

Histamine is determined either by the high performance liquid chromatographic (HPLC) method or by spectroflurometric method.

d. Indole as index of quality

Indole production is an indication of spoilage in shrimp. Indole is currently used by the USFDA to validate the sensory evaluation of shrimp decomposition. Prawns with less than 25 microgram / 100g indole are organoleptically acceptable.

Indole is usually determined by spectroflurometric or spectrophotometric method of the AOAC.

e. Nucleotide based method

Chemical quality tests accurately reflect the edibility or sensory quality of the product. The nucleotide degradation products, especially IMP, Hx or K-value clearly reflect the quality loss in fish. The presence of higher levels of IMP in the muscle indicates relatively high quality, while accumulation of inosine and hypoxanthine is an indication of poor quality.

(i) Hypoxanthine, an index of quality

Hypoxanthine content has been used for evaluating fish quality; the value increases with spoilage in many species of fish. Fish with Hx content more than 2.5 micromoles / g is regarded as spoiled.

Hypoxanthine content can be determined by the silver salt method (Johns, 1968) or by the enzymatic method. Nowadays, the enzyme immobilisation technique and HPLC method are also used to measure Hx content in fish.

(ii) K-value to assess fish quality

K-value which has been defined as the ratio of the sum of inosine and hypoxanthine to total concentration of other nucleotides, has been

regarded as the most appropriate index of fish quality. It is usually expressed as percentage. K-value in fish muscle is determined by the method of Ryder (1985) using HPLC. K. value of very fresh fish is in the range of 20 - 25% and at rejection it is usually above 50 - 60%.

f. Rancidity tests

Peroxide value (PV)

It measures peroxides and hydroperoxides. The most common method is based on iodometric titration which measures the iodine produced from potassium iodide (KI) by the peroxide present in the oil. The PV is a good guide to quality of fat. Fresh oil should have PV 1 milli equivalent O_2/kg; on storage it may increase up to 10 milli equivalent/kg.

The oil from fish muscle is extracted with chloroform after dehydrating the muscle with anhydrous Na_2SO_4. The solution is filtered. Ten ml of the chloroform extract is taken in a conical flask and mixed with 3-5 ml of aldehyde free acetic acid. One ml of saturated KI solution is added and the solution kept in the dark for 10 minutes. It is then titrated against standard (0.002 N) thiosulphate solution using starch as indicator.

PV = ml of 0.002 N thio/g. Fat.

Thio - barbituric acid value (TBA value)

TBA measures the malonaldehyde produced during fat oxidation. TBA reacts specifically with malonaldehyde to give a red chromogen which can be determined spectrophotometrically. The test can be carried out in two ways, either directly in food, followed by steam distillation and the distillate allowed to react with TBA reagent or by preparing an extract of the muscle followed by colour development. The absorbance of the red coloured pigment is measured at 538 nm against a reagent blank. The TBA number is then calculated as mg. of malonaldehyde per kg. of sample, which is equal to 7.8 times the optical density (O.D).

TBA number = O.D. x 7.8

The PV is a measure of the first stage of oxidative rancidity and TBA value, the second. It can be said that if the PV is above 10 - 20 or TBA value above 1 - 2, then the fish will, in all probability, smell and taste rancid.

Instrumental method for assessing seafood quality

a. Freshness meters

Based on the changes taking place in the electrical properties of fish muscle (such as conductance and capacitance) a freshness meter has been developed at Torry Research Station (U.K.) known as Torrymeter (TM) which has readings from 0 to 16. A similar meter with a wider range (0 to 100) has been developed in Germany, known as Intelectron Fish Tester (IFT). These meters give quick and reliable indication of fresh fish quality in tropical fish. In GR Torrymeter, highest value (16) is obtained for very fresh fish and the readings decrease with spoilage and is characteristic of the species. The RT-Freshness Grader developed by Iceland is a commercial success in this series. The grade measurement is faster and correlates well with sensory (odour) and chemical (TMA-value) assessment of freshness.

b. Texture measurement

The texture of the fish is often a good measure of the quality and might be determined most often in shear or compression test. The Universal Testing Machine (UTM) and other commercial texturometers (RHEO TEX, Japan) are used to measure objective textural quality.

Microbial methods

Bacteria largely determine the quality of fresh and lightly preserved fish products. The amount of bacteria in foods serves as a general indicator of hygiene. Determination of total bacterial count is widely used to assess the bacterial quality of fish. In freshly landed fish / shrimp, total bacterial count is in the range of 10^3 - 10^6 and when the levels rise above 10^7 organisms/g, fish flesh starts visibly to spoil.