# Biochemical indices of seafood quality & determination of adulterants in seafood

Laly S. J.* and Priya E. R.
Quality Assurance and Management Division
ICAR- Central Institute of Fisheries Technology, Cochin
*lalyjawahar@gmail.com

## Introduction

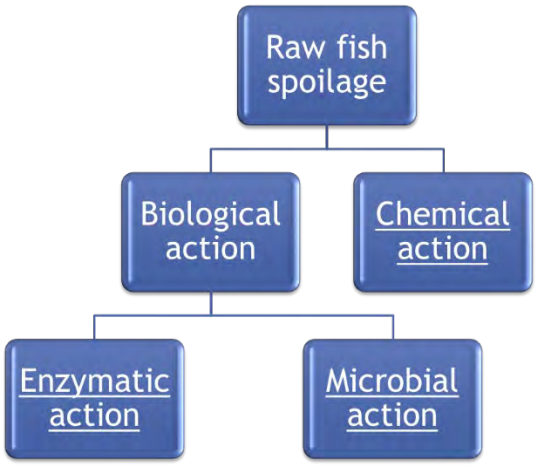
Fish and other seafood are highly important as they cover a part of protein demand for humans. The nutrient composition of fish is rich in health beneficial polyunsaturated fatty acids, vitamins and minerals. Fresh fish spoilage can be very rapid after it is caught. Freshness makes a major contribution to the quality of fish and fishery products. Nutritional values, color, texture, and edibility of foods are susceptible to spoilage. Improper pre and post-harvest handling conditions can enhance exacerbation of indigenous bacteria that could cause spoilage of fish.

Freshness is the most important attribute when assessing the quality of seafood and is of great concern. The quality of seafood degrades after death due to the chemical reactions [changes in protein and lipid fractions, the formation of biogenic amines and hypoxanthine (Hx)] and microbiological spoilage. This leads to the deterioration of sensory quality of seafood during inadequate storage. The factors contributing to spoilage of fish are

- High fat content
- High protein content
- High moisture content
- Weak muscle tissue
- Extent of bacterial contamination
- Unhygienic handling etc.

## Spoilage of fish

Seafood is highly perishable food commodity and spoilage of fish involves three separate processes such as enzymatic spoilage, bacterial spoilage and chemical decomposition. "Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture."



Spoilage of fish is also called “Putrefaction”. It refers to the contamination of fish, resulting in an undesirable change in the colour, texture, flavour, odour, appearance, etc.

**Enzymatic spoilage**

Shortly after capture of fish chemical and biological changes take place in dead fish due to enzymatic breakdown of major fish molecules. The changes textural quality during early stages of deterioration but did not produce the characteristic spoilage off-odors and off-flavors. The digestive enzymes cause extensive autolysis which results in meat softening, rupture of the belly wall and drain out of the blood water which contains both protein and oil. During improper storage of whole fish, proteolysis is responsible for degradation of proteins and is followed by a process of solubilization. Belly bursting is caused by leakage of proteolytic enzymes from pyloric caeca and intestine to the ventral muscle.

Table 1. Enzymes involved in spoilage of fish

| Enzyme(s) | Substrate | Effect | Prevention |
|---|---|---|---|
| Glycolytic enzymes | Glycogen | Lactic acid production resulting in pH drop. | Avoid pre-rigor stress |
| Autolytic enzymes involved in nucleotide breakdown | ATO, ADP, AMP, IMP | Gradual production of Hypoxanthine | Avoid pre-rigor stress and improved handling. |
| Cathepsins | Proteins, peptides | Softening of tissue | Avoid rough handling during storage |
| Chymotrypsin, trypsin, carboxy-peptidases | Proteins, peptides | Belly-bursting | Problem increased with freezing/ thawing or long-term chill storage |
| Calpain | Myofibrillar proteins | Softening | Removal of calcium |
| Collagenases | Connective tissue | Softening and gaping of tissue | Time and temperature of chilled storage |
| Trimethylamine Oxide (TMAO) demethylase | TMAO | Formaldehyde | Storage temperature less than -30°C, physical abuse, freeze/thawing |

*FAO 2005

**Oxidative spoilage**

Lipid oxidation is a major cause of deterioration and spoilage for the pelagic fish species such as mackerel and herring with high oil/fat content stored fat in their flesh. Fish lipids which consist of polyunsaturated fatty acids are highly susceptible to oxidation. Lipid oxidation involves a three-stage free radical mechanism: initiation, propagation and termination. Initiation involves the formation of lipid free radicals through catalysts such as heat, metal ions and irradiation. This free radical which reacts with oxygen to form peroxyl radical. During propagation, the peroxyl radicals reacting with other lipid molecules to form hydroperoxides and a new free radical. Termination occurs when a buildup of these free radicals interacts to form non-radical products. In fish, lipid oxidation can occur enzymatically or non-enzymatically. Enzymatic hydrolysis by lipases is called as lipolysis (fat deterioration) in which lipases split the glycerides forming free fatty acids resulting off flavor. Non-enzymatic oxidation is caused by hematin compounds (hemoglobin, myoglobin and cytochrome).

**Microbial spoilage**

Composition of the micro flora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish micro flora includes bacterial species such as *Pseudomonas*, *Alcaligenes*, *Vibrio*, *Serratia* and *Micrococcus*. Microbial growth and metabolism are a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors. For unpreserved fish, spoilage is a result of Gramnegative, fermentative bacteria (such as *Vibrionaceae*), whereas psychrotolerant Gram-negative bacteria (such as *Pseudomonas* spp. And *Shewanella* spp.) tend to spoil chilled fish.

**Methods of Assessing Freshness Quality**

**Sensory methods**

Sensory evaluation is the most important method in freshness assessments. Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze, and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch, and hearing. Sensory evaluation provides rapid measurements of freshness of seafood.

Freshness makes a major contribution to the overall quality of fish and fishery products and is greatly influence by both pre-harvest conditions and post-harvest handling practices. There are sensory and Non-sensory or instrumental methods are available. Non-sensory methods include chemical, physical and microbiological methods. Non-sensory assessment is based mainly on measuring major physical or chemical alterations from the original condition of the fish.

Sensory responses can be variously measured and can be assigned to sensory impression in different ways: nominal data, ordinal data, interval data and ratio data. In sensory evaluation of seafood, grading, ranking and scaling methods are the most frequently used methods. However, difference tests can be relevant to use in selected cases. Grading is a useful method of evaluation and is often used in commerce. It depends on one or two product experts. Graders usually learn the scale from other graders. The EU-scheme is an example of a grading scheme. In ranking, three or more samples are arranged in order of intensity or degree of some specific attribute. A category scaling is a method where the panellists are asked to rate the intensity of a particular stimulus by assigning a value on a limited numerical scale.

***Torry scale***

The first scoring method for use with fish and fishery products was developed at the Torry Research Station in the UK. The Torry scale is a 10-point scale originally developed to assess the eating qualities of cooked fish samples. Scores are given from 10 (very fresh in taste and odour) to 3 (spoiled). Scores below a 3 are considered unnecessary, as the fish is then not fit for human consumption. The average score of 5.5 may be used as the limit for consumption. The Torry scale has been developed for lean, medium fat, and fatty fish species.

***European Union Scheme***

In EU scheme there are three quality levels in which E (Extra) is the highest quality; A is acceptable quality; and B is the level beyond which fish are not admitted for human consumption. The EU scheme is criticized for its limitations in that it does not consider the differences between species (uses only general parameters) and mixes both subjective and objective sensory methods in the assessment scheme.

***Quality Index Method***

The QIM was developed at the Tasmanian Food Research Unit (TFRU) of the Commonwealth Scientific and Industrial Research Organization (CSIRO) QIM schemes are developed for individual species. Each attribute is scored from 0 to 3 by novice or experienced assessors with low scores indicating the best quality. The sum of all attributes is called demerit points, or QIM index points. This value increases linearly with storage time in ice of a given fish Using the QIM system, the linear relationship between the quality index (QI) and storage time on ice, makes it easy to calculate the remaining shelf-life of fish.

**Biochemical indices**

Measuring the concentration of indicator compounds within the sample, which are closely related to the level of a specific sensory attribute of the fish (primarily odor or flavor). These

compounds are produced by autolytic enzymes, putrefactive microorganisms or by chemical reactions like lipid oxidation. These compounds accumulate gradually in the flesh and are found most useful quality indices and they include

- **Volatile bases** – Ammonia, Trimethylamine oxide (TMAO), trimethylamine (TMA), Dimethylamine (DMA) etc
- **Nucleotides** – Degradation products of ATP
- **Lipid oxidation** – Peroxides, hydroperoxides, aldehydes etc.

**Total volatile base nitrogen (TVBN)**

TVBN is a useful index of spoilage in different fresh and lightly preserved seafood. Most widely used method for assessing fish quality. TVB contains ammonia, trimethylamine (TMA) and dimethylamine (DMA). TVBN along with TMA is the most common index of spoilage of fish. In case of very fresh fish TVBN is < 20 mg %. A range of 35 – 40 mg TVB-N / 100 g of fish muscle is usually considered as limit of acceptability, beyond which the fish can be regarded as spoiled. TVB-N values identify the latter stages of spoilage as limited significant changes during the early stages of spoilage. Conway microdiffusion method and steam distillation method are commonly used method for estimation TVBN.

**Trimethylamine (TMA)**

TMA is a microbial metabolite and it can only be used as an index of spoilage and not as an index of freshness. Marine fish is characterized by the presence of an odourless compound called trimethylamine oxide (TMAO). TMAO appears to be part of the system used for osmoregulation. Th e TMAO content of seafood varies with species, age, fi sh size, time of year, and environmental factors [152]. Seawater fish have 1–100 mg TMAO in every 100 g muscular tissue, whereas freshwater fish generally contain only 5–20 mg %. TMA is produced by reduction of spoilage bacteria from Trimethylamine oxide (TMAO). Limit of acceptability in case of TMA is 10 – 15 mg%. Fresh fi sh has a very low amount of TMA with values less than 1.5 mg TMA/100 g TMA values increases with storage temperature. With different initial levels of TMAO, trimethylamine accumulates at different rates in different species. Trimethylamine usually does not indicate a change in quality until the fish have been stored in ice for approximately 6-10 days. This indicator is primarily suitable for evaluating samples of medium to poor freshness quality. Trimethylamine (TMA) levels are used universally to determine microbial deterioration leading to fish spoilage.

**Dimethylamine (DMA)**

TMAO is degraded to form dimethylamine (DMA) and formaldehyde by the enzyme TMAO demethylase in the absence of oxygen. DMA increase at a constant rate, even during the first few days of iced storage and, therefore, is a superior chemical indicator of freshness quality and is restricted to cod-like species and hakes, which contain TMAOases in their muscle tissue. There is no effect on the flavor or texture of the fish. It gives an indirect indication of formaldehyde-induced toughening of the muscle during frozen storage.

**Ammonia**

Ammonia is formed by the bacterial degradation/deamination of proteins, peptides and amino-acids. Significant increase in ammonia content occurs only after spoilage. Urea present in sharks & rays is degraded to ammonia by bacterial action. Thus, high level of ammonia in these species is an indication of spoilage.

**Nucleotide degradation**

One of the most extensively investigated methods of measuring odor and flavor aspects of the freshness quality of fish. ATP (Adenosine tri phosphate) is degraded into ADP (adenosine diphosphate), AMP (adenosine monophosphate), IMP (Inosine monophosphate), Ino (Inosine) and Hx (Hypoxanthine) during processing and storage of fresh and lightly preserved seafood. IMP is formed by autolytic enzymes. Spoilage bacteria contribute to Ino and Hx formation. Hx has a bitter taste which may be part of the off-flavor in stale fish. K value is an excellent index of freshness. In most fish, K-values increase linearly during the first days of chilled storage.

$$\text{K value (\%)} = \frac{(HxR+Hx)}{(ATP+ADP+AMP+IMP+HxR+Hx)} \times 100$$

K value is usually expressed as %. K value of Very fresh fish is 20 – 25 %. At rejection, the value will be above 50-60 %. ATP, ADP and AMP are almost completely converted to IMP within 24 hours post-mortem.

k1 value = [Ino + Hx]/ [IMP + Ino + Hx]

K value increase varies considerably between fish presumably due to differences in the species-dependent optimum pH of IMP-degrading enzymes. In general, K value increases more rapidly in cold-water fish. Increase has been observed to be five times faster in dark than in white muscles It may depend upon the location of fillet and is strongly influenced by the physiological effects of fish harvest and death struggle.

**Hypoxanthine content**

Hypoxanthine content is used for evaluating fish quality and the value increases with spoilage. In case of spoiled fish, the value will be > 2.5 micromoles/g.

**Histamine**

Many seafood spoilage bacteria produce one or more of the biogenic amines agmatine, cadaverine, histamine, putrescine, spermidine, spermine, and tyramine. Production of biogenic amines in seafood depends on concentrations of the free amino acid substrates and is, therefore, strongly species dependent. In fishes like mackerel, tuna, bonito, herring sardine etc histamine formation is an indication of spoilage. Dark fleshed fish will have high histidine content and will be converted to histamine by bacteria namely *Morganella morganii*, *Klebsiella pneumonia*, *Hafnia alvei*. Histamine is heat stable biogenic amine and cadaverine & putrescine act as potentiators of histamine formation. As per USFDA guideline the toxicity and defect action level established are 50 mg/100g and 5 mg/100g respectively. According to EU regulation No 2073/2005 mean value all samples (nine) must not exceed 10 mg/100g, two samples may be > 10 mg/100g but < 20 mg/100g and no sample may exceed 20 mg/ 100g. According to USFDA guideline for the control of histamine production a core temperature of 4.4 ºC or less should be achieved and maintained throughout handling, processing and distribution of susceptible species.

**Indole**

Indole is a useful freshness index of non-frozen shrimp which is produced by degradation of tryptophan by microbial enzymes. High level of indole indicates decomposed shrimp and temperature abuse is not toxic at high level. As per USFDA the acceptable limit is <25 microgram/100g.

**Lipid oxidation indices**

Compounds derived from the oxidation of the highly unsaturated fatty acid moieties in fish lipids have been used to quantify the extent of oxidative rancidity. The major chemical indices of oxidative rancidity are peroxide value (PV) and thiobarbituric acid-reactive substances (TBA-RS).

***Peroxide value***

The primary oxidation products (peroxides and hydroperoxides) are estimated by peroxide value and is a good guide to quality of fat. PV is a measure of first stage of oxidative rancidity. When peroxide value is > 10 – 20 milliequivalent Oxygen/Kg it smells and tastes rancid.

***TBARS***

The secondary oxidation products comprise carbonyl compounds yielding the fishy and rancid character associated with oxidized fish lipid. It measures malonaldehyde produced during fat oxidation. TBA react with malonaldehyde and gives a red chromogen and is measured spectrophotometrically. TBA above 1-2 mg malonaldehyde /Kg fat indicates rancidity.

**Free fatty acid value**

Free fatty acid value is a measure of hydrolytic rancidity. Prior to the appearance of oxidative rancidity in lean fish, there is rise in lipid hydrolysis that leads to build up of FFA. It is non-esterified fatty acids in “free” form. It is more readily oxidized than esterified fats. FFA can act as pro-oxidants in oils by speed up the rate of hydroperoxide decomposition. Thus, high FFA content in the oil may cause further oxidation and lead to development of offensive taste and flavor in the Fish.

**Total volatile acids**

Total volatile acids include formic acid and acetic acid formed during spoilage. It is formed only after putrefaction and can be used as a quality index. In case of fresh muscle, the content is low and it increases rapidly after a few days in ice. TVA content cannot increase or decrease during canning process hence can be used for checking quality of canned raw material.

**pH**

Natural pH of live fish is above 7 and (typically 7.3). pH Falls after death as it goes through rigor and glycogen is converted to lactic acid. Post mortem pH is 6-6.8 in case of most species. In Tuna, it is below 6, due to high initial glycogen level. pH increases as the spoilage progresses.

**Determination of adulterants in seafood**

There are so many components of food safety that still remain unattended, and make a major concern to consumer health. Economically motivated adulteration is such an activity. For thousands of years, fish is considered a healthy human diet due to its rich protein content, vitamins, minerals, and highly unsaturated fatty acids. Since fresh fish is highly perishable in nature, there is an emerging risk of economically motivated adulteration to enhance the keeping quality of fish. India’s domestic fish market is reported to be selling formaldehyde adulterated fishes, especially in markets located far away from landing centers or production sites. According to Indian and International regulations, fresh fish and shellfish should be preserved only by means of ice made out of potable quality water. The use of substances other than ice to extend the keeping quality is a fraudulent practice. Apart from direct application of

adulterants, even adding ammonia like substance during ice manufacture to slow down the melting of ice or to cut down the cost of ice, or even adding approved preservatives of the processed commodity such as benzoate, to fresh fish to control the microbial activity are illicit in nature and have potential to cause health problems to consumers.

**Formaldehyde**:

Formaldehyde is a colourless, flammable, strong-smelling chemical, well known for its preservative and anti-bacterial effects. This chemical is generally used in building materials and to produce household products, in pressed-wood products, glues, and adhesives, paper product coatings, as an industrial fungicide, germicide, and disinfectant, and as a preservative in mortuaries and medical laboratories. Formaldehyde also occurs naturally in the environment. It is produced in small amounts by most living organisms as part of normal metabolic processes. Short-term health effects of formaldehyde exposure include watery eyes, burning sensations in the eyes, nose, and throat, coughing, wheezing, nausea, and skin irritation. In 1987, the U.S. En vironmental Protection Agency (EPA) classified formaldehyde as a probable human carcinogen under conditions of unusually high or prolonged exposure. The International Agency for Research on Cancer (IARC) also classified formaldehyde as a human carcinogen.

**Ammonia**:

Ammonia is a naturally occurring chemical in the atmosphere, as well as a synthetically made chemical. At room temperature, ammonia is a colourless, pungent-smelling gas and is lighter than air. Ammonia is an essential element for the plant, animal, and human life. It is found in water, soil, and air, and is a source of much-needed nitrogen for plants and animals. Ammonia is also present in fertilizers, power plants, mobile sources, and other manufacturing emissions. Ammonia levels in the air at 5 ppm can be recognized by odor. An average person detects ammonia by odor at around 17 ppm. Continuous ingestion of ammonia can lead to many health issues like the mucous membrane of the mouth, throat, esophagus, and stomach. Ammonia readily dissolves in water and forms ammonium hydroxide, and the ingestion of ammonium hydroxide can result in corrosive damage to the mouth, throat, and stomach.

**Benzoate**:

Benzoate is a white solid that is slightly soluble in water. Benzoic acid and sodium benzoate are used as food preservatives and are most suitable for foods, fruit juices, and soft drinks that are naturally in an acidic pH range. Their use as preservatives in food, beverages, toothpaste, mouthwashes, dentifrices, cosmetics, and pharmaceuticals is regulated. Sodium benzoate is a permitted additive used in processed fish and fishery products to inhibit the growth of mold, yeast, and many bacteria. The acceptable daily intake (ADI) fixed by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) for sodium benzoate is 0-5 mg/Kg body weight and the maximum allowable limit is 0.1% as per EU regulations. Benzoic acid is slightly irritating to the skin and irritating to the eye, while sodium benzoate is not irritating to the skin and is only a slight eye irritant. In humans, the acute toxicity of benzoic acid and sodium benzoate is low. However, both substances are known to cause non-immunological contact reactions.

**"CIFTest"- Rapid detection kits for detection of adulteration of formaldehyde and ammonia in fresh fish**

The ICAR-CIFT developed two different kits containing chemically treated paper strips that can react with the adulterant – Formaldehyde/Ammonia present in the tissue of the fish. Adding one drop of reagent solution to the swabbed paper strip can result in colour development within one minute to a maximum of 2 minutes. The development of blue colour indicates the adulteration of fish with formaldehyde/ammonia. With the use of CIFTest kits, the illicit use of chemical substances like Formaldehyde and ammonia in fresh fish can be effectively controlled being the consumers are empowered to check the commodity they are purchasing. ICAR-CIFT had transferred the CIFTest technology to M/s HIMEDIA Laboratories Pvt. Ltd., Mumbai and it is commercially available in India under the trade names- HiRapid Formalin test kit (for fish) and HiRapid Ammonia test kit (for fish).