SPOILAGE INDICES OF FISH AND SHELLFISH

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1. INTRODUCTION:

Fish is a highly perishable commodity. Spoilage begins as soon as the fish dies. In tropical conditions, fish spoils quit rapidly, within a few hours of fish landings, if it is not properly preserved at low temperature. Chemically, fish and shellfish have four major components: proteins, lipids, carbohydrates, and moisture. The relative proportions of all these components give fish and shellfish their characteristic structure, flavor, texture, color, and nutritional value. Besides these main classes of compounds, there are other minor components such as nucleotides and other non-protein nitrogen compounds, some of which are important in the spoilage process. Once fish dies, microorganisms present in the gills, gut, and skin, in conjunction with the activities of endogenous enzymes, begin to metabolize the above compounds. Compositional changes during fish spoilage result in lipid oxidation and protein degradation as well as the loss of other valuable molecules, resulting in off-flavors, texture deterioration, discolorations, and other changes characteristic of fish spoilage. Off odours and off flavour, slime, gas production, discolouration and soft texture are the obvious sign of fish spoilage.

In addition, the following factors contribute to spoilage of fish,

- High moisture content
- High fat content
- High protein content
- Weak muscle tissue
- Extent of bacterial contamination
- Ambient temperature
- Unhygienic handling
- Rigor mortis hastened – struggling of fish, lack of oxygen, warm temp.
- Use of an antibiotics, ice or dip.

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. The rate of spoilage is temperature dependent and lowing the temperature will reduce the spoilage rate. Also, the rate of spoilage of fish may be reduced by following good handling practices.

2. QUALITY AND FRESHNESS:

Most often "quality" refers to the aesthetic appearance and freshness or degree of spoilage which the fish has undergone. It may also involve safety aspects such as being free from harmful bacteria, parasites or chemicals. It is important to remember that "quality" implies different things to different people and is a term which must be defined in association with an individual product type. Therefore, quality can be defined as the degree of excellence to which a product meets all of the attributes, characteristics and features that the buyer or consumer of the product, and the regulatory agencies expect. Consequently, quality as related to seafood involves availability, safety (chemical and microbiological), convenience, freshness, integrity, and nutritional value.

Freshness, defined in terms of odor, flavor, texture and appearance, makes a major contribution to the overall quality of fish. Today, food safety remains a major concern facing the seafood industry, and it is a critical component in ensuring food and nutrition security worldwide. The production and consumption of safe food are central to any society, and they have a wide range of economic, social and, in many cases, environmental consequences. Chemical deterioration and microbial spoilage are responsible for loss of 25% of gross primary agricultural and fishery products every year (Baird-Parker, 2000). One-fourth of the world's food supply (Huis in't Veld, 1996) and 30% of landed fish (Amos, 2007) are lost through microbial activity alone. Fish lose due to spoilage is estimated to be 10 to 12 million tons per year which accounts 10% of total production of fish (Getu *et al*., 2015).

The relationship between quality and freshness is depicted in Figure 1. Quality is a function of freshness; freshness is essential for quality but it is not a priori a quality factor. The 'quality' circle

comprises the factors that contribute to quality, and the 'freshness' circle details the various approaches used to evaluate fish freshness.

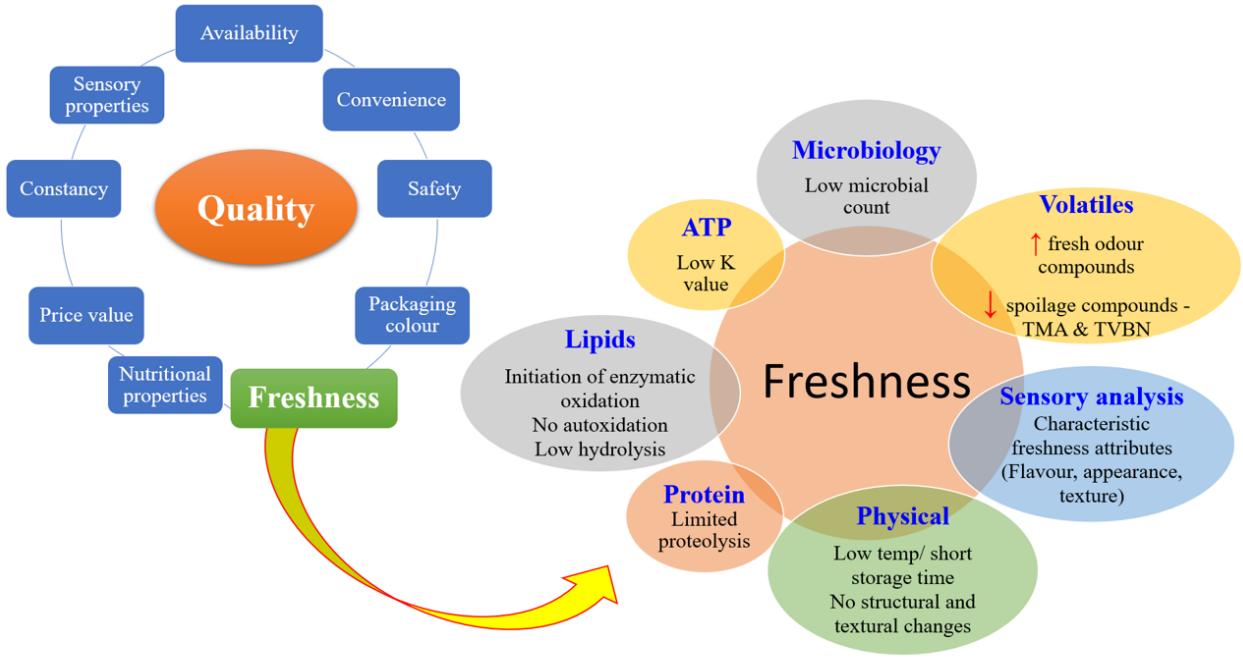


Figure 1. Relationship between quality and freshness (Olafsdbttir *et al*., 1997)

3. FISH SPOILAGE:

In raw fish, spoilage takes place mainly due to three reasons namely,

1. Enzymatic Spoilage
2. Bacterial Action
3. Chemical Decomposition – oxidation

Enzymes and bacteria do not cause any deteriorative changes in living cell due to the natural defensive mechanism.

3.1. Enzymatic Spoilage:

Shortly after capture, chemical and biological changes take place in dead fish due to enzymatic breakdown of major fish molecules. Autolytic spoilage is responsible for early loss of quality of fresh fish. Autolytic enzymes reduced textural quality during early stages of deterioration but did not produce the characteristic spoilage off-odors and off-flavors. This indicates that autolytic

degradation can limit shelf-life and product quality even with relatively low levels of spoilage organisms. The autolytic Changes in Chilled Fish are presented in Table 1. (FAO, 2005). The first enzymatic changes in fish mussel is the gradual hydrolysis of glycogen to lactic acid which is known as glycolysis. On the other hand, peptides and free amino acids can be produced as a result of autolysis of fish muscle proteins, which lead towards the spoilage of fish meat as an outcome of microbial growth and production of biogenic amines (Fraser and Sumar, 1998). During improper storage of whole fish, proteolysis is responsible for degradation of proteins and is followed by a process of solubilization. A number of proteolytic enzymes are found in muscle and viscera of the fish after catch. These enzymes contribute to post mortem degradation in fish muscle and fish products during storage and processing. There is a sensorial or product associated alteration that can be contributed by proteolytic enzymes.

Enzymatic spoilage causes bally bursting in fish, especially during a periods of high food intake. These fishes will have a large contain of digestive enzymes in the digestive tract. Such fish will degrade quickly and spoil easily soon after they are caught. Belly bursting is caused by leakage of proteolytic enzymes from pyloric caeca and intestine to the ventral muscle. 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. The rate of degradation by proteolytic enzymes was reduced when the fish was kept at 0°C.

Table 1. Autolytic Changes in Chilled Fish (FAO, 2005)

Enzyme(s)	**Substrate**	**Changes Encountered**	**Prevention/Inhibition**
Glycolytic enzymes	Glycogen	Production of lactic acid, pH of tissue drops, loss of water-holding capacity in muscle. High temperature rigor may result in gaping	Fish should be allowed to pass through rigor at temperatures as close to 0°C as practically possible. Pre-rigor stress must be avoided.
Autolytic enzymes, involved in nucleotide breakdown	ATP ADP AMP IMP	Loss of fresh fish flavour, gradual production off bitterness with Hx (later stages)	Same as above Rough handling or crushing accelerates breakdown

Cathepsins	Proteins, Peptides	Softening of tissue making processing difficult or impossible	Rough handling during storage and discharge
Chymotrypsin, trypsin, carboxy-peptidases	Proteins, Peptides	Autolysis of visceral cavity in pelagics (belly- bursting)	Problem increased with freezing/thawing or long- term chill storage
Calpain	Myofibrillar proteins	Softening, molt-induced softening in crustaceans	Removal of calcium thus preventing activation
Collagenases	Connective tissue	Gaping of fillets softening	Connective tissue degradation related to time and temperature of chilled storage
TMAO demethylase	TMAO	Formaldehyde-induced toughening of frozen gadoid fish	Store fish at temperature <= -30°C physical abuse and freezing/thawing accelerate formaldehyde-induced toughening

3.2. 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 (Figure 2). Oxidation typically involves the reaction of oxygen with the double bonds of fatty acids. Chain reaction (initiation) involves the production of lipid free radicals through catalysts (such as heat, metal ions and irradiation) by removal of a hydrogen atom from the alpha methyl group. Production of hydroperoxide takes place in the propagation sequence of reaction. In this reaction, free radicals react with oxygen to form peroxyl radicals, which in turn react with other lipid molecules to form hydroperoxides and a new free radical. Which can initiate chain of event again. Termination occurs when peroxide decompose or when a buildup of these free radicals interacts with one another or one other oxidation products resulting in formation of non-radical products such as carboxylic

acid, carbonyl compounds and condensation products. These end products are responsible for rancid odour and flovour.

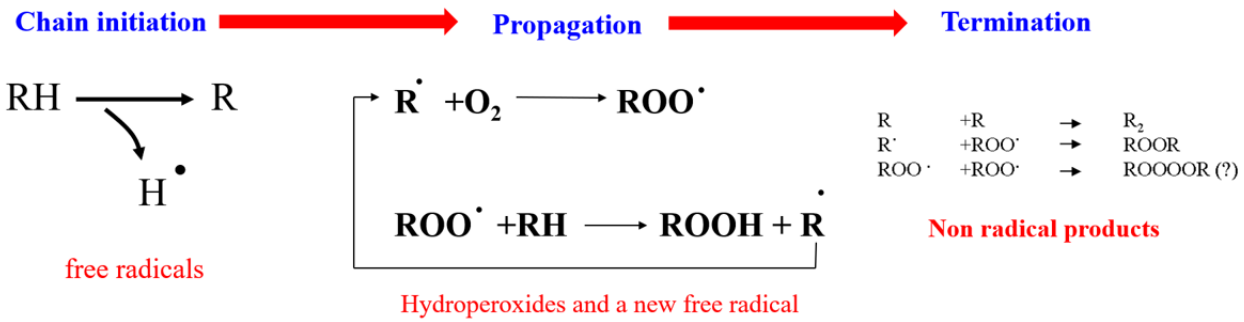


Figure 2. Three-stage free radical mechanism of lipid oxidation

In fish, lipid oxidation can occur enzymatically or non-enzymatically. The enzymatic hydrolysis of fats by lipases is termed lipolysis (fat deterioration). During this process, lipases split the glycerides forming free fatty acids which are responsible for: (a) common off flavour, frequently referred to as rancidity and (b) reducing the oil quality. While, non-enzymatic oxidation is caused by hematin compounds such as hemoglobin, myoglobin and cytochrome. The fatty acids formed during hydrolysis of fish lipids interact with sarcoplasmic and myofibrillar proteins causing denaturation. Lipid oxidation can occur in fish muscle due to the highly pro-oxidative Hemoglobin (Hb), specifically if it is deoxygenated and/or oxidized.

3.3. Microbial Spoilage:

Composition of the microflora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish microflora 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. The compounds formed during spoilage through microbial metabolism are listed in Table 2.

Table 2. Bacterial spoilage compounds (Church, 1998)

Specific Spoilage bacteria	Spoilage compounds
Shewanella putrifaciens	TMA, H_2S, CH_3SH, $(CH_3)_2S$, HX
Photobacterium phosphoreum	TMA, HX
Pseudomonas spp	Kenones, aldehudes, esters, non-H2S sulphides
Vibrionacaea	TMA, H_2S
Aerobic spoilers	NH_3, actic, butyric and propionic acid

4. METHODS OF ASSESSING FISH FRESHNESS AND QUALITY:

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. The methods for evaluation of fresh fish quality may be conveniently divided into two categories. They are: Sensory method of quality assessment and non-sensory or instrumental method of quality assessment. Non-sensory assessment of freshness and quality of fish includes 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.

4.1. METHODS FOR SENSORY EVALUATION OF FISH:

In sensory analysis, the scientific means of quantifying and interpreting the variations in the sensory characteristics of food such as appearance, odour, flavour and texture are evaluated through the human senses of sight, smell, taste, touch and hearing. Most sensory characteristics can only be measured meaningfully by humans. However, advances are being made in the development of instruments that can measure individual quality changes. With some practice, the pattern of changes in sensory characteristic between very fresh and very spoilt food can be easily and quickly by sensory means and the degree of freshness can be accurately determined. Seven quality factors are the most important and reliable in the Organoleptic examination of fish factors,

1. General appearance

2. Appearance of flesh
3. Texture of raw fish
4. Odour of raw fish
5. Odour of cooked fish
6. Flavour of cooked fish
7. Texture of cooked fish

Application of sensory analysis includes quality control of raw materials and finished products, storage teats, development of new products, tand off flavour, aroma research, consumer test and hedonic test. There are two kinds of assessment generally followed,

a) Organoleptic testing (Subjective method) and
b) Sensory testing (Objective method)

4.1.1. Grading Schemes:

Grading is the process of applying a categorical value to a lot or group of fish and fishery products. Grading has the advantage that it offers the possibility of selecting products for different qualities. There are several grading methods used to assess freshness in fish and fish products such as:

a) The torry scoring system
b) The European Union schemes
c) The quality index method

The Torry Scoring System:

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. Scores are given from 10 (for very fresh in taste and odour) to 3 (for spoiled fish) (Table 3). 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.

Table 3. Sensory score sheet for Cod (cooked) from gutted fish stored in melting ice

Score	Odour	Flavour	Texture, mouth feel and appearance	Score
10	initially weak odour of sweet, boiled milk, starchy, followed by strengthening of these odours	watery, metallic, starchy; initially no sweetness but meaty flavours with slight sweetness may develop	dry, crumbly with short tough fibres	**10**
9	shellfish, seaweed, boiled meat, raw green plant	sweet, meaty, creamy, green plant, characteristic		**9**
8	loss of odour, neutral odour	Sweet and characteristic flavours but reduced in intensity	succulent, fibrous; initially firm going softer with storage; appearance originally white and opaque going yellowish and waxy on storage.	**8**
7	wood shavings, woodsap, vanillin	neutral		**7**
6	condensed milk, caramel, toffee-like	insipid		**6**
5	milk jug odours, boiled potato, boiled clothes-like	slight sourness, trace of 'off' flavours		**5**
4	lactic acid, sour milk, 'byre-like'	slight bitterness, sour, 'off' flavours		**4**

3	lower fatty acids (eg acetic or butyric acids), composted grass, soapy, turnipy, tallowy	strong bitter, rubber, slight sulphide	**3**

European Union Schemes:

In this scheme, three grades of freshness are established: E, A and B, corresponding to various stages of spoilage. E (Extra) is the highest possible quality; A is acceptable quality; while below B is the level where fish is considered unfit for human consumption (Table 4). This method gives rather limited information about the condition of the fish, as it is not species-related and does not take into account the differences between species. The EU-scheme is commonly accepted at auction levels however its use has been disputed.

Table 4. Criteria of EU schemes

	CRITERIA			
	Freshness Category			**Not Admitted**
	Extra	**A**	**B**	
Skin	Bright, iridescent pigment or opalescent, no discolouration	Pigmentation bright but not lustrous	Pigmentation in the process of becoming discoloured and dull	Dull pigmentation
Skin mucus	Aqueous, transparent	Slightly cloudy	Milky	Yellowish, grey, Opaque mucus
Gills	Bright colour, no mucus	Less coloured, transparent mucus	Brown/green becoming discoloured, thick opaque mucus	Yellowish, milky mucus
Peritoneum on gutted fish	Smooth, bright, difficult to detach from flesh	Slightly dull, can be detached from flesh	Speeckled comes away from flesh	Does not stick

Smell of gills and abdominal activity	Seaweedy smell	No smell of seaweed, neutral smell	Fermented, slightly sour	sour
Flesh	Firm and elastic, smooth surface	Less elastic	Slightly soft, less elastic	Soft, scales easily detached from skin, surface rather wrinkled.

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 (Table 5). 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, therefore, the linear relationship between the quality index (QI) and storage time on ice, makes it easy to calculate the remaining shelf-life of fish.

Table 5. Quality Index Method (QIM) schemes

Quality Parameters		**Description**	**Points**
Whole fish	Skin colour/appearance	Pearls-shiny, iridescent pigmentation	0
		Less pearl-shiny, yellowish, strips still distinct	1
	Odour	Neutral, pond, fresh fish, seaweed	0
		Melon, cucumber, green grass	1
		Cardboards, fishy, putid, rotten	2
	Texture	In rigor	0
		Firm, resilient, finger mark disappears immediately	1
		Soft, finger mark still persists after 3 seconds	2

Eyes	Pupil	Black, clear, bright, iridescent	0
		Dark gray, meat, dull	1
		Milky, cloudy, hazy, light, gray	2
	Shape	Convex, bulging	0
		Flat	1
		Concave, sunken	2
Gills	Mucus	Transparent, clear, none	0
		Milky, clotted	1
	Colour/appearance	Bright red, red, burgundy	0
		Pale red, pink, light brown	1
		Brown, dull	2
	Odour	Pond, fresh fish, fresh rain	0
		Melon, cucumber, metallic	1
		Musty, fishy, putrid, rotten	2
Quality index (total score)			**0-14**

4.2. CHEMICAL METHODS OF QUALITY ASSESSMENT:

Chemical methods of quality assessment primarily focused on 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 in fish muscle by autolytic enzymes, putrefactive microorganisms or by chemical reactions like lipid oxidation. During the spoilage these compounds accumulate gradually in the meat and hence their

determination offers a measure of the progress of spoilage. The compounds found most useful as quality indices are,

- Volatile bases: Ammonia, Trimethylamine oxide (TMAO), trimethylamine (TMA), Dimethylamine (DMA) etc
- Nucleotides: Degradation products of ATP (adenosine triphosphate)

  Ex. Inosine monophosphate (IMP), Hypoxanthine (Hx) etc.
- Lipid oxidation: Peroxides, hydroperoxides, aldehydes etc. prodcuts.

Total volatile basic nitrogen (TVB-N):

Total volatile basic nitrogen (TVB-N) is a useful index of spoilage in different fresh and lightly preserved seafood. The TVBN value along with the TMA is the most common index of quality used method for deciding the state of freshness of fish. Some TVB is present in very fresh fish (<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 too spoiled for most uses. TVB-N values identify the latter stages of spoilage due to Lack of significant changes during the early stages of spoilage. Conway microdiffusion method and steam distillation method are commonly used method for estimation TVBN.

Trimethylamine (TMA):

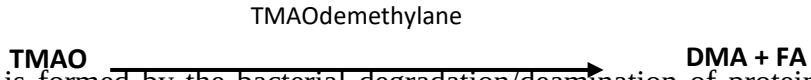
TMA is a microbial metabolite and it can only be used as an index of spoilage and not as an index of freshness. Trimethylamine oxide (TMAO) is found largely in most marine fish; in contrast, its presence is negligible or nil in freshwater fish. TMAO is reduced to TMA partly by intrinsic and mostly by bacterial enzymes of the group reductases. TMA-specific gas sensor technology is now available for routine rapid assay. For a good quality fish, TMA nitrogen value of l.25–2.00 mg% is recommended, and levels of 10–15 mg% can be considered as the safety limit beyond which most chilled fish become spoiled. Trimethylamine is associated with fatty substance and is responsible for the fishy smell of spoiled fish. Production of TMA is exponential, slow initially and increasing rapidly after a few days of chilled storage. Trimethylamine (TMA) levels are used universally to determine microbial deterioration leading to fish spoilage.

$$TMAO + 2H^{+} + 2e \xrightarrow{\text{TMAO Oxydoreductase}} TMA + H_2O$$

Dimethylamine (DMA):

The methyl group of TMAO is removed to form dimethylamine (DMA) and formaldehyde by the enzymes TMAO demethylase in the absence of oxygen. DMA increase at a constant rate, even during the first few days of iced storage and therefore, it is a superior chemical indicator of freshness quality. However, DMA is restricted to cod-like species and hakes, which contain TMAOases in their muscle tissue. They do not affect on the flavor or texture of the fish. However, Formaldehyde production is responsible for the increase in the firmness of the fish muscle under frozen storage. So, it is an Indirect indication of formaldehyde-induced toughening of the muscle during frozen storage. The amount of DMA and formaldehyde can be related to the freshness of fish.

Ammonia:



Ammonia is formed by the bacterial degradation/deamination of proteins, peptides and amino- acids. It is also produced in the autolytic breakdown of adenosine monophosphate (AMP). Ammonia has been found to be an excellent indicator of squid quality. However, ammonia would appear to be a much better predictor of the latter changes in quality insofar as finfish are concerned. Hence significant increase in ammonia content occurs only after spoilage. the ammonia levels increase far more quickly than trimethylamine (TMA) levels which have traditionally been used to reflect the growth of spoilage bacteria on lean demersal fish species. Thus, ammonia has potential as an objective quality indicator for fish which degrades autolytically rather than primarily through bacterial 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

Nucleotide degradation is one of the most extensively investigated methods of measuring odor and flavor aspects of the freshness quality of fish. Adenosine triphosphate (ATP) is degraded into Adenosine diphosphate (ADP), Adenosine monophosphate (AMP), Inosine monophosphate (IMP), Inosine (Ino) and hypoxanthine (Hx) during processing and storage of fresh and lightly preserved seafood. Various enzymes are involved in the breakdown of ATP to the end product, urea. Initially the tissue enzymes are prominent in their action to form hypoxanthine (Hx) from

ATP, but at the later stages, bacterial enzymes play a significant role. Thus, it can be considered as a measure of both autolytic deterioration and bacterial spoilage.

Hypoxanthine (Hx):

Hx has a bitter taste which may be part of the off-flavour in stale fish. Hypoxanthine content has been used for evaluating fish quality, the value increases with spoilage. Hx value progressively increases from near zero in extremely fresh fish to levels as high as >2.5 mol/g when the fish is considered spoiled. Nowadays, Hx can be determined by HPLC methods.

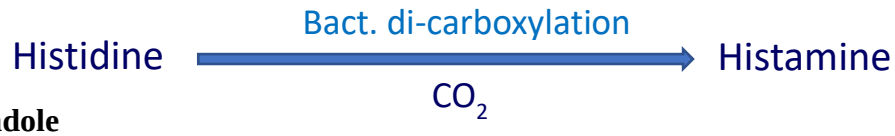
K-value

K-value is considered to be one of the best indices of spoilage in seafood. *K*-value is calculated from the values of Hx, inosine (I), and total nucleotide levels in fish at the point of measurement. The *K*-value measurement takes into account the role of most enzymes in the ATP breakdown. Hence, it is a more accurate index of loss of fish freshness. *K*-value could be as low as zero, 20-25% for moderate-quality fish, and at rejection it is usually above 50-60%. K-value is determine by HPLC methods.

$$K\% = \frac{[\mathrm{I} + \mathrm{Hx}]}{[\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP} + \mathrm{IMP} + \mathrm{I} + \mathrm{Hx}]}\ X\ 100$$

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. Most pelagic and scombroid fish contain a good amount of histidine in free state as well as with proteins. Histamine production occurs in fresh fish hew hours after death in tropical conditions when fish is not chilled properly. It causes food poisoning known as scombroid poisoning as it is linked with eating tuna, mackerel, and other species of the Scombroidea family. 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 converted to histamine. Certain bacteria under favorable conditions are able to produce histamine from histidine by a decarboxylation process. USFDA enforces a Maximum level of 50 mg/100g of histamine in fish tissue. Histamine is determined eighter by HPLC method or by spectrophotometer method.



Indole

Conversion of tryptophan to indole by microbial enzymes is another consequence of amino acid decomposition. Indole production is an indication of spoilage in shrimp and it a Useful freshness index of non frozen shrimp. USFDA used indole level along with sensory evaluation for the measurement of shrimp decomposition. High level of indole indicates decomposed shrimp and temperature abuse. However, it is not toxic at high level. The shrimp with <25 microgram/100g indole is acceptable (USFDA). Determined by spectrofluorometric and spectrophometric methods of the AOAC.

Lipid oxidation

Fish lipid is characterized by a high level of polyunsaturated fatty acids and hence undergoes oxidative changes. In fatty fish in particular, lipid oxidation gives rancid flavour and odour as well as discoloration. Lipid oxidation takes place into 2 processes,

- Autooxidation: action of O2 to the unsaturated fatty acids
- Lipid hydrolysis: an enzymatic hydrolysis with free fatty acids (FFA)

Oxidative rancidity is one of the great concerned in fatty fish storage. At first, hydroperoxides are formed, which further degrade to form aldehydes and ketones with typical rancid flavour. 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, peroxide value (PV) and thiobarbituric acid-reactive substances (TBA-RS)

Peroxide value

It measures the primary oxidation products such as peroxides and hydroperoxides. The peroxide value is a good guide to quality of fat and PV is a measure of first stage of oxidative rancidity. Fresh oil should have 1 milliequivalent Oxygen/Kg and on storage it may reach to >10.

TBARS

The secondary oxidation products comprise carbonyl compounds yielding the fishy and rancid character associated with oxidized fish lipid. TBA measures malonaldehyde produced during fat oxidation. TBA react with malonaldehyde to gives a red chromogen and is measured spectrophotometrically. PV is a measure of the first stage of oxidative rancidity whereas TBA value measures the second stage of oxidative rancidity. When PV is > 10 – 20 milliequivalent Oxygen/Kg and TBA above 1-2 mg malonaldehyde /Kg fat indicates rancidity in fish and gives smell and taste rancid.

Free fatty acid value

Lipid hydrolysis is the dominant reason for the generation of FFA when the fish lipid entered the second stage of lipid oxidation. It is a measure of hydrolytic rancidity. It is a Non esterified fatty acids in "free" form and 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. Prior to the appearance of oxidative rancidity in lean fish, there is rise in lipid hydrolysis that leads to build up of FFA.

Total volatile acids (TVA):

Formic acid and acetic acid formed during spoilage; they are volatile in nature. They formed only after putrefaction and can be used as a quality index. In Fresh muscle FFA is low, while, FFA value Increases rapidly after a few days in ice. TVA content not increase or decrease during canning process, so, can only be used for checking quality of canned raw material.

pH:

Change in pH of the fish muscle is a usual good index for freshness assessment. Natural pH of live fish above 7 (typically 7.3). Ph Falls after death as it goes through rigor and glycogen is converted to lactic acid, dropping the pH further. Post mortem pH is 6-6.8 (most species), In Tuna it is below 6 (high initial glycogen). pH increases as the spoilage increases

Microbiological methods:

The number of bacteria in food determine the general indicator of hygiene. Determination of APC or total viable count (TVC) are the most common method for determination of bacteriological quality of fish. In fresh fish/shrimp, Aerobic plate count (APC) is in the range of 10^3 – 10^6 cfu/g and during spoilage it rise above 10^7 cfu/g. Spoilage bacteria can generate unpleasant odours and flavours. They produce TMA from TMAO. Also, producers of hydrogen sulphide.

- Presence of *E. coli* in fish in an indication of unhygienic handling of fish – E. coli should be <20 cfu/g
- *Faecal streptococci* - < 100 cfu/g
- *Staphylococcus aureus* - < 100 cfu/g
- Salmonella – Absent in 25 g
- *Vibrio* spp. – Absent in 25 g
- *Listria* spp. – Absent in 25 g