BIOCHEMICAL QUALITY ASSESSMENT OF SEAFOOD

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The quality changes during preservation and storage of fish and fishery products is of great significance, since the important attributes are influenced by the post harvest handling practices. During spoilage of fish, a number of chemical reactions take place in the fish muscle. In the chemical assessment of quality, various compounds formed are quantitatively determined and correlated with sensory characteristics. These compounds are produced in fish muscle by autolytic enzymes, putrefactive microorganisms or by chemical reactions such as lipid oxidation. During the course of spoilage, these compounds gradually get accumulated in the flesh and their quantification is an important means to measure the progress of spoilage. The appeal of biochemical and chemical methods for the evaluation of seafood quality is related to the ability to set quantitative standards. The establishment of tolerance levels of chemical spoilage indicators would eliminate the need to base decisions regarding product quality on personal opinions. The following is an overview of some of the most widely used chemical indices of seafood quality.

1. Total volatile base nitrogen (TVB-N)

TVBN measures the amount of volatile bases formed from solubilised nitrogen derivatives. It is a measure of decomposition of proteins. TVB-N in fish is mainly composed of ammonia and primary, secondary and tertiary amines. Bacterial catabolism of aminoacids in fish muscle results in the accumulation of ammonia and other volatile bases. Ammonia and primary amines are bound by formalin, therefore this fraction is called the formalin bound nitrogen (FBN). The trimethyl amine (TMA) represents the fraction, which is not bound by formalin. The TVB-N value is used as an index of quality for deciding the state of freshness of fish (along with TMA). A level of 35-40 mg 1VB-N /100g of fish muscle is usually regarded as the limit of acceptability, beyond which the fish can be regarded as spoiled. Generally, there is an increasing trend in TVBN values as the fish gets spoiled.

2. Trimethylamine (TMA)

Trimethylamine (TMA) is used to assess the freshness in marine fish. TMA is derived from trimethylamineoxide (TMAO) which is critical for osmo regulation in marine fish. TMAO is a tasteless non-protein nitrogen compound whose content varies with the season, size and age of fish. During spoilage, TMAO is reduced by enzymes to TMA. The concentration of amines in fish tissues is both time and temperature dependent and is related to the deterioration of fish. The determination of TMA as an indicator of freshness (actually of decay) has been a useful criterion for evaluating the quality of fish. TMA-N between 10-15 mg / 100g muscle is considered as the limit of acceptability for round, whole chilled fish. This index is not suitable for freshwater fish and heat treated fish products.

3. Biogenic amines as an index of spoilage

Biogenic amines have been proposed as markers to evaluate fish freshness. Fish muscle has the ability to support the bacterial formation of a wide variety of amine compounds which result from the direct decarboxylation of amino-acids. Most spoilage bacteria possessing decarboxylase activity do so in response to acidic pH, presumably so that the organisms may raise the pH of the growth medium through the production of amines. Biogenic amines are non-volatile compounds, which are found at very low level in fresh fish and their accumulation is related to bacterial spoilage which are thermally stable and can be used as indicator of poor quality of raw material in preserved products.

Histamine, putrescine, cadaverine and tyramine are produced from the decarboxylation of histidine, ornithine, lysine and tyrosine, respectively. Histamine has received most of the attention since it has been associated with incidents of scombroid poisoning in conjunction with the ingestion of tuna, mackerel, mahi-mahi. It is interesting tonote that most of the biogenic amines are stable to thermal processing, so their presence in finished canned products is a good indication that the raw material was spoiled prior to processing. Although the biogenic amines have been associated with fish spoilage legal limit has been established for histamine only. The European Union set a maximum average content of 100 mg/kg fish for canned products and trace for ripened products. The US Food and Drug administration lowered the limit from 100 to 50 mg/kg, recommending that not only histamine level but also other biogenic amine content had to be taken

into account. India has also limited maximum permissible level for histamine content in frozen fish 200 mg/kg. Less than 5 mg/kg is considered safe for consumption; 5 – 20 mg/kg is safe; 20-100 mg/kg is probably safe and > 100 mg/kg is toxic and unsafe for consumption. Incidence of histamine poisoning after eating fish are mainly due to poor quality of raw material.

4. Nucleotide Catabolites

After the death of fish, ATP is broken down over a period of days by enzymes present in the flesh, to different substances. The final stages of this process is the formation of a compound called hypoxamthine, which gradually increase with time and can be used as a measure of quality of fish. The nucleotide degradation products especially inosine monophosphate (IMP), hypoxantine (H_x) or K value clearly reflects the quality loss in fish. The presence of higher levels of IMP in the muscle indicates relatively high quality, whereas accumulation of inosine and hypoxathine is an indicator of poor quality. The amount of nucleotide degradation products is measured by the enzymic method or by High Pressure Liquid Chromatography (HPLC) method. K value is one of the most appropriate indicators of freshness. It is the percentage of the intact ATP present at death that has been converted by enzymic action into hypoxathine and its immediate precursor called inosine in the chain of decomposition of ATP. HPLC method is used to determine the K value. K value as an index of estimating the freshness of fish has become widely used in Japan.

K value can be defined as,

$$K = \frac{H_xR + H_x}{H_xR + H_x + ATP + ADP + AMP + IMP} \times 100$$

Where H_xR = Inosine

H_x = Hypoxathine

ATP = Adenosines triphosphate

ADP = Adenosines diphosphate

AMP = Adenosines monophosphate

IMP = Iosines monophosphate

5. Peroxide value

The highly unsaturated fatty acids found in fish lipids are very susceptible to oxidation. The primary oxidation products are the lipid hydroperoxides. These compounds can be detected by chemical methods, generally by making use of their oxidation potential to oxidize iodide to iodine or to oxidize iron(II) to iron(III). The concentration of the hydroperoxides may be determined by titrimetric or by spectrophotometric methods, giving the peroxide value (PV) as milliequivalents (mEq) peroxide per 1 kg of fat extracted from the fish. The most common method is based on iodometric titration which measures the iodine produced from potassium iodide (KI) by the peroxide present in fat. PV is a good guide to assess the quality of fat. Fresh oil should have PV 1 mg.oxygen/kg. On storage it may increase to 10 mg/kg.

6. Free fatty acids (FFA)

Fish muscle contains lipase, which is able to catalyse the hydrolysis of short chain triglycerides. Free fatty acids are suspected of deriving primarily from phospholipids, as the latter disappear with time of storage which can be affected by the action of bacteria, enzymes or non-enzymic catalysis. During spoilage, the amount of free fatty acids increases, which can be measured by reacting with alkali and is expressed as %oleic acid.

7. Thiobarbituric Acid Value (TBA Value)

TBA index is the most used indicator for advanced lipid oxidation. The peroxides formed may break down to carbonyls, form polymers, or react with protein, vitamins, pigments etc.. Lipid oxidation frequently contributes to flavour changes that occur during the storage of food and is one of the major degradative processes responsible for losses in quality of high-fat foods. The most widely used test for measuring extent of oxidative deterioration of lipids in muscle foods is the 2-thiobarbituric acid test or TBA test, which expresses lipid oxidation in mg of malonaldehyde/Kg of the sample . Malonaldehyde was shown to be a secondary oxidation product of polyunsaturated fatty acids. TBA measures the malonaldehyde produced during fat oxidation. TBA reacts specifically with malodadehyde to give a red chromogen which can be determined spectrophotometrically. The test can be performed in two ways either directly in

food followed by steam distillation and allowing the distillate to react with TBA reagent or by preparing an extract of the muscle followed by colour development. A reagent blank is also run simultaneously and is is measured at 538 nm. The TBA number is calculated as milligram of malonaldehyde per kg of sample, which is equal to 7.8 times of the optical density.

Suggested Books for reading:

Handbook of Natural Toxins. (1988) Anthony T.U. Marcel Dekker, INC.

Principle of total quality On Achonu, V.K. and Ross, J.E. (1994) St. Lucie Press, Florida.

Quality Assurance in Seafood processing (2002) T.S.G. Iyer, M.K. Kandoran, Mary Thomas and P.T. Mathew, Society of Fisheries Technologists (I), Cochin

Quality Assurance in the Fish Industry (1992), H.H. Huss *et al (eds)* Elsvier Science Publishers, BV

Seafood Quality Determination (1986), DE Kramer and J. Liston, Elsiver Science Publishers B.V. Amolerdam.