Chapter 18

Quality assessment of fishery products: Notified NABL labs, Referral Labs and Reference Labs in India for fishery products

Dr. A.A. Zynudheen and Dr. S. Remya
Quality Assurance & Management Division, ICAR-CIFT
Email: zynu@rediffmail.com

Demand for seafood has consistently increased during recent years with fish protein being the major animal protein consumed in many parts of the world. According to the Food and Agriculture Organization, fresh seafood represents 40.5 % of the world's seafood production, while processed products (frozen, cured, canned, etc.) represent 45.9 %. To assure the quality of raw material used for processing, fish has to be treated carefully before and after harvest. The quality assessment of fish and other seafood is hampered by the immense variation between species and by the considerable variability between specimens of each species. The challenge, when assessing aquatic animals is that immediately after capture or harvest an alteration in intrinsic properties is initiated firstly by metabolic (autolytic) and secondly by microbiological processes, which last until the final state of spoilage is reached. Several analytical methods (chemical, physical, microbiological and sensory) can be used to determine spoilage depending on its state.

Freshness and quality of seafood

Freshness is the major attribute on quality of fish and of fishery products. For all kinds of products, freshness of the raw material used for processing is essential for the overall quality of the final product. It is impossible to process a high-quality product from raw material with poor freshness characteristics. Besides freshness, many other parameters also contribute to the complex quality of a seafood-based product such as the sensory properties (taste, odour, texture, appearance); the availability/disposability over a long time period; the safety; desired convenience characteristics; appealing packaging, colours and pictures; an attractive price; consistency, uniformity and conformity with standards and legislation; nutritional properties; ethical (stunning and slaughter); and ethnic (halal, kosher) constraints.

Indicators for freshness determination of fish

The properties, which wet fish in ice (fresh, unprocessed, not frozen) should have, when regarded as perfect fresh, can be considered as freshness indicators.

Rigor mortis: Fish regarded as fresh should be pre-rigor, in rigor or should just have passed rigor; it must be 'new', meaning that the time passed between harvesting or catch and marketing (auction) and consumption or processing into final products (including freezing) should be as short as possible.

Sensory quality: The sensory assessment of a cooked sample of the edible part should give high scores for all attributes – appearance, flavour, odour, taste, colour and texture.

Volatiles: The concentrations of basic volatiles like trimethylamine (TMA), dimethylamine (DMA) or total volatile basic nitrogen (TVB-N) are low, and that of trimethylamine oxide (TMAO) is high. Fresh odours (of plants, cucumber and mushroom character) are high in concentration whereas spoilage odours (amines) are insignificant; typical fishy odour is lacking.

Physical methods: The pH is clearly below 7.0 and the impedance measured is high. The microstructure is intact. Mechanical properties of fresh fish are commonly measured post rigor. Elasticity decreases and flesh is softening.

Microorganisms: Only a few microorganisms are present on skin and gills, the muscle is sterile; the proportion and activity of specific spoilage bacteria in microbiological flora determines remaining shelf life (freshness).

ATP and ATP breakdown products: k-value should be low. k-value increases with time.

Proteins: Low proteolysis rate in proteins.

Lipids: The muscle is in a status, where lipid oxidation is inhibited.

Fish spoilage

Seafood deteriorates very quickly due to various spoilage mechanisms. Spoilage can be caused by the metabolic activity of microorganisms, endogenous enzymatic activity (such as autolysis and the enzymatic browning of crustaceans' shells) and by the chemical oxidation of lipids.

- Self-digestion by enzymes (Autolytic changes)
- Bacteria (Microbial changes)
- Oxidation & hydrolysis (Chemical changes)

Seafood flesh has a high amount of non-protein nitrogenous (NPN) compounds and a low acidity (pH > 6), which support the fast growth of microorganisms that are the main cause of spoilage. The growth and metabolic activity of the spoilage microorganisms, especially specific spoilage organisms (SSOs), result in the production of metabolites that affect the organoleptic properties of the product. Immediately following death, autolysis resulting from the action of endogenous enzymes, initially causes loss of the characteristic fresh odour and

taste of fish and then softens the flesh. The main changes that take place are initially the enzymatic degradation of adenosine triphosphate (ATP) and related products and subsequently the action of proteolytic enzymes. Enzymes are also responsible for colour changes. Chemical oxidation of lipids (oxidative rancidity) is one of the most important spoilage mechanisms, especially in fatty fish.

Quality assessment of seafood by chemical methods

The traditional methods, which have been using for evaluating the quality of seafood are as follows.

1. **TMA & TVB-N**: Formation of amines increases when spoilage microorganisms have entered the muscle tissue and microbial degradation of low-molecular substances and proteins starts. Depending on the fish species and on the storage temperature, it takes about 10 days before microbial action on muscle tissue leads to an increase of amines. From this point on, amine formation follows an exponential function and can be used as a measure for spoilage. TVB-N is still the most frequently used method for quality assessment of fish. Conway's microdiffusion method is the routine method used for checking the TMA and TVB-N limit.
2. **Biogenic amines/Histamine**: Histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine and spermidine are the most relevant biogenic amines in seafood. Quality assessment using biogenic amines content such as histamine, cadaverine, putrescine and agmatine was reported by many researchers. Histamine is determined by HPLC method/Spectroflurometric method.
3. **Indole**: Spectroflurometric/ Spectrophotometric method
4. **Hypoxanthine**: HPLC method/Enzyme immobilization technique
5. **K-value**: The extent of breakdown of ATP (adenosintriphosphate) and related products was expressed as K-value, defined as a percentage of the amount of inosin and hypoxanthin to the total amount of ATP-related compounds. HPLC method is used for K-value determination.
6. **Peroxide value**: Iodimetric titration
7. **TBA value**: Steam distillation/Spectrophotometric method

Physical and Instrumental methods for assessing seafood quality

1. Freshness meter: E.g., Torry meter, Intelectron fish tester. As Fish tester and Torry meter measurements are based on the existence of intact cell membranes, they fail when the cells are disrupted or broken (damage, bruising, frozen/thawed fish).
2. Texture measurement: Texturometers/ Universal testing machines
3. Colour determination: Colourimeter
4. pH: Measuring pH in an aqueous muscle homogenate or using an injection electrode directly in the muscle tissue is the simplest method for quality assessment of seafood. A general rule is that when a pH of 7.0 in fish muscle is reached or exceeded, the borderline of edibility is reached though it is considered by many as not a good indicator of freshness and/or spoilage.

Microbial methods for assessing seafood quality

- Determination of total bacterial count
- Determination of specific spoilage bacteria
- Determination of pathogenic bacteria

Sensory quality assessment

At present, the commonly used methods of sensory evaluation include Quality Index Method (QIM), Tasmanian Food Research Unit schemes, and the Torry schemes. Among them, QIM is the most commonly used, which is based on objective assessment of some attributes of raw fish.

Novel Methods for fish quality assessment

1. **Biosensors**
    - Amine gas sensor: Detect the volatile amine gas (ammonia, TMA and DMA) in a short time (60 s) from raw fish flesh
    - Electronic nose: Most widely used technique are based on metal oxide gas sensor
2. **Spectroscopic techniques**
    - Visible/near-infrared (Vis/NIR) spectroscopy: Analyse food quality due to the interaction between food components and electromagnetic radiation emitted from lights

Quality control programmes

The traditional quality control program was based on establishing effective hygiene control. Confirmation of safety and identification of potential problems was obtained by end-product testing. Control of hygiene was ensured by inspection of facilities to ensure adherence to established and generally accepted Codes of Good Hygiene Practices (GHP) and of Good Manufacturing Practices (GMP). Below are listed the most well-known methods to manage quality and/or safety.

- Good Hygienic Practices (GHP) / Good Manufacturing Practice (GMP) or Sanitation Standard Operating Procedures (SSOP) or prerequisite programmes
- Hazard Analysis Critical Control Point (HACCP)
- Quality Assurance (QA) / Quality Management (QM) - ISO standards
- Total Quality Management (TQM)

Food Safety and Standards Authority of India (FSSAI): Ensuring food quality

The Food Safety and Standards Authority of India (FSSAI) was established under the Food Safety and Standards Act, 2006 as a statutory body for laying down science-based standards for articles of food and regulating manufacturing, processing, distribution, sale and import of food so as to ensure safe and wholesome food for human consumption. Various central acts including the erstwhile Prevention of Food Adulteration Act (1954) were merged under this act. Ministry of Health & Family Welfare, Government of India is the governing Ministry of FSSAI. It has the main Headquarters in Delhi.

The FSSAI is responsible for maintaining the food quality & security in India. FSSAI role in food quality is crucial for food security. The FSSAI's role in food quality is to ensure safety and providing satisfaction to every customer. Food Testing and analysis is an essential part of the food safety ecosystem to assure that the food is safe to consume. For the same, FSSAI recognizes and notifies NABL accredited food laboratories under Section 43 of FSS Act, 2006. FSSAI approved notified laboratories as National Reference Laboratories (NRLs) and as ancillary facility of NRLs (ANRLs) for specific purpose.

1. **Primary food laboratories**: The Food Authority notifies food laboratories and research institutions accredited by National Accreditation Board for Testing and Calibration Laboratories or any other accreditation agency for the purposes of carrying out analysis of samples by the Food Analysts. Presently there are 187 notified food testing laboratories.
2. **Referral food laboratories**: The Food Authority recognizes referral food laboratories for the purposes of carrying out analysis of appeal samples. Presently there are 18 referral food laboratories.
3. **National Reference Laboratories**: FSSAI has recognised National Reference laboratory (NRL) to set up a country wide standard for routine procedures, validation of such standard procedure / testing methods, development of new methods and ensuring proficiency in testing across the food laboratories with special reference to the risks or food categories. Either a primary food laboratory or a referral food laboratory can be considered for declaration as NRL. Presently there are 12 NRLs and 2 ANRLs.

FSSAI Notified Referral Laboratories under section 43 (2) of FSS Act, 2006

1. Central Food Laboratory, 3 Kyd Street, Kolkata- 700016
2. Food Safety & Analytical Quality Control Laboratory, C/o Central Food Technological Research Institute, Mysore-570013
3. State Public Health Laboratory, Stav ely Road, Cantonment Water Works Compound, Pune-411001
4. National Food Laboratory, Ahinsa Khand-II, Indirapuram Ghaziabad-201014
5. Indian Institute of Horticultural Research, Hessaraghatta lake post, Bangalore-560089
6. Quality Evaluation Laboratory, Spices Board, Palarivattom P.O. Kochi-682025
7. Quality Evaluation Laboratory, Spices Board, Chuttugunta Center, GT Road, Guntur-522004
8. Quality Evaluation Laboratory, Spices Board, Plot No. R-11, Sipcot Industrial Complex, Gummidipoondi, Thiruvallur Dt., Chennai-601201
9. Quality Evaluation Laboratory, Spices Board, First Floor, Banking complex II, Sector 19A, Vashi, Navi Mumbai-400703

10. Centre for Analysis and Learning in Livestock in Food (CALF), National Dairy Development Board (NDDB), Anand-388001, Gujarat
11. CSIR-Indian Institute of Chemical Technology, Uppal Road, Tarnaka, Hyderabad - 500007
12. National Research Centre on Meat, Chengicherla, Buduppal, Hyderabad – 500092
13. Indian Institute of Food Processing Technology, Food Safety and Quality Testing Laboratory, Pudukkottai Road, Thanjavur – 613005, Tamil Nadu
14. ICAR- Central Institute of Fisheries Technology, Indian Council of Agricul tural Research, Willingdon Island, CIFT Junction, Matsyapuri P.O., Cochin – 682029, Kerala
15. ICAR-National Research Centre for Grapes, P.O. Manjiri Farm, Solapur Road, Pune - 412307
16. Pesticide Formulation and Residue Analytical Centre, National Institute of Plant Health Management, Rajendranagar, Hyderabad - 500030
17. Punjab Biotechnology Incubator, Mohali SCO7 & 8, Phase-5, SAS Nagar, Mohali 160059, Punjab
18. CSIR-Indian Institute of Toxicology Research, Vishvigyan Bhawan, 31, Mahatma Gandhi Marg, Lucknow - 226 001, Uttar Pradesh, India

Referral Food Laboratory

The Laboratory having competence to carry out the analysis as per "The Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011" and "Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011". In addition, the Referral laboratory must have the competence to meet the following requirements:

- **R & D Capabilities**: Laboratories having documentary evidence for carrying out R&D in food sector
- **Training Facilities**: The laboratory should have training centre for capacity building by way of organizing professional training, workshops and seminars.
- **Other Facilities**

1. Analysis of samples of food sent by any officer or authority authorized by the Food Authority for the purpose and submission of the certificate of analysis to the authorities concerned;
2. Investigation for the purpose of fixation of standard of any article of food;
3. Investigation in collaboration with the laboratories of Food analysts in the various States and such other laboratories and institutions which the Food Authority may approve on its behalf, for the purpose of standardizing methods of analysis.

4. Ensuring that the laboratory follows the scientific protocols laid down for handling/testing the articles of food.
5. Maintaining high standards of accuracy, reliability and credibility in the operation of the laboratory and achieving and maintaining the required levels of accreditation and reliability.
6. Laying down mechanism for ensuring that personnel of the laboratory adhere to high professional standards and discipline.
7. Such other conditions, as the Authority may lay down for Referral Laboratories such as coordinating proficiency testing programmes in the country etc.

Reference Laboratory

The Food Authority may recognise any notified food laboratory or referral food laboratory as a reference laboratory for the purpose of developing methods of testing, validation, proficiency testing, and training.

The reference laboratory shall carry out the following functions, namely

i. be a resource centre for provision of information for certified reference materials and reference materials
ii. develop standards for routine testing procedures and reliable testing methods
iii. provide technical support in the area of competence
iv. evaluate the performance of other notified laboratories
v. coordinate exchange of information amongst notified food laboratories
vi. collaborate for data generation among the network of notified food laboratories and referral food laboratories and collate the data related to their specific domain
vii. such other functions as may be specified by the Food Authority

Criteria for notifying and recognising food laboratories

For being recognised and notified, every food laboratory shall have-

- Accreditation against ISO/IEC 17025 by the National Accreditation Board for Testing and calibration laboratories or such other equivalent accreditation agency as may be approved by the Food Authority.
- Adequate capability and competence for testing of food safety and quality parameters as per the requirements of the Act.
- Person possessing qualification and experience required for being appointed as Food Analyst under rule 2.1.4of the Food Safety and Standards Rule, 2011: Provided that a food laboratory accredited by an accreditation body having authorised signatory designated by such accreditation body, shall also be considered for being notified subject to the condition that such authorised signatory shall, within one year from the date of such notification, acquire the qualification and experience required for being appointed as Food Analyst under the said rule.

- The infrastructure and facilities including equipment required for carrying out the analysis as per the scope applied for.

ICAR-CIFT: National Reference Laboratory for Fish and Fish Products

ICAR-Central Institute of Fisheries Technology, Cochin has been conferred with a status of "National Reference Laboratory (NRL) for Fish and Fish Products" by Food Safety and Standards Authority of India (FSSAI), Ministry of Health and Family Welfare, Govt. of India under Regulation 3 of Food Safety and Standards (Recognition and Notification of Laboratories) Regulation, 2018 on 19th March, 2019 vide Order No. 12013/02/2017-QA. ICAR-CIFT is the only research Institute under SMD (Fishery), ICAR to be adorned with such a high-profile recognition. The Institute had already been notified as National Referral Laboratory vide Government of India Gazette Notification S.O. 97(E) of Ministry of Health and Family Welfare (Food Safety and Standards Authority of India) dated 10th January, 2017.

Under the NRL notification, ICAR-CIFT has earmarked with the following research activities on emerging issues pertaining to:

- Risk assessment of dietary exposure of persistent organic pollutants and emerging contaminants such as brominated flame retardants and pharmacologically active substances to Indian population from fish and fisheries products.
- Research on ingression of specific migration of chemicals from plastic packaging materials to fishery products
- Research on incidence of biotoxins in finfish/shellfis

Presently there are 12 NRLs. Including ICAR-CIFT, seven laboratories in Government sector and five laboratories in private sector have been given the status of National Reference Laboratory in specific areas.

List of national reference laboratories (NRLs) approved by FSSAI

Sl. No.	Name of the laboratory/ institution/organisation	Specific area for which declared as NRL
Government laboratories		
1.	Central Food Technological Research Institute, Mysore	Nutritional information and labelling
2.	Export Inspection Agency, Kochi	GMO (genetically-modified organism) testing (subject to implementation of GMO regulations)
3.	Punjab Biotechnology Incubator, Mohali	Sweets and confectionery, including honey
4.	ICAR-National Research Centre For Grapes, Pune	Pesticide residues and mycotoxins
5.	Central Institute of Fisheries Technology, Kochi	Fish and fish products
6.	Centre for Analysis and Learning in	Dairy and dairy products

	Livestock and Food-National Dairy Development Board, Anand	
7.	CSIR-Indian Institute of Toxicology Research, Lucknow	Toxicological evaluation/risk assessment of nutraceuticals, functional foods and novel/emerging foods/food ingredients
Private laboratories		
8.	Trilogy Analytical Laboratory Pvt. Ltd., Hyderabad	Mycotoxins in cereals and pulses, spices and condiments and related PT activities
9.	Edward Food Research and Analysis Centre Limited, Kolkata	Veterinary drug residues, antibiotics and hormones
10.	Vimta Labs Limited, Hyderabad	Water, alcoholic and non-alcoholic beverages
11.	Fare Labs Pvt. Ltd., Gurugram	Oils and fats
12.	Neogen Food and Animal Security (India) Private Limited, Cochin	Food allergens

Conclusion

Seafood is a very perishable product and the risk of contamination of seafood products by biological hazards is very high. Processing and preservation is necessary to assure the prolonged shelf life and safety of seafood. Increasing demands from legislation and from the consumer for better quality and safer products have to be taken into account. Seafood now has to be high quality, nutritious, safe and have the convenience of an extended shelf life. To meet these criteria, seafood processing had to assimilate all the new advances in food science and technology and in quality and safety assurance. Advanced quality and safety methods, such as modern and rapid techniques for assessing quality and safety, species identification techniques and risk assessment tools, all have significant applications in the seafood sector.

Food testing and analysis is an essential part of the food safety ecosystem to assure that the food is safe to consume. This includes strengthening the network of food testing laboratories, assuring quality of food testing, investing in human resources, carrying out surveillance activities and educating consumers. As an essential part of the food safety ecosystem, the Food Safety and Standards Authority of India has created a network of 232 laboratories to fulfil its mandate on food testing and analysis. The network comprises of 142 accredited primary food-testing laboratories from both government and private sphere, 72 state food testing laboratories and 18 referral laboratories of which two are under the direct control of FSSAI. FSSAI's role in food quality is important for smooth functioning. As a resultant of this, every customer receives an equal level of assurance of food safety.

Reference/Website

1. https://www.fssai.gov.in/
2. https://cift.res.in/national-reference-laboratory
3. Huss, H.H., 1988. *Fresh fish--quality and quality changes: a training manual prepared for the FAO/DANIDA Training Programme on Fish Technology and Quality Control* (No. 29). Food & Agriculture Org..