Training Manual

On

QUALITY ASSURANCE OF FISH AND FISHERY PRODUCTS

(Under ITEC, Ministry of External Affairs, Govt. of India)

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FOREWORD

Quality of food plays a significant role in the development of healthy life and its safety remains utmost importance in the food production system. Today, lot of concern has been raised globally by consumers on different food safety related issues. Food contamination in the food chain is happening on regular basis and proper identification of hazards associated with the food production systems is the need of the hour as hazards may be introduced at any stage. Food-borne outbreaks have been occurring due to the consumption of contaminated food. Frequent outbreaks have been reported which are caused by different human pathogenic bacteria such as *Salmonella*, *Vibrio parahemolyticus*, *E. coli* O157:H7 and *Listeria monocytogenes* etc. Apart from this contamination is also happening due to the adulteration of food with different chemical substances and also due to accumulation of heavy metals as a result of increase in pollution.

ICAR-Central Institute of Fisheries Technology (CIFT) being a premiere government organization has been continuously working on the quality and safety of fish, fishery products and water since its inception. In India, ICAR-CIFT was involved in the formulation of several international and national standards pertaining to the quality of fish and fishery products, their marketing technologies, export and import of seafood. Apart from this, ICAR-CIFT was the driving force for setting the standards for packaged drinking water in India. Also, Central Institute of Fisheries Technology initiatives on the seafood quality management remains as a milestone in the history of pre-shipment system in India. A significant achievement in the ICAR-CIFT's relation to the seafood processing sector has been associated with the introduction and implementation of Hazard Analysis and Critical Control Point (HACCP) based quality assurance system in the processing industries throughout the nation. In collaboration with Food Safety Standards Authority of India (FSSAI), ICAR-CIFT is working to set standards for the quality of Fish and Fish products and its consumption domestically. Based on the pioneering work carried out by Central Institute of Fisheries Technology, FSSAI recognised ICAR-CIFT as the National Referral Laboratory as well as National Reference Laboratory of for testing the quality parameters for Fish and fishery products. ICAR-CIFT has extended its support to FSSAI in formulation of development of food safety standards, food testing protocols, guidance on establishment of testing laboratories, empowering of technical personnel and assessment on the functioning of seafood processing industries.

Indian Technical & Economic Cooperation Programme (ITEC), Ministry of External Affairs, Government of India in collaboration with ICAR-CIFT, Cochin organized the international training on 'QUALITY ASSURANCE OF FISH AND FISHERY PRODUCTS' on hybrid mode to disseminate the technical expertise developed over many decades by the institute could be shared with researchers and officials from different countries. During the training days, 10 participants from 6 countries talks pertaining to quality issues in different fish and fishery products, different hazards associated with seafood, HACCP & ISO 22000: 2018 implementation, regulatory requirements, traceability and validation & verification of testing methods. The topics for the training programme were selected to give a comprehensive knowledge on quality assurance of fish and fishery products. This training manual consists of 38 chapters covering different aspects of quality assurance of fish and fishery products. I am sure that this training manual will be very useful for the researchers working in the areas of quality assurance of fish and fishery products. The knowledge about quality assurance of fish and fishery products will help to ensure food safety along the entire food chain, and safe food to consumers.

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PREFACE

Food safety is a global concern due to its direct effect on human's health. Fish and fishery products constitute a significant component of human diet. Contribution of fish and other aquatic products in the average animal protein consumed worldwide is around 15 percent. Fish and other seafood in daily diet is a smart choice for health-conscious consumers. There are proven health benefits of consumption of aquatic products that out-weigh risks. Though fish provide many health benefits, seafood can be compromised by different chemical contaminants which are harmful to consumers, if they are harvested from waters contaminated with industrial chemicals, heavy metals, pesticides and antibiotics residues. These contaminants may accumulate in fish at levels that are harmful for human health (e.g. carcinogenic and mutagenic effects). Food can become contaminated with contaminants at any point during production, distribution and preparation. Everyone along the production chain, from producer to consumer, has a role to ensure the safety of seafood. The seafood may get contaminated with various pathogenic bacteria due to unhygienic handling practices, cross contamination of raw foods with cooked or ready-to eat foods, and lack of proper time - temperature control. Bacterial and viral Pathogens including parasites which occur naturally are the primary food safety concern with regard to seafood. The vital tools commonly used to define the requirements for an effective Food Safety Management System are ISO 22000 and HACCP (Hazard Analysis and Critical Control Points). These are the basis for Food Safety principles defined by Codex Alimentarius Commission of World Health Organization. HACCP is an internationally recognized risk management tool, which is proactive in nature, while ISO 22000 is a complete food safety management system, enabling continual improvement of performance. The training programme on 'QUALITY ASSURANCE OF FISH AND FISHERY PRODUCTS' is an attempt to make clear picture about role of ISO 22000/HACCP in food safety management system to ensure safe food to consumers. This book, through its various chapters discusses quality issues in different fish and fishery products such as live, fresh, chilled, frozen, dried, smoked, thermally processed, fermented, different fishery by products and seaweed-based products. The book also covers different hazards associated with seafood processing, HACCP & ISO 22000: 2018 implementation, regulatory requirements, traceability and validation & verification of testing methods. We would like to acknowledge the Ministry of External Affairs and ICAR for giving us an opportunity to conduct this training programme. We acknowledge the entire resource persons for immensely contributing for this manual. Infact the entire manual was prepared during the training programme itself and without the support of all faculties it would not have been possible. We would like to acknowledge Dr. George Ninan, Director, ICAR-CIFT for all the support, guidance and encouragement given for the successful completion of this training programme as well as training manual. We hope that this publication will serve as guide for academicians, technologists and entrepreneurs engaged in seafood quality assurance and food safety management system.

Course Directors &

Course Coordinators

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Chapter 1

Overview of Quality Aspects in Fish: Traditional and Modern Approach

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The desire for quality product is the major factor providing a boost towards the operational efficiency in any industry. As far fish quality is concerned, it includes parameters that pertain to the species, sensory expectations of consumers, health and safety aspects, nutritional value, functional properties, and conformation to product standards regarding composition, proportions of components, appearance, ingredients, packaging, labeling, and shelf-life. Most of these attributes can be quantitatively determined for assessing the quality and grade of the fish. Most often quality refers to the aesthetic appearance and freshness of the fish. The term quality may also involve the safety aspects also. Quality is a subjective concept. As per the International Organization for Standardization (ISO) the term quality is defined as "degree *to which a set of inherent characteristics that fulfills requirements*".

Different quality attributes of freshly harvested fish depend on the species' characteristics, seasonal biological changes in the fish, feeding habits, parasite infestation etc. The species-related properties like size, appearance, maturity stage, shape, yield of edible parts, content and distribution of dark muscles, flavour and texture of the meat, contents of nutritional components etc are important parameters while valuing the quality.

Quality of seafood differs from other type food stuffs due to its compositional pattern. Since the moisture content of sea food is more than 70%, it is prone to easy spoilage, if not preserved immediately after catch. Presence of high quantity of simple protein, highly unsaturated lipid and high moisture content make it highly prone to microbial decomposition and oxidation. These processes will result in the release of various compounds affecting the quality of fish. Unscientific handling practices like washing with near shore water and harbour water gives easy entry for pathogenic and spoilage bacteria into the fish and speed up the quality degradation process.

Quality and freshness of fish can be ascertained by following physical, chemical, microbiological, instrumental and sensory methods. Even though sensory methods are highly subjective, experienced persons can give reliable results on the quality by sensory methods instantly. ICAR-CIFT has developed quality index methods and mobile application based

systems for the field level assessment of quality of fish and shell fish to enable the customers for selecting quality fish easily and reliably. The suitability of fish for the preparation of the indented product and process largely depend on the color, flavor, and texture of the meat. This in turn depends on the degree of freshness and the primary storage method followed after catch.

Quality Assurance and Quality Control

The minimum requirement for a quality assurance system is to prevent any hazard to the consumer. Industry needs routine tools of quality assurance (QA) linked with HACCP plan and quality control functions to perform necessary analysis to evaluate the safety of the process/products. As per ISO 8402, Quality Assurance can be defined as all those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality. While Quality Control (QC) is defined as the operational techniques and activities that are used to fulfill requirements for quality, Hazard Analysis and Critical Control Point (HACCP) is a quality assurance approach based on prevention, rather than correcting the occurrence of the potential hazards that may cause illness/injury to the consumer during the manufacturing process. Total Quality Management (TQM) is a theory of management based on the principles of quality assurance. There are nine TQM practices adopted for food manufacturing such as cross-functional product design, process management, supplier quality management, customer involvement, information and feedback, committed leadership, strategic planning, cross functional training, and employee involvement.

All these quality assurance systems are intended to provide confidence to the management, customer and regulatory agencies that the company meets all the relevant food quality and safety requirements.

Quality and safety issues in fish products:

Quality issues	**Safety issues**
Live/fresh/chilled/frozen fishes	
Belly bursting Discoloration Blackening/ melanosis in crustaceans Pink discoloration in squid and cuttlefish Freezer burn/ dehydration Off flavors	Pesticide residues and Other Persistent organic pollutants Residues of veterinary drugs and extra label chemicals Unapproved additives Presence of adulterants Growth of pathogenic bacteria Allergens

Dried fish	
Shrinkage Casehardening Protein denaturation and rehydration Maillard reaction Rancidity Dun, Pink/Red Insect infestation Fragmentation	Growth of pathogenic bacteria *Clostridium botulinum* toxin production (for uneviscerated products) *Staphylococcus aureus* toxin Pesticide residues Unapproved additives Allergens
Fish mince and surimi	
Dehydration Presence of foreign matter Denaturation of protein	Parasites Growth of pathogenic bacteria Pathogenic bacteria survival Heavy metals Natural toxins Allergens and Food intolerance substances Metal inclusion
Smoked fish	
Presence of pathogens Decomposition Parasites	Growth of pathogenic bacteria *Clostridium botulinum* toxin production Pathogenic bacteria survival Allergens and Food intolerance substances Metal inclusion Natural toxin Polyaromatic hydrocarbons
Canned fish	
Struvite formation Sulphide blackening Blue discoloration Curd and adhesion	Growth of pathogenic bacteria *Clostridium botulinum* toxin production Pathogenic bacteria survival

Honey combing Retort burn Case hardening Softening and mush	Allergens and Food intolerance substances Metal inclusion
Convenient products	
Discoloration Rancidity Protein denaturation Loss of nutrients	Growth of pathogenic bacteria *Clostridium botulinum* toxin production Pathogenic bacteria survival Allergens and Food intolerance substances Metal inclusion
Coated products	
Shelling Blow off Poor adhesion Gummy interface	*Clostridium botulinum* toxin production (Reduced Oxygen Packaging -ROP) *Staphylococcus aureus* toxin (ROP & other than ROP) Allergens and Food intolerance substances Metal inclusion
Fish pickles	
Soft, slippery slimy/dark appearance Shriveled/bitter tasty pickle Yeast and mold growth Presence of pathogenic bacteria	Growth of pathogenic bacteria *Clostridium botulinum* toxin production Allergens and Food intolerance substances Metal inclusion Glass inclusion
Fermented fishery products	
Parasites Natural toxins Histamine	Growth of pathogenic bacteria *Clostridium botulinum* toxin production

Presence of pathogenic bacteria Rancidity Dehydration/ dryness and discoloration Presence of extraneous matter	Allergens and Food intolerance substances Metal inclusion Glass inclusion

A practical knowledge on the commonly occurring quality issues including the naturally occurring biotoxins during different seasons and their probable locations can help in non-procurement during the specific period and thereby controlling its consumption to avoid health risks. The most important factors deciding the quality and safety of fish are the time temperature tolerance. The rigor period starts immediately after death depend on various factors such as temperature, stress and species. If the fish is properly iced and kept at 0°C, the rigor can last up to 2-4 days. Most of the consumers, except those who are in proximity to fish landing centers/harbors or fishermen, prefer taste and texture of post-rigor fish only. So, this pre-rigor and rigor period can be used for transportation purpose, so that high quality fish can be served to consumers. Along with that if there is an effective quality assurance system in practice, the safety of the product also can be assured.

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Chapter 2

Post - Mortem Quality Changes in Fish

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Information about the post mortem changes of fish is imperative to appreciate the processors involved in the spoilage and quality changes. The knowledge is beneficial for better control of the quality of raw material. Understanding the factors that cause changes in quality helps to find ways to prevent the changes and maintain the quality and freshness of the raw material.

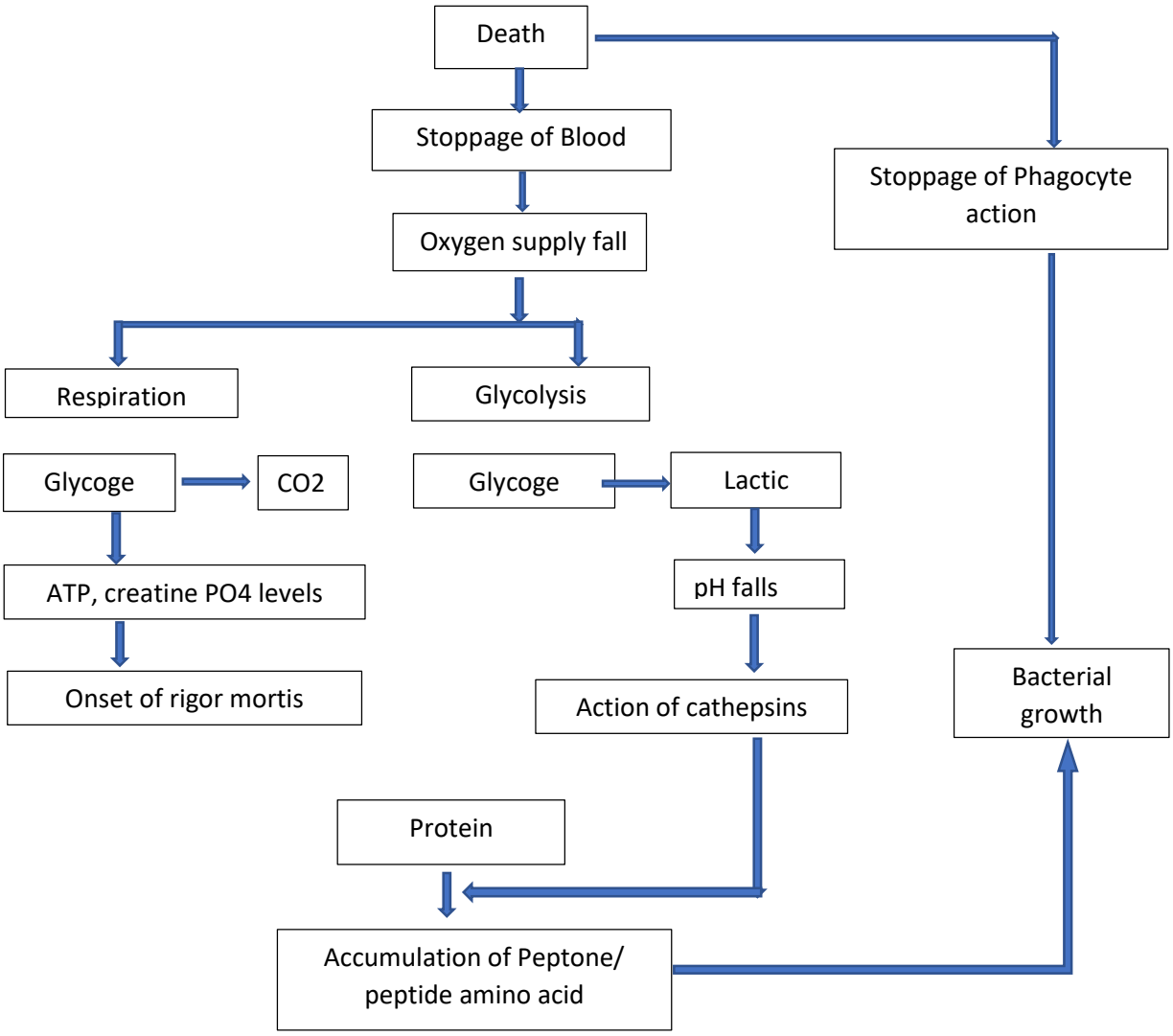
Fish is a food item of good acceptability and nutritional value. But it is a highly perishable item and quality deterioration very fast if not preserved properly. The changes leading to spoilage of fish are highly complex. Both biochemical and microbiological processes contribute the quality deterioration. The enzymes naturally present in the system are primarily responsible for the post-mortem biochemical changes.

During the period after death till its consumption a large number of physicochemical changes take place, which can be classified into three stages.

1. Pre-rigor state –in which the meat is soft and pliable and is characterised biochemically by a fall in ATP and creatine phosphate and glycolysis.
2. Rigor mortis-stiff and rigid condition, which extends from 1 to 7 h and is affected by a number of factors.
3. Post rigor- a stage during which meat tenderisation takes place making the meat organoleptically acceptable.

Pre rigor state

It is the stage of the fish immediately after death and before the onset of rigor mortis. The first sign of dead fish is the unusually high mucus on the body. In live fish mucus, a glycoprotein formed of mucin, is secreted at a controlled rate and plays a role in preventing the entry of microbes by its anti-bacterial and lysosomal action. However, after death the properties of the mucus are altered or lost and it no longer acts in controlling microbial invasion.



Following death, the circulation of bold to body ceases leading to depletion of oxygen supply to the tissues is cut off. This results in the inability of body tissues to synthesise adenonsine-5' triphosphate (ATP) as electron transport chain (ETC) and oxidative phosphorylation mechanisms are no longer operative. This results in the depletion of ATP and creatine phosphate. Alongside this the anaerobic conversion of glucose to lactic acid takes place, leading to drop in pH. This accelerates rigor mortis and protein denaturation. The drop in pH accelerates action of cathepsins and other proteolytic enzymes. This leads to accumulation of various metabolites, flavour, bacterial growth and ultimately spoilage.

Rigor mortis

Normal aerobic oxidation of glucose through oxidative chain produces 39 molecules of ATP per molecule of glucose. After death as anaerobic condition is created and glycogen can no longer be converted in to CO_2 and water. The major supply of ARP is, thus, cut off. Anaerobic

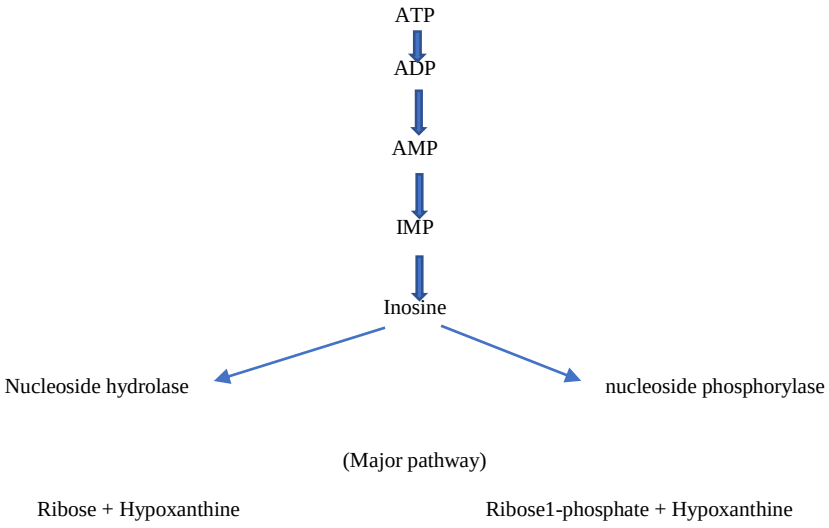
conversion of glycogen to lactic acid takes place, leading to the production of just 3 molecule of ATP.

Besides, the ATP available is also depleted by sarcoplasmic ATPase for the phosphorylation of glycogen to glucose-1-phosphate. For some time after death the ATP concentration is maintained at the expense of creatine phosphate by the action of the enzyme creatine kinase. The ADP released as a result of ATPase activity is rephosphorylated to ATP and free creatine is formed. Thus, in the early post mortem stages the concentration of ATP is maintained and the concentration of creatine phosphate decreases rapidly. Eventually due to the continued activity of ATPase, the concentration of ATP as well as creatine phosphate falls.

Drop in ATP level initiates combination of actin and myosin leading to the formation of actomyosin. This results in a rigid condition of the muscle, called rigor mortis. In cod it was reported that a 5% drop in ATP level leads to onset of rigor mortis.

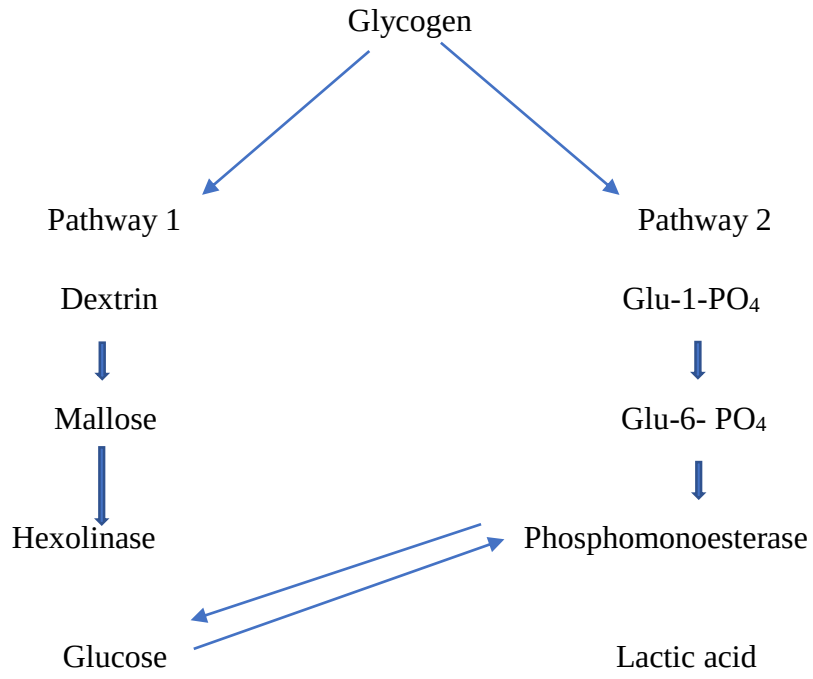
Post mortem metabolism of ATP

ATP is converted in to adenosine-5' –diphosphate (ADP) by sarcoplasmic ATPase, which is by myokinase. Conversion of adenosisne-5' monophosphate (AMP) to IMP is effected out by a deaminase action. IMP, which plays a role in the flavour of good quality fish, is dephosphorylated to inosine. Decomposition of inosine produces hypoxanthine, which is considered to be a quality index for freshness and quality of fish.



Post mortem glycolysis

On cessation of oxygen supply to the muscle tissues, glycogen the main carbohydrate source of muscle, is no longer oxidized to Co2 and water but broken down to lactic acid by anaerobic glycolysis, which is reported to take place by two pathways- hydrolytic or amylolytic pathway, and phosphorolytic pathway.



Post mortem pH

As lactic acid accumulates in the system, the pH of the system falls form the initial physiological pH of 7.2-7.4 to the ultimate post mortem pH of 5.3 - 5.5 in well fed and rested animals which have high levels of tissue glycogen. In fish relatively high pH is required to prevent toughness of meat and the final pH is attained in 24 hrs. Fish which was made to struggle just before death have a lower glycogen level and hence the ultimate pH would be around 6 – 6.6.

Time course of rigor mortis

The development of rigor is closely related to temperature. The length of time between death and onset of rigor is determined by the relative activities of enzyme systems responsible for ATP degradation. This in turn is controlled by the relative concentrations of ATP, creatine phosphate and glycogen in the muscle tissue at the time of death. In well-fed and well –rested animals the levels of these chemicals are high so that a longer delay period is observed prior to development of rigor, producing meat of low pH and high quality. Subjecting the fish to starvation or struggling would inevitably result in a much shorter delay period producing meat of inferior quality.

A prominent post-mortem change is the joss of water bound to protein molecules due to the loss of water holding capacity falls. This is related to the drop in pH to 5.3-5.5 which is almost close to the isoelectric pH of fish meat. During post rigor aging of meat, the water holding

capacity of meat was found to increase. This is attributed to an increased osmotic pressure within the fibres or alterations of the electrical charges on protein molecules involved and is related to the movement of ions to and from the muscles.

Changes in muscle proteins

The muscle myofibrillar protein, particularly actin and myosin changes in relation to rigor mortis, the actin and myosin are dissociated in the pre rigor stage. Depletion of ATP gradually associates the two leading to the formation of actomyosin. The fish sarcoplasmic proteins are far more stable than myofibrillar protein. They possess better thermo stability and solubility than their counterparts in other meats and not appear to play roles in muscle texture.

Following the resolution of rigor, a gradual tenderization of meat occurs and the post rigor meat is organoleptically well accepted compared to that in rigor.

Mechanism

Meat tenderization following rigor mortis is an important process because it imparts to the meat its final texture and flavour immediately before consumption. The important changes takin place during the process includes the following

1. The water holding capacity is increased
2. The level of water insoluble non-protein nitrogen namely peptides and amino acid level increase duo to the action of proteolytic enzymes.
3. It is also shown that the resolution of rigor is not related to separation of actin and myosin form actomyosin but is the result of the weakening and disintegration of z-line of A-band of muscle fibre.

Changes produced by naturally occurring bacteria

Microorganisms are found on all the outer surfaces (skin and gills) and in the intestines of live and newly caught fish. The total number of organisms vary enormously. A normal range of 10^2-10^7 cfu (colony forming units)/cm2 on the skin surface. The gills and the intestines both contain between 10^3 and 10^9 cfu/g.When the fish gets into the processing area the bacterial count on the skin is often high. If the fish is not washed well with clean water a lot of bacteria can get in the processing area and contaminate the fish flesh during filleting. The flesh can also get contaminated with mesophilic bacteria from the people in the working area. So personal hygiene is also very important. Highest numbers of bacteria of fresh fish are present in digestive tract, but numbers in outer surface can be increased upto 10^8 during spoilage. Temperature plays very important role in controlling microbial growth. Higher temperatures (around 37°C) can increase the microbial growth.

Main Spoilage bacteria found in fish are *Pseudomonas, Shewanella putrefaciens, Photobacterium phosphoreum, Enterobacteriacea.* Usually Pathogens found in natural environment of fish are *Clostridium botulinum, Listeria monocytogenes, Vibrio parahaemolyticus, V. vulnificus, Aeromonas hydrophila* and *Plesiomonas shigelloides*. Other pathogens that can contaminate from the environment are *Staplylococcus aureus*, *Salmonella* species, *Escherichia coli etc.* In post-rigor stage, bacterial spoilage becomes very fast. Growth of bacteria contributes to the development of spoilage odours because of production microbial metabolites.

The rise of muscle pH from acidic to alkaline range for accumulation of volatile bases like ammonia and trimethylamine produced by spoilt fish enhances bacterial growth. Trimethylamine is produced by the reduction of trimethylamine oxide by bacterial enzyme as well as by tissue enzymes.

Chapter 3

Sensory Evaluation & Hygienic Handling of Fish

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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.

Methods for Assessing Freshness 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. There are different methods for assessing the fish freshness. They are

- Sensory and Non-sensory or instrumental.
- Non-sensory – 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 methods

Sensory evaluation is the most important method in freshness assessments. They are the most convenient method for testing seafood quality in field level. Sensory tests involve using senses to evaluate the quality of seafood. Typical sensory tests include the evaluation of appearance, texture, odor, flavor and other attributes. This method is very subjective since everyone has their own likes and dislikes.

Sensory evaluation of food is defined as the scientific means of quantifying and interpreting the variations in food characteristics (odour, taste, tactile, appearance) by using human senses of sight, smell, taste, touch and hearing. Sensory evaluation provides rapid measurements of the freshness of seafood. Sensory methods are capable of giving objective and/reliable results when assessments are done under controlled conditions. They can be very fast, reliable, non-destructive on raw fish and no expensive instruments are needed. There are several grading methods used to assess freshness in fish and fish products. Vision is very important to see defects such as bloodstains, bones and parasites. The appearance of the fish, including the gills and eyes, gives information about the freshness of the fish. Odour of both raw and cooked fish is also important in sensory evaluation of freshness. People are very sensitive to various compounds produced in fish during storage, and especially spoilage, such as several sulphur and nitrogen compounds. Flavour is the olfactory perception caused by volatile substances released from a product in the mouth as well as the basic tastes caused by soluble substances in the mouth. There are four classical basic tastes: sour, salt, bitter and sweet. In addition to these others are metallic, astringent and umami. In sensory evaluation of fish, the tactile sense is mainly used to evaluate the texture of fish flesh, for example by pressing a fingertip on the fish flesh to observe if the fish is still stiff or soft. The texture of seafood can also be sensed through chewing.

Selection and training of sensory panels

- Selection of participants shall be based on basic sensory acuity and ability to describe perceptions analytically.
- Personal characteristics are very important when selecting people for the sensory group
- Panellists must also be healthy and normally sensitive (taste and odour senses).
- Basic selection tests and training guidelines can be found in Meilgaard *et al.* (2006), ISO 8586-1 (1993), The Codex guidelines for sensory evaluation of fish and shellfish in laboratories (Codex Standards 1999)
- The training of the sensory panel should begin by describing the procedures of the sensory evaluation

- Samples should be coded and randomly presented among the panellists

Procedures for sensory evaluation

- ❑ COLLECTING AND TRANSPORTING SAMPLES –
- ✓ Acceptance or rejection decision is made on the basis of an examination of a sample drawn from the batch according to guidelines (regulatory or commercial)
- ✓ Samples if not evaluated immediately, should be stored under appropriate conditions.
- ✓ Fresh and chilled products should be examined on the day they are received.
- ✓ Products in either chill or frozen storage should be appropriately wrapped to prevent drying out or desiccation
- ❑ PREPARATION OF SAMPLES FOR EXAMINATION
- ✓ Procedures for the preparation of samples should be appropriate for the product types - fresh or frozen
- ✓ Frozen products should be first examined in the frozen state
- ✓ then be thawed for sensory evaluation
- ✓ Thawing can be accelerated by immersion of the material in water with wrapping
- ❑ COOKING

A small portion is sectioned from the sample unit and the odour and flavour or gelatinous condition confirmed by cooking without delay by cooking method. The product must not be overcooked.

Procedures for the assessment of products

Samples should be assessed relative to the characteristics of the species concerned

1. Assessment of Raw Products
2. Assessment of Frozen Products -Frozen fish should be examined in the frozen state.
3. Assessment of Cooked Samples - Cooked samples should be held in a closed container, allowed to cool to a comfortable tasting temperature, and kept warm unless they are assessed immediately. Products which have already been cooked, for example cooked shrimps, should be warmed up slightly.

Training of assessors

- **Objective -** effects of personal influence are minimized, used to distinguish between two or more products, based on certain attribute of raw fish (skin, eyes, gills, texture, etc.)
- **Subjective –** acceptance is freely expressed, often called hedonic, used in product development and market research, what ordinary consumers think

Sensory evaluation of seafood: methods

In sensory evaluation of seafood, grading, ranking and scaling methods are the most frequently used methods.

- **Difference tests**

Difference tests can be used to determine whether a difference exists in a single sensory attribute or in several. They determine whether there exists a perceptible difference in a given attribute, and the specification of the direction of difference.

- ✓ ranking (ISO 8587, 1988) - arrange in order according to the degree, used for preliminary screening
- ✓ triangle test (ISO 4120, 2004) – select one out of three coded samples
- ✓ paired comparison test (ISO 5495, 2005) – select from two set of samples

- **Grading schemes**

Grading is the process of applying a categorical value to a lot or group of products. Sensory grading most often involves a process of integration of perceptions by the grader.

Examples : EU quality grading scheme (EU-scheme) and the Torry scheme

European Union Scheme

The EU scheme its main advantage is that the fluctuation between assessors is diminished. 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, while below B is the level where fish is considered unfit for human consumption. The method only uses general parameters to describe freshness quality, it does not take different sensory characteristics of different species into account, nor does it provide useful information about the past or remaining storage time as it is too general.

The EU scheme is criticized for its limitations in that it does not take into account the differences between species (uses only general parameters) and mixes both subjective and objective sensory methods in the assessment scheme.

	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
Eyes	Convex,black,bright pupil,transparent cornea	Convex and slightly sunken, black,dull pupil	Flat,opalescent cornea, opaque pupil	Concave in the centre ,grey pupil, milky cornea
Gills	Bright colour,no mucous	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	Speckled, 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

Torry scale

- ✓ The Torry scale is the first detailed scheme developed for evaluating the freshness of cod (Shewan *et al.* 1953).
- ✓ 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 the most frequently used industry scale for evaluating the freshness of cooked fish.
- ✓ It is used both by producers and buyers.
- ✓ It is a descriptive 10-point scale that has been developed for lean, medium fat and fat fish species. Scores are given from 10 (very fresh in taste and odour) to 3 (spoiled). It is considered unnecessary to have descriptions below 3, as the fish is then not fit for human consumption.
- ✓ The average score of 5.5 has been used as the limit for 'fit for consumption'

- ✓ Members of the sensory panel detect evident spoilage characteristics, such as sour taste and hints of 'off' flavours.
- ✓ The Torry scale has been developed for lean, medium fat, and fatty fish species.

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.

- ✓ QIM is widely accepted as a reference method in research.
- ✓ In quality management it is important to be able to apply a sensory system that reflects the different quality levels in a simple, reliable and documented way.
- ✓ QIM has those advantages, in addition to being rapid, cheap to use, non-destructive and objective compared with other sensory methods.
- ✓ Further, it is easy to work with as it includes instructions. It is a convenient method to teach inexperienced people to evaluate fish, and to train and monitor performance of panellists.
- ✓ In QIM, a demerit score is employed to assess the quality of fish by a panel of experts. The demerit score consists of different quality attributes, whose quality can be assessed by giving demerit score to each attributes/factors
- ✓ As the quality index (the total sum of scores, referred to as the QI) is designed to increase linearly with storage time
- ✓ QIM is based on characteristic changes that occur in seafood with storage time in ice. A score from 0 to 3 points is given for changes of parameters

  A demerit score system developed by Branch and Veil (1985) having a total demeit score of 39 is given below

Factor being assessed	Observation	Demerit points
Appearance of surface	Very bright Bright Slightly dull Dull	0 1 2 3
Skin	Firm Soft	0 1
Scales	Firm Slightly loose Loose	0 1 2

Slime	Absent Slightly slimy Slimy Very slimy	0 1 2 3
Stiffness	Pre rigor Rigor Post rigor	0 1 2
Eyes clarity	Clear Slightly cloudy cloudy	0 1 2
Shape	Normal Slightly sunken Cloudy	0 1 2
Iris	Visible Not visible	0 1
Blood	No blood Slightly bloody Very bloody	1 1 2
Gill colour	Characteristic Slightly dark Slightly faded Very dark/very faded	0 1 2 3
Mucus	Absent Moderate Excessive	0 1 2
Smell	Fresh oily Seaweedy Fishy Stale/Spoiled	0 1 2 3
Belly discolouration	Absent Detectable Moderate Excessive	0 1 2 3
Firmness	Firm Soft Burst	0 1 2
Vent condition	Normal Slight break/exudes Excessive	0 1 2
Smell	Fresh Neutral Fishy Spoiled	0 1 2 3
Bell y cavity stains	Opalescent Greyish Yellow brown	0 1 2
Blood	Red Dark red Brown	0 1 2

Total demerit point		0-39

FISHQCheQ App

- ICAR – CIFT has developed a demerit score based fish quality index (FQI) system for assessing/ evaluating quality/ freshness of fresh fish.
- The developed FQI system considered five general quality characteristics viz.
- Appearance of fish outer surface, fish eyes and gills, condition of fish belly and vents.
- This web based system would help the consumer to check/ evaluate the quality of fresh fish instantly.
- This system provides the instructions for evaluating the quality/ freshness of fish.
- After evaluating the fish in terms of quality characteristics description, the consumer or user has to select appropriate score given in the element score sheet.
- After complete selection of demerit score, a final fish quality index (FQI) would be computed automatically along with the quality description of fish.
- Both web and mobile application (Google play store) is available

Hygiene handling of fish

A high level of care is required while handling the harvested fish as the fish is highly perishable compared to any other food commodities. Implementing good post-harvest handling practices is essential to keep the fish safe and in good condition till it reaches the consumers. Bacterial spoilage is the most important factor affecting fish quality. Bacterial growth on fish is slowed by proper cleaning, dressing and chilling. Chemical changes, including oxidation, causes a fish to have a fishy odour. Chemical changes can be slowed if the fish is kept out of sunlight, is kept covered and moist, and is chilled properly. Fish muscle tissue is fragile compared with muscle tissue of other animals. Bruising, gaping, and mushy flesh can all be reduced if fish are handled gently and chilled quickly. Dehydration, or drying out can be avoided if fish are chilled quickly and kept covered with ice or chilled seawater. Contaminants should be kept away from fish and from surfaces that come in contact with fish. Fuel, oil, paint, cleaners, and other such chemicals should never be stored in a fish hold. Only clean ice made from potable water should be used to chill fish.

Onboard handling practices

- A good supply of clean or potable water at adequate pressure should be available.
- Non potable waterlines should be clearly identified and separated.

- Ice prepared from potable water under hygienic conditions should be available to preserve fish.
- Fish receiving deck shall be smooth, clean and free from engine oil, grease, etc. Adequate facilities should be provided for washing and disinfecting equipment, where appropriate.
- Objectionable substances, which could include bilge water, smoke, fuel oil, grease, drainage and other solid or semi-solid wastes, should not contaminate the fish and shellfish.
- Containers for offal and waste material should be clearly identified, covered and made of impervious material.
- Separate and adequate facilities should be provided to prevent the contamination of fish and shellfish by poisonous or harmful substances; offal and waste materials.
- Adequate hand washing and toilet facilities, isolated from the fish and shellfish handling areas, should be available where appropriate.
- The artificial lights provided on the deck and in the hold shall have protective covers. Facilities to prevent the entry of birds, insects or other pests, animals or vermin should be provided, where appropriate.

Fish Landing Site/ Harbour

Location and Surroundings

- The Landing Site / Fishing Harbour shall be located at a site ideal for the purpose and shall be free from undesirable smoke, dust, other pollutants and stagnant water. The premises shall be kept clean.

Design and Construction

- Suitable covering shall be given at the fish landing site/harbour to protect fish and shellfish from environmental hazards such as sun light, rain, wind blown dust etc.
- Adequate working space shall be provided for hygienic handling of fish and shellfish.
- Floor and walls shall be smooth and easy to clean and disinfect. The floor shall have sufficient slope for proper drainage and to avoid stagnation of water.
- Drainage lines of adequate size and slope shall be provided to remove waste water, the outlet of which shall not open to the sea near the landing berth.
- Landing site shall be constructed in such a way to avoid entry of exhaust fumes from vehicles.

- Preferably, separate auction hall(s) may be provided, which is well protected from the entry of pests/insects, for display and sale of fish and shellfish.
- Fish and shellfish shall not be kept directly on floor. Raised platforms shall be constructed for display, which are smooth, easy to clean and disinfect.

Equipments and Containers

- The containers and equipment used to handle fish and shell fish shall be smooth, impervious and made of corrosion free material, which is easy to clean and disinfect and kept in a good state of repair and cleanliness.

Facilities and Utilities

- Provision for adequate quantity of potable water or clean sea water shall be available in the landing sites for cleaning and sanitation. Non potable waterlines should be clearly identified and separated
- Facilities for hygienic handling and storing of sufficient quantity of good quality ice and provision for crushing the ice hygienically shall be provided, as applicable.
- Adequate facilities should be provided for washing and disinfecting equipment, where appropriate.

Marketing

Location

- The facility shall be located in the areas not subjected to regular and frequent flooding, and shall be free from undesirable odour, smoke, dust, pest, and other contaminants.
- The facility shall have adequate drainage and provision for easy cleaning.
- The area should provide ease of transportation of fish towards the market and outwards

Premises Requirements and Construction

- Facility shall be constructed to enable hygienic processing and sale of fish and fish products to ensure food safety
- Sufficient parking facility, loading and unloading facility for fish, cleaning facility for fish transportation vehicle, fish storage crates, chilled fish storage, solid waste disposal facility, effluent treatment plant etc may be provided.
- In wholesale fish markets raised platforms with drainage facility
- A sign board indicating the type of fish and fish products sold shall be displayed prominently.
- The surfaces of walls, partitions and floors of retail area shall be made of impervious materials for easy cleaning and sanitation

- adequate space for the fixtures, fittings and equipment used
- Fish laid down for sale shall not come into direct contact with floors, walls or other fixed structures
- Doors, windows and floors shall be constructed for effective cleaning & sanitation
- provide protection to avoid entry of flies, other pests and stray animals
- There shall be an adequate supply of potable water.
- Fish handlers shall be provided facilities for cleaning their hands and toilet facilities.

Reference

1. Codex guidelines for sensory evaluation of fish and shellfish in laboratories
2. Food Safety Management System (FSMS) Guidance Document for Fish and Fish Products

Chapter 4

Quality Issues in Live/Fresh/Chilled/Frozen Fish and Fish Products

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Fish and fishery products constitute an important component of human diet. Contribution of fish and other aquatic products in the average animal protein consumed worldwide is around 15%. Fish and other seafood items in daily diet is a smart choice for health conscious consumers. There are proven health benefits of consumption of aquatic products that out-weigh risks. Some of them are high content of omega-3 long-chain (>C20) polyunsaturated fatty acids that are found as high as 2-3% in tropical fishes. Documented proven health benefits of omega-3 fatty acids include aiding infant development, reduction of childhood asthma, lowering risk of breast cancer, protection against coronary heart disease and acute coronary syndrome, reduction of age-related macular degeneration, slow progression of Alzheimer's disease, reduction of depression and alleviation of symptoms of rheumatoid arthritis. Compared to marine fishes, freshwater fishes are characterized by elevated levels of omega-6 PUFA, especially linoleic (18:2) and arachidonic acids (22:4). Although PUFA content of freshwater fishes are lower than their marine counterparts, the levels are substantial to impart nutritional value. Apart from that fish is a valuable source of minerals such as calcium, phosphorus, iron, copper and selenium which are essentially required for human nutrition. Compared to other animal proteins, fish proteins are highly digestible with a balanced source of essential amino acids. Further, cholesterol levels in fish are also quite low (24-85 mg/100g) making it more amenable to present generation of health conscious consumers. Although fishes are not known to be good source of vitamins, levels of niacin, B12 and B6 are comparable to other protein rich foods. Some freshwater fishes like Salmon and Trout are known be good source of Vitamin D.

In aquacultured fish, the nutritional parameters are heavily influenced by the feeding regime as well as culture conditions. It also offers scope of artificially enriching cultured species with PUFA or similar nutrients.

Ensuring Quality in live/fresh/chilled/frozen fishes

The major reasons behind low processing of freshwater fish are lack of quality control measures at the production site and absence of cold chain network. Main quality concerns in live, fresh, chilled and frozen fishes are as follows:

Pesticide residues: As most of the waterbodies such as reservoirs and village ponds used for fish culture are multi-purpose in nature, there is a definite possibility of contamination with pesticides from anthropogenic sources. Riverine and lacustrine environments receive pesticide load from discharge of sewerage and industrial wastewaters. Pesticides also come from agricultural runoff and seepage through ground water contamination. After getting released into the environment, they are transformed into a range of different products based upon their susceptibility to biotic and abiotic degradation. These compounds are mobile, more persistent, and often more toxic to non-target organisms than the precursor parental pesticides. The organochlorine pesticides which are mostly detected in freshwater fishes are DDT, DDE, DDD, HCB, HCHs, CHLs, Aldrin, Dieldrin and Endrin.

Various herbicides, weedicides and insecticides are also used in aquaculture farms as a part of farming practices, especially during pond preparation. Presence of higher levels of DDT, HCH, Aldrin, Dieldrin, Endosulfan, Chloropyrifos and malathion in some aquatic water bodies has been reported. Lower residues of DDT and HCH in tropical fishes compared to temperate countries are ascribed to rapid volatilization of these organochlorine pesticides in tropical environments.

Presence of other persistent organic pollutants (POPs): As natural water bodies like rivers and lakes bear the onslaught of industrial dishrags, presence of persistent organic pollutants in fish tissue has raised concern. Important among them are dioxin and dioxin like compounds (PCDD/PCDF and PCBs), brominated flame retardants (BFRs), polychlorinated naphthalenes (PCNs) and polyaromatic hydrocarbons (PAHs). Incomplete combustion during waste incineration is the main reason behind loading of PCDD and PCDFs into the environment. The most toxic compound is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a group 1 carcinogen which if present in food can cause severe reproductive and developmental problems, apart from the cancer.

Extra-label use of chemicals and drugs in aquaculture: As aquaculture has become a commercial venture, chemicals and drugs are increasingly used to boost production. It has also brought into focus which the extra-label use of drugs i.e drugs meant for human medicine are increasingly used in treating fish diseases. As fish is a food commodity, the residue of the drugs passes on to the consumers and pose serious health hazard. Use of pharmacologically active

substances like antibiotics, hormones, steroids, and anti-parasitic agents are reported in aquaculture practices.

Use of unapproved additives for preservation: In order to gain commercial advantage and extra shelf life, sometimes many un-approved/prohibited substances are used in freshwater fish preservation. Although health implications for many of these substances are poorly studied, use of these additives either not listed as GRAS or unspecified, bring challenges for domestic trade and regulatory agencies.

Adulteration with ammonia and formaldehyde: In order to give a false façade of freshness, freshwater fishes are often adulterated with varying concentration of ammonia. Higher ammonia content is not only hazardous to fish handlers, it also downgrades the organoleptic attributes to a large extent. Similarly, formaldehyde is used to mask spoilage in some parts of India. Presence of formaldehyde poses a serious health hazard for the consumer.

Presence of human pathogenic bacteria: As most of fish farms are situated close to human habitation and large waterbodies face the problem of dumping of un-treated domestic waste, presence of human pathogenic bacteria are often noticed in freshwater ecosystems. Presence of *Vibrio cholerae* and *Salmonella* has been reported in aquaculture ponds. Unhygienic handling at the farm site or onboard fishing boat and subsequent handling during auction or resale results in unhindered proliferation of pathogenic bacteria like *Staphylococcus aureus*, *Salmonella*, *Shigella* and *Escherichia coli*.

Off flavours: Many cultured freshwater fishes and prawns face the problem of muddy, earthy and mouldy off flavours. Geosmin and 2-methylisoborneol are two primary compounds responsible for musty or earthy flavours which are secondary metabolites produced by various actinomycetes and cyanobacteria. Geosmin is rapidly absorbed through gills and temperature plays an important role in rate of absorption and depuration from fish body.

Ushering quality in fresh/chilled and frozen fish

The term quality has no standard definition; it is used as a qualifier in describing some product or service. ISO defines quality as "*degree to which a set of inherent characteristics that fulfills requirements*". American Society of Quality (ASQ) defines quality as "*the totality of characteristics of a product or service that bear on its ability to satisfy stated and implied needs*". The different dimensions of quality include performance, features, reliability, conformance, durability, serviceability, aesthetics and perception. Although all these dimensions are not applicable for fishery products, pursuit of quality at every stage of value chain has been a priority requirement for all stakeholders.

The term 'quality control' and 'quality assurance' is used interchangeably without understanding the basic difference between the two. According to ISO (ISO 8402 – Terminology), quality assurance (QA) is defined as *all those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality*. Hence, QA is a strategic management function that establishes policies, adapts programs to meet established goals, and provides confidence that these measures are being effectively applied. Quality Control (QC), on the other hand, is defined as *the operational techniques and activities that are used to fulfill requirements for quality* (ISO 8402 — Terminology), i.e., a tactical function that carries out the programs established by the QA.

Quality assurance approach got its inception with the advent of HACCP (Hazard Analysis and Critical Control Point) concept, which was based on preventing rather than correcting the occurrence of defects and hazards or the presence of foreign substances during product manufacture. Further changes have taken place in QA with the development of the concepts and applications of Total Quality Management (TQM). Total Quality Management (TQM) is a theory of management based on the principles of quality assurance. As defined by British Standard (BS7850-1), *TQM is a management philosophy and company practices that aim to harness the human and material resources of an organization in the most effective way to achieve the objectives of the organization*. The nine common TQM practices adopted for food manufacturing are cross-functional product design, process management, supplier quality management, customer involvement, information and feedback, committed leadership, strategic planning, cross functional training and employee involvement.

Function of quality assurance programme in freshwater fish processing can be as follows:

1. Development and implementation of a good hygiene and sanitation programme
2. Implementation of food safety QA prgramme
3. Internal audit of all QA programmes

Quality assurance systems are intended to provide confidence to a food company's management, its customers and to government regulatory agencies that the company is capable of meeting the food quality and food safety requirements.

Measures to retain quality

The international standards ISO 9001:2000 and ISO 9004:2000 have been formally used by food processing industries worldwide as a quality management standard. The ISO standard on food safety management system (ISO 22000:2018) was later on developed to

specifically cater to food industry. This standard is developed with the key elements of interactive communication, system management, pre-requisite programmes and HACCP principles. In India fish processing industries are gradually adopting this standard to strengthen international acceptance of Indian fishery products. Apart from ISO 22000:2018, many private food safety standards are being adopted. All these standards are formulated keeping intact the principles of HACCP (Hazard analysis and critical control point). Hence any domestic or international food safety standard can be very well implemented if HACCP principles are well understood.

Hazard Analysis and Critical Control Point (HACCP)

Hazard Analysis and Critical Control Point (HACCP) evolved as a quality assurance approach in late 1950's has been embraced as a food safety management tool throughout the world. Compared to traditional end product testing based food safety programmes, HACCP is a dynamic, preventive system of food control with a prior anticipated risk-response approach.

HACCP is a preventive system to control significant identified hazards. It also functions by designing food safety into a product and controlling the process by which the product is produced. However, it should be noted that HACCP does not rely on end product testing or lot acceptance criteria. HACCP is a core component in all national and international food safety standards such as IS 15000, ISO 22000:2005, USFDA Seafood HACCP regulation (CFR 123, Title 21), Dutch HACCP, BRC Gloabal Standard for Food, SQF 2000, IFS, etc.

HACCP is a system that identifies, evaluates, and controls hazards that are significant for food safety. As described by Codex Alimentarius Commission (CAC/RCP 1-1969; Rev. 4 - 2003) HACCP can be implemented by 12 logical steps that include five preliminary steps and seven principles.

Step 1.	Assemble HACCP team	Preliminary Steps
Step 2.	Describe product	
Step 3.	Identify intended use	
Step 4.	Construct flow diagram	
Step 5.	On-site confirmation of flow diagram	
Step 6.	Conduct hazard analysis	HACCP Principle I
Step 7.	Determine Critical Control Points (CCP)	HACCP Principle II
Step 8.	Establish critical limits for each CCP	HACCP Principle III
Step 9.	Establish a monitoring system for each CCP	HACCP Principle IV
Step 10.	Establish corrective actions	HACCP Principle V
Step 11.	Establish verification procedures	HACCP Principle VI
Step 12.	Establish Documentation and Record Keeping	HACCP Principle VII

Conclusion

Fish and fishery products have an edge over other animal products in terms of plethora of health benefits. But it's an arduous task to maintain quality starting from subsistence farming to commercial processing activity. Incorporation of modern food safety management tools coupled with emphasis on basic hygiene and sanitation measures throughout the food chain is the only solution. Then only we can claim fish as not only nutritious, but also safe.

Chapter 5

Chemical Quality Indices and Freshness Indicators for Fish and Shellfish

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Products with no proven quality find little space in the market. The term quality represents the consumer acceptability of the commodity. The eating quality of fish determines the acceptability of fish as food to the consumer. The quality of fish begins to deteriorate immediately after catch. Therefore, the fish needs to be preserved well soon after the harvest to maintain its freshness and prevent deterioration. The process of quality deterioration or change in fish or fish product that renders it less acceptable, unacceptable, or unsafe for human consumption is known as spoilage. Spoilage can be

1. Microbial
2. Physical
3. Chemical

The most commonly used method for the quality evaluation of raw fish is sensory evaluation. Although the method is simple and rapid, the main disadvantage is the lack of objectivity. During spoilage, a number of chemical reactions are taking place in the fish muscle. Various compounds are formed during these reactions, which are quantitatively determined, correlated with sensory characteristics, and used as spoilage indices.

During spoilage, various compounds are produced in the fish muscle by autolytic enzymes, putrefactive microorganisms, or chemical reactions and gradually get accumulated in the flesh. Hence the quantitative determination of these compounds will provide a measure of the spoilage process. The spoilage indices for fish and shellfish are as follows:

1. Volatile bases
2. Nucleotides
3. Lipid oxidation products

Total Volatile Bases:

Volatile bases are produced by spoilage bacteria in fish. They are basic nitrogenous compounds such as ammonia, trimethylamine (TMA), Trimethylamine oxide (TMAO), and Dimethylamine *etc*., The most commonly used index of quality for the freshness of fish is the Total Volatile Base Nitrogen value (TVBN) along with Trimethylamine. Fish with a TVBN

value of 20mg/100g is considered very fresh. The limit of acceptability of TVBN is 35-40 mg/100g beyond which the fish is considered as spoiled.

Trimethylamine (TMA):

Trimethylamine is the specific index used for assessing the freshness of marine fish. In most cases, the TMA concentration is extremely low, normally under 1mg N/100g. Studies indicate that in a few bivalves, the TMA content is about 20mg N/100g. In elasmobranchs and marine teleosts, the viscera, especially the spleen, liver, and kidney contain the most TMA and the muscle the least. The midgut gland has the highest level of TMA in squid.

TMA is derived from TMAO which is critical for osmoregulation in marine fish.

Two types of enzymes are considered to be responsible for the reduction of TMAO to TMA and to DMA and formaldehyde (FA)- endogenous enzymes in fish, and exogenous enzymes produced by spoilage bacteria. The strains of bacteria capable of reducing TMAO to TMA have been found in most species of the Enterobacteriaceae including *Escherichia coli, Achromobacter, Micrococcus, Flavobacterium*, nonfluorescent *Pseudomonas, Clostridium, Alcaligenes,* and *Bacillus* spp. TMAO is reduced by bacterial enzymes to TMA while the endogenous enzymes reduce TMAO to DMA and then to FA. During frozen storage, the production of DMA is greater than that of TMA. Hence DMA can be used as an index of enzymatic deterioration during frozen storage and TMA as an index of pr-freezing quality. The formation of DMA is accompanied by the equimolar formation of formaldehyde (FA), which can cause the denaturation of myofibrillar protein in fish flesh.

A level of 10-15 mg TMA-N/100g muscle is considered as the limit of acceptability. This level increases with storage time during iced storage hence TMA can be used as a good index of spoilage.

Ammonia:

Bacterial spoilage of fish generates small amounts of ammonia from the free amino acids. The ammonia content can be used as an indication of the extent of spoilage. A greater amount of ammonia is produced during the spoilage of elasmobranchs due to the high content of urea in their flesh. Shellfish can also produce a large amount of ammonia than marine fishes at the early stages of spoilage.

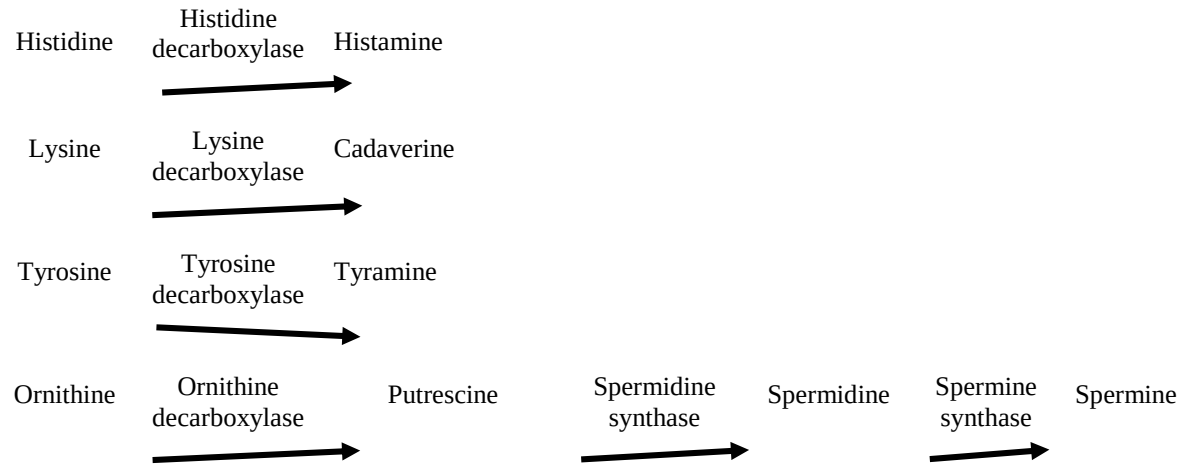
Biogenic amines:

Biogenic amines are non-volatile compounds, found at very low levels in fresh fish. Important biogenic amines are histamine, cadaverine, putrescine, tyramine, tryptamine, spermine and spermidine. Histamine is known to be the causative factor of scombroid poisoning/histamine poisoning in histamine-forming fishes such as mackerel, tuna, sardine, bonito, herring, anchovy

etc., Food Safety and Standards Authority of India has identified the following family of fishes as histamine forming fish species.

1. Carangidae – 30 species of fishes including jacks, scads, pompanos, queen fishes, kingfishes, and trevallies
2. Chanidae (Milkfish)
3. Clupeidae – 33 species of fishes including Sardine and Shad
4. Coryphaenidae (Mahi Mahi/Dolphin fish)
5. Engraulidae – 9 species of anchovy
6. Istiophoridae – 9 species of Marlin/Sailfish
7. Mugilidae (Mullet)
8. Pristigasteridae – 2 species of Ilisha/Pellona
9. Scombridae – 32 species of fishes including Mackerel, Tuna, Bonito, and Seer fish
10. Xiphiidae (Swordfish)

These fishes are found to be having high free histidine content which gets converted into histamine during spoilage. The biogenic amines formed during the spoilage of fish are found to be thermally stable and thus can be used as an indicator of poor quality of raw material in preserved/processed fishery products. Cadaverine and putrescine are found to be potentiators of histamine. The direct precursors of histamine, cadaverine, and putrescine are histidine, lysine, and ornithine respectively. Putrescine is also an intermediate of a metabolic pathway that leads to the formation of spermidine and spermine.



Although biogenic amines have been associated with fish spoilage, the legal limit has been established for histamine only. As per Food Safety and Standards Regulation (FSSR, 2011), the maximum permissible level of histamine content in fish and fishery products is 200mg/Kg.

Fishes with histamine content up to 20mg/kg are considered to be safe for consumption, 20-100mg/Kg is probably safe while ≥100mg/kg is toxic and unsafe for consumption.

Studies also indicated that cadaverine and putrescine can also be used as freshness indices for fish and shellfish respectively. Fish and fishery products containing cadaverine below 15mg/100g are considered as good for consumption, 15-20mg/100g indicates potential decomposition, and over 20mg/100g advanced decomposition. The quality Index (QI) and Biogenic Amine Index (Bai) are also used to indicate the freshness of fish.

$$QI = \frac{Histamine + Putrescine\ + Cadaverine}{[1 + (Spermidine + Spermine)]}$$

$$BAI = (Histamine + Putrescine + Cadaverine + Tyramine)$$

Indole

Indole is a spoilage indicator in shrimp and crab. Indole (2,3-benzopyrene) is a degradation product of tryptophan. Indole is highly volatile and soluble in different solvents such as hot water, alcohol, ether, and benzene. Shrimp with indole content <25mg/100g is organoleptically acceptable.

Nucleotide degradation products:

Nucleotide degradation is one of the earliest indices to assess freshness. It reflects both the action of autolytic enzymes and bacterial action. The nucleotide degradation products – Inosine Monophosphates (IMP), Hypoxanthine (H_x) or K value clearly reflects the quality loss in fish. After the death of the fish, Adenosine triphosphate (ATP) is degraded by endogenous enzymatic action and forms Adenosine diphosphate (ADP), Adenosine monophosphate (AMP), IMP, Inosine (Ino), and H_x successively. Hypoxanthine is further degraded by xanthine oxidase to xanthine and uric acid. The degradation of ATP up to IMP is very fast, but the degradation of IMP is relatively slow. IMP imparts a pleasant, sweet taste and flavor (Umami taste especially in crabs. Degradation of IMP to inosine and hypoxanthine results in bitter taste and progressive loss of desirable flavor. The sequence of nucleotide catabolism in fish as given below:

ATP

↓

ADP

↓

AMP

↓

IMP

↓

Ino

↓

H_x

↓

Xanthine

↓

Uric acid

K value is a biochemical index for fish quality assessment based on nucleotide degradation. K value includes intermediate breakdown products, and it varies with species of fish. K value is the percentage of the intact ATP present at death that has been converted by enzymatic action into hypoxanthine and its immediate precursor called inosine in the chain of decomposition of ATP.

$$K\ (\%) = [\frac{Ino + Hx}{ATP + ADP + AMP + IMP + Ino + Hx}] \times 100$$

Lipid oxidation products:

During processing and storage, enzymatic and non-enzymatic lipid oxidation occurs. Lipid oxidation is the limiting factor in fatty fish during storage, which results in rancidity (development of off-flavor and off-odor). The factors affecting the onset and development of rancidity are

1. Degree of unsaturation
2. Type and concentration of antioxidants
3. Pro-oxidants
4. Moisture content
5. Oxygen availability
6. Temperature
7. Degree of exposure to light

The major chemical indicators for the determination of the extent of oxidative rancidity are anisidine value (AV), peroxide value (PV), and thiobarbituric acid value (TBA). Peroxide value is also known as hydroperoxide value, used as a measure of the extent of oxidation in the early stages. It measures the primary products of lipid oxidation, which break down into secondary products of oxidation or reacts with protein. An increase in PV is most useful as an index of the earlier stages of lipid oxidation; as the oxidation proceeds the PV start to fall. AV and TBA measures the secondary product of lipid oxidation. TBA measures the malonaldehyde produced during lipid oxidation. It can be assessed that if the PV value is 10-20 mg oxygen/kg or TBA is above 1-2 mg of malonaldehyde per kg of sample, then the fish will in all probability smell and taste rancid. During prolonged storage of fish, PV, AV and TBA values may increase reaching a peak and decline.

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Chapter 6

Quality Issues in Traditional/Ethnic Fishery Products and its Control Measures

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Introduction

Traditional fishery products hold a unique place in the country's rich culinary heritage. For generations, these products have not only served as a source of nourishment but have also been an integral part of cultural and regional identity. The major traditional fishery products include dried/salted fish products, smoked fish products, pickled/marinated fish products, fermented fish products, etc. Traditional fishery products also play a critical role in addressing food security and providing livelihoods to millions of people by providing employment opportunities, particularly in rural areas. They are a valuable source of protein, vitamins, and essential nutrients, making them essential for a balanced diet, especially in regions where alternative sources of protein are limited. While traditional fishery products hold immense cultural and economic value, they also face significant challenges in maintaining quality. The production, preservation, and distribution of traditional fishery products come with their fair share of challenges, leading to various quality issues. Quality issues in traditional fishery products poses a significant concern in the domestic and international markets causing huge economic loses. Traditional fishery products, often valued for their cultural heritage and artisanal production methods, face challenges related to product consistency, safety, and adherence to international quality standards. One of the primary challenges is the lack of standardized quality control measures. Unlike modern fishery practices, where quality can be closely monitored throughout the supply chain, traditional fishery products often pass through multiple intermediaries, increasing the risk of quality degradation. These issues can encompass various aspects, including the handling, processing, and storage of fishery products, as well as concerns regarding traceability and sustainability. Addressing these quality issues is crucial not only for the preservation of traditional culinary practices but also to ensure consumer confidence and access to safe and high-quality fishery products in an evolving global market. In this context, exploring the factors contributing to quality problems in traditional fishery products and developing strategies to overcome them is essential for the industry's sustainability and the satisfaction of consumers worldwide.

Common quality issues encountered in traditional fishery products

The common quality issues that afflict traditional fishery products that compromise their safety and quality attributes are discussed below.

1. Spoilage

Traditional fishery products are highly perishable, and improper handling, storage, or transportation can lead to rapid spoilage. Several factors are responsible for causing spoilage and affecting the quality of fish. Factors such as improper storage conditions, inadequate salting, drying, or smoking techniques, and exposure to contaminants can accelerate spoilage. The consequences of spoilage extend beyond the loss of product quality; they can also pose health risks to consumers. Foul odours, off-flavours, and changes in texture are common indicators of spoilage. To combat this issue, it's crucial to educate producers and consumers on proper preservation techniques and storage practices. Additionally, implementing modern technologies for monitoring and controlling the environmental conditions during production and distribution can help extend the shelf life of traditional fishery products, ensuring that they remain safe, enjoyable, and true to their cultural heritage.

2. Rancidity

Rancidity is a significant concern in traditional fishery products, particularly those subjected to long-term storage and preservation methods. The primary cause of rancidity in these products is the breakdown of fats and oils, which are abundant in fish. When exposed to air, light, and temperature fluctuations, the fats in fish can undergo oxidation, leading to the development of off-flavours and odours, ultimately rendering the product unpalatable. Traditional fishery products like salted fish, dried fish, or fermented fish are especially susceptible to rancidity if not properly stored in a cool, dark, and dry environment. To mitigate rancidity, traditional methods of preservation should be complemented with modern packaging and storage techniques, such as vacuum-sealing and refrigeration. Additionally, educating producers and consumers about the importance of proper storage and handling can help extend the shelf life of these valuable traditional products while preserving their unique flavours and cultural significance.

3. Contamination with physical, chemical and biological agents

Traditional fishery products can be susceptible to various forms of contamination, including physical, chemical, and biological sources. Physical contamination occurs when foreign objects, such as pieces of metals, glass, ceramics or quartz materials, come into contact with the fish during processing. These contaminants can compromise the safety and quality of the fishery products, leading to potential health risks for consumers. Chemical contamination is another concern, and it can arise from various sources. Pesticides and herbicides from agricultural runoff, heavy metals

like mercury and lead from polluted water bodies, and chemicals used in fishing practices such as preservatives or cleaning agents can all find their way into traditional fishery products. These chemical contaminants can accumulate in the fish and pose health risks when consumed by humans. Biological contamination involves the presence of pathogen like bacteria, viruses, and parasites in traditional fishery products. These pathogens can thrive in unhygienic processing environments or when fish are stored improperly. Consuming fish contaminated with harmful microorganisms can lead to foodborne illnesses, making it essential to maintain strict sanitation practices throughout the supply chain. To ensure the safety and quality of traditional fishery products, it is crucial to implement rigorous quality control measures, adhere to food safety regulations, and promote sustainable fishing practices that minimize contamination risks. Monitoring and testing for physical, chemical, and biological contaminants are vital steps in safeguarding the integrity of these products and protecting the health of consumers.

4. Textural changes

Textural changes in traditional fishery products are a critical aspect of their overall quality and consumer appeal. Freshly caught fish often have firm and resilient textures, but over time, post-harvest processes such as drying, salting or smoking can alter these textures. Drying or smoking removes moisture from the fish, leading to a firmer and denser texture, which is highly prized in some traditional products like salted fish or smoked salmon. Maintaining the desired texture in traditional fishery products is a delicate balance that requires precise control of processing parameters and careful handling. Texture plays a pivotal role in determining the overall sensory experience and consumer satisfaction.

5. Flavour changes

Over time, traditional fishery products may undergo aging and fermentation processes, intensifying their flavours and creating a more pronounced umami profile. The maturation of these products can be highly valued by regular consumers, as it adds depth and complexity to their taste. But at the same time, some undesirable flavours changes do occur due to improper handling and storage condition leading to poor consumer preference.

6. Discolouration

Discoloration is a common issue that traditional fishery products may encounter during their production and storage. It can affect both the appearance and, in some cases, the quality of these prized culinary items. Discoloration in fishery products can manifest in various ways, often due to factors such as exposure to oxygen, enzymatic reactions, or microbial activity. One of the most noticeable forms of discoloration is the browning or darkening of fish flesh when exposed to air. Additionally, fish tissues can result in colour changes due to the enzymatic breakdown of

pigments. Microbial activity can also contribute to discoloration, as bacteria can produce pigments that alter the appearance of fishery products. This is often seen in the form of slimy or off-coloured surfaces, which can be indicative of spoilage and pose food safety concerns. To mitigate discoloration in traditional fishery products, various strategies are employed, including vacuum sealing, packaging in a protective atmosphere, or the use of antioxidants to prevent oxidation. Maintaining proper temperature and hygiene throughout the supply chain is crucial in minimizing the risk of discoloration and preserving the product's visual appeal and quality. Addressing discoloration not only ensures the aesthetics of traditional fishery products but also helps maintain consumer trust by delivering products that are not only safe but also visually appealing and appetizing.

7. Disintegration

The issue of disintegration in traditional fishery products can be a significant concern, affecting their overall quality and consumer satisfaction. Disintegration refers to the loss of structural integrity in the product, leading to it falling apart or breaking down prematurely. This problem can manifest at various stages, from production to consumption. During processing, traditional fishery products may be susceptible to disintegration if they are not handled with care. Overly aggressive handling can result in disintegration issues. This not only affects the product's appearance but also its texture and eating experience. Traditional dishes often rely on the texture and structural integrity of these products, so disintegration can compromise the authenticity of the cuisine. To mitigate disintegration issues, producers can focus on optimizing processing techniques to maintain the product's structural integrity.

8. Loss of Nutritional value

The loss of nutritive value in traditional fishery products is a concern that can arise from various factors, including processing methods and storage conditions. Traditional fishery products, when processed and preserved improperly, can experience a reduction in their nutritional content. Processing techniques, like smoking or drying, may lead to nutrient loss due to the exposure of fishery products to high temperatures or extended drying periods. Another consideration is the impact of storage conditions. Traditional fishery products that are not adequately stored can be susceptible to oxidation, which can lead to the degradation of healthy fats like omega-3 fatty acids. Moreover, improper storage can result in nutrient loss through the action of enzymes or the growth of microorganisms. To address the issue of lost nutritive value, traditional fishery product producers can ensure proper storage conditions and distribution channel.

9. Packaging issues

Packaging issues in traditional fishery products present a significant challenge to both producers and consumers alike. One key concern is the preservation of product quality and safety. Traditional packaging methods often lack the protective properties needed to keep fishery products free from contamination during storage and transportation. Inadequate packaging can lead to spoilage, bacterial growth, and loss of product integrity, ultimately impacting the product's marketability and consumer satisfaction. Moreover, traditional packaging may not provide proper labeling and information for consumers regarding product origin, ingredients, and expiration dates, which is essential for making informed choices and ensuring food safety. Additionally, the environmental impact of traditional packaging materials, such as plastic and non-biodegradable materials, is a growing concern as they contribute to pollution and harm marine ecosystems. Addressing these packaging issues is crucial to maintain the quality and safety of traditional fishery products, support the livelihoods of fishermen, and meet the growing demand for these products in domestic and international markets.

Factors contributing to quality issues in traditional fishery products

Several factors are responsible for the deterioration of quality in traditional fishery products. These factors are given below.

1. ***Improper handling practices***: Improper handling of fishery products leads to physical damage, bruising, or contamination. These practices can significantly impact product quality.
2. ***Poor hygiene and sanitation***: Poor hygiene and Sanitation during processing and packaging can introduce bacteria and pathogens, leading to spoilage and potential health risks for consumers.
3. ***Temperature and humidity***: Drying or smoking, may not provide adequate protection against environmental factors like humidity, temperature fluctuations, or pests, which can compromise product quality and safety.
4. ***Water activity***: High water activity (aw > 0.75) in traditional fishery products create a favourable environment for the growth of microorganisms, including bacteria, molds, and yeasts. As a result, traditional fishery products with high water activity are at an increased risk of spoilage, which can lead to off-odours, flavours, and textures.
5. ***Environmental contaminants***: Contamination with dust, dirt, and others pollutants are common issues found in traditional fishery products.
6. ***Processing methods***: Traditional fishery products often rely on time-tested processing methods, such as drying, smoking, salting, or fermenting. While these methods can impart unique flavours and characteristics to the products, they also come with specific challenges. For

example, inadequate drying or smoking can result in uneven product quality, leading to variations in taste and texture. Improper salting can result in overly salty or under-preserved products. Fermentation, if not controlled carefully, can lead to spoilage or off-flavours.

7. ***Use of chemical additives***: Traditional fishery products may be treated with preservatives, antioxidants, or antimicrobial agents to extend their shelf life. However, if these additives are not used in accordance with regulatory guidelines or exceed permissible limits, they can pose health hazards to consumers. Ingredients like salt and vinegar have been used for centuries to preserve fishery products, such as salted fish or pickled herring. In modern times, synthetic preservatives like sodium nitrite are also used to prevent bacterial growth and maintain product safety. Common antioxidants include ascorbic acid (vitamin C) and tocopherols (vitamin E). While chemical additives serve important functions in traditional fishery products, their use is subject to regulatory standards to ensure safety.
8. ***Poor storage conditions***: The way traditional fishery products are stored can significantly impact their quality and shelf life. Improper and unhygienic storage conditions can lead to issues such as mold growth, spoilage, or loss of flavour and texture.
9. ***Cross-contamination***: Traditional fishery products produced in less controlled environments has a higher risk of cross-contamination. This can occur when different types of fish or seafood, or even non-seafood items, come into contact with each other, potentially transferring harmful bacteria, allergens, or flavours. Cross-contamination can happen during handling, processing, storage, or even transportation, and it poses a significant risk to product quality and consumer safety.
10. ***Variability in raw materials***: Traditional fishery products often depend on seasonal availability, which can lead to fluctuations in product quality and quantity throughout the year.
11. ***Lack of quality standards***: In some cases, traditional fishery products may not be subject to the same regulatory oversight as their modern counterparts, allowing for quality control issues to persist.
12. ***Inadequate packaging system***: Traditional packaging materials and methods might not offer the same level of protection and preservation as modern packaging, making the products susceptible to moisture, air, and light exposure.
13. ***Traditional knowledge***: Traditional knowledge and practices may not always align with best practices for ensuring product quality and safety, creating a gap in quality control.

QUALITY ISSUES IN DRIED FISH

- Shrinkage- due to moisture loss

- Case hardening occurs in dried fish when high air temperature but low Relative Humidity (RH) forming dry impervious layer and preventing further diffusion of moisture. This make the final product into brittle.
- Denaturation of protein caused loss of juiciness of muscle leading to toughening of texture.
- Poor rehydration in dried fish- denatured protein-loss of WHC
- Changes in colour and Flavor- pigments & fats oxidized-non enzymatic browning (Maillard reaction)-free sugars react with free amino groups- produce brown colour.
- Contamination- Physical, Chemical, Biological
- Loss of Nutritional Value- Protein, lipid, vitamin, etc.

Spoilage of fish during drying and storage

- Infestation with flies (sundried), commonly blowflies- Chrysomya spp., Lucilia spp., Sarcophaga spp., etc.
- Infestation with insects-beetle- Dermestes spp. Can grow even at 15% moisture content.
- Moulds growth (both salted and unsalted) if moisture content is high; RH is > 75%, and temp. is in 30-35°C range.
- Rancidity-if fatty fish are used for drying

Control Measures - Hygiene and Sanitation

- Implementation of PRPs (GMP, GHP, SSOP)
- Implementation of HACCP (Hazard Analysis Critical Control Points)
- Effective cleaning and sanitizing procedures
- Regular staff training

Control Measures - Quality Control

- Case hardening can be controlled by maintaining sufficient high RH in drying atmosphere (mechanical) and controlling drying temperature
- Rancidity can be controlled by air tight packaging
- Use of flies traps and mesh at ventilators
- Regular Inspection and monitoring during drying
- Hygienic Handling and Processing
- Proper sanitation of the facilities
- Use of approved additives and preservatives
- Trained quality assurance team.

Control Measures - Storage and Packaging

- Maintaining proper storage temperature and RH.

- Used of appropriate packaging materials- resistant to mechanical abrasion and puncture; impermeable to moisture, oxygen & insects- e.g. Polypropylene.
- Vacuum Sealing and Oxygen Absorbers
- Proper Labelling and Date Coding

SPOILAGE IN SALTED FISH

Microbial spoilage:

a. Pink or Red

- Surface becomes covered with red/pink slime that gives off and unpleasant odour.
- Caused by halophiles (Temp. > 42°C and salt>10%) such as Halobacterium salinaria, H. cutirubum, Sarcina morrhuae, S. littoralis and Micrococcus rosens

b. Dun

- Develops in heavily salted fish-coloured spots-black, grey or brown on the surface.
- Caused Sporendonema epizoum

c. Saponification

- Malodorous slime on the surface caused by aerobic micro-orqanisms active even at low temperature.
- Occur in light salted fish, commonly boxed herring when in contact with air.

d. Putrefaction

- Flesh near backbone becomes ‘tanned’ or ‘reddened’ accompanied by offensive putrid smell.

Control Measures – Pink/Red and Dun

- To prevent Pink- Keep the fish out of contact with air. Store at lower temperature (<10°C).
- Dip treatment in sodium metabisulphite/sodium or calcium salts of propionic acid are also effective for Pink.
- For Dun- dip in 0.1% sorbic acid will provide some protection
- Used of good quality salt as it is main source of contamination.
- Storage at optimum RH, ventilated and cool & dry place.

Control Measures – Saponification and putrefaction

- To prevent saponification, the fish can be kept in brine for some time containing vinegar.
- Any pre-salting operation which can accelerate penetration of salt to the interior of the flesh such as gutting, splitting, etc. can prevent development of tanning.

Non-microbial spoilage

a) Maggots infestation

- Cheese flies Drosophila spp. are commonly encountered.

b) Rust

- Appearance of colour similar to that of rusted iron on surface due to oxidation of lipid.
- Beside colour, unpleasant taste and rancid odour are seen.
- Fatty fish such as sardine, mackerel, etc. are prone to rusting.

c) White spots

- White spots occur on surface occasionally due to disodium hydrogen phosphate derived from enzymatic breakdown of nucleotide.
- Occurs when initial spoilage of fish took place prior to salting.
- And also exposure to dry air during storage.

d) Fragmentation

- Becomes brittle and break during storage and transportation.
- Occurs when protein get denatured, hollowing of fish by insect attacks, used of spoiled fish for processing, etc.

Control measures of non-microbial spoilage

- To prevent maggots, dip in brine is effective.
- Flies trap installation
- Maintaining processing premises
- Rust can be reduced by washing using dilute solution of sodium bicarbonate.
- Best is to prevent from occurrence by keeping away fish from contact with air.
- White spots and Fragmentation can be prevented by using fresh fish for processing and proper packaging.

COMMON QUALITY ISSUES IN FERMENTED FISHERY PRODUCTS

The common quality issues found in fermented fishery products are given below.

- Histamine formation in favourable environment
- Mycotoxin formation in poorly stored products
- Botulinum toxin production in favourable condition
- Contamination with foodborne pathogens when handled unhygienically
- Growth of parasites
- Strong odour and flavour
- Production of high volatile nitrogen compounds
- Rancidity
- Dehydration and dryness

- Occurrence of sand particles
- Discolouration

Potential food safety hazards in fermented fishery products

- Histamine - chemical hazard
- Pathogenic Escherichia coli - biological hazard
- Coagulase positive Staphylococci aureus and its enterotoxin - biological hazard
- Salmonella - biological hazard
- Botulinum poisoning - biological hazard
- Parasites (in low salted product)- biological hazard
- Heavy metals and chemical residues - chemical hazard
- Biotoxins (if marine reef fishes are used) - chemical hazard

Preventive measures for histamine formation

Use fresh raw material transported at chilled condition. Gutting and gilling of susceptible fish. Refrigerated storage and freezing of unused raw martial. Using suitable starter cultures and/or their enzymes. When fresh fish was used for ripening, histamine formation in anchovy products did not occur (Herrero et al., 1999). FSSR (2011) notified for fermented fishery products that out of 9 samples only 2 samples may have 200 mg/kg histamine and no sample should possess equal to or more than 400 mg/kg histamine.

Preventive measures for botulinum toxin

Maintaining pH 4.5 or below, or having NaCl content of 15% and above would prevent growth of C. botulinum and formation of toxin. Therefore, the low level of incidence of C. botulinum poisoning in fermented fishery products may be mainly attributed to the high level of salt usage, activities of proteolytic enzymes and cooking before consumption.

Preventive measures for foodborne pathogen

Adoption of effective Good Manufacturing Practises (GMP) and Sanitation Standard Operating Procedure (SSOP) in the manufacturing unit will prevent the cross contamination of the fermented fishery products with foodborne pathogenic bacteria. Examples such; Food handlers must wash hand thoroughly after using the lavatory. Food handlers must maintain personal hygiene, etc.

Prevention measures for Biotoxin (Ciguatera)

To ensure that incoming fish have not been caught in an area for which there is a CFP advisory or for which there is knowledge that CFP is a problem.

QUALITY ISSUES IN SMOKED FISH

Biological hazards

- Listeria monocytogenes-cold smoking (22-28°C)
- Clostridium botulinum
- Parasites-nematodes, cestodes, trematodes

Chemical hazards

- Polycyclic Aromatic Hydrocarbons (PAHs): Accumulation of carcinogenic compound-benzopyrene on the surface of fish.
- Histamine
- Biotoxins

Control Measures

- Listeria monocytogenes- can be prevented by sufficient heat treatment, proper hygienic handling and cold chain maintenance during distribution
- Preventing C. botulinum- proper salt concentration, proper refrigeration, and reduced oxygen packaging like MAP can prevent the occurrence of C. botulinum in smoked fish and fishery products.
- Salt along with smoke effectively prevents the toxin formation.
- To prevent parasite- attention needs to be paid to cold smoked or smoke-flavoured products, which should be frozen before or after smoking if a parasite hazard is present.
- The PAH contamination in smoked products can be significantly reduced by using indirect smoking process instead of direct smoking of the fish. Use of liquid smoke is a better option.

Quality issues in fish pickle

Pickling imparts unique and characteristic taste, flavor and texture to fish, but the chang occurring during storage should be carefully monitored.

PROBLEMS	CAUSE
Soft, slippery slimy pickles (discard pickles, spoilage is occurring)	Hard water, acid level too low, cooked too long or at too high a temperature, bacteria not destroyed, jars not airtight, jars in too warm a resting place

Shrivelled, tough pickles	Pickles overcooked, syrup too heavy, too strong brine or vinegar solution
Dark, discoloured pickles	Iron utensils used, copper, brass, or zinc cookware used, Hard water, Metal lid corrosion, High quantity of powdered and dried spices used.
There is white sediment on the bottom of the jar	Harmless yeasts have grown on the surface and then settled to the bottom, Additives in table salt.
Pickles have a strong, bitter taste	Spices were old, they were cooked too long in the vinegar or the quantity was excessive, vinegar used was too strong, salt substitutes contain potassium chloride, which is naturally bitter

Other quality issues in fish pickle

- Another issue in fish pickle is biogenic amines such as histamine, tyramine, tryptamine, putrescine, cadaverine, spermidine and spermine.
- The outbreaks of foodborne pathogens such as E. coli O157:H7 and Salmonella sp. in acidified foods were reported recently.
- Clostridium botulinum is another food born pathogen of concern in pickled products.
- Pickling can reduce parasite hazard in fish but cannot eliminate it.
- For example, nematode larvae can survive for 28 days in 21% salt by weight (FDA, 2020).

Control Measures

- Time-temperature (<4°) control of raw material during processing inhibit histamine formation.
- In fermented pickles, starter culture or probiotic strains (e.g., L. plantarum, L. casei, E. faecium and Pediococcus sp.) may reduce the biogenic amine formation.
- By controlling the pH level to 4.6 or below, salt to 5% wps(water phase salt) or more, water activity to 0.97 or below, or combination of these barriers sufficiently prevents the growth of C. botulinum.

Regulatory standards

Regulations and standards specific for dried/salted and fish pickles are as following:

- CODEX STAN 167-1989 – Standard for salted fish and dried salted fish of the Gadidae family of fishes
- CODEX STAN 236-2003 - Standard for Boiled Dried Salted Anchovies

- CODEX STAN 244-2004 Standard for salted Atlantic herrings and salted Sprats
- Indian Standard (IS 14950:2001)- Dried and dry salted fish
- Indian Standard (IS 14515:1998)- Fish pickles
- Food Safety and Standards Regulations (FSSR), 2011

Conclusion

Ensuring quality is not only a matter of preserving culinary traditions but also vital for economic sustainability. High-quality traditional fishery products can command premium prices in both domestic and international markets, contributing to the livelihoods of local fishing communities. Addressing quality issues is essential from a public health perspective, as sub-standard products can pose risks to consumers. By implementing measures to enhance the quality and safety of traditional fishery products, we not only safeguard cultural heritage but also promote economic prosperity, food safety, and consumer trust, ultimately benefiting both producers and consumers alike. One key approach as control measures is to establish quality standards and regulations that encompass various aspects of production, from sourcing and handling to processing and packaging. Regular inspections and audits by relevant authorities can help ensure compliance with these standards. Additionally, promoting good manufacturing practices and providing training to fishermen and processors can enhance their knowledge and skills, leading to improved product quality. Emphasizing proper hygiene, temperature control, and traceability measures in the supply chain is essential to prevent contamination and ensure product integrity. Encouraging sustainable fishing practices also contributes to quality by maintaining the health of fish stocks. Overall, a comprehensive approach involving regulation, education, and sustainability efforts is essential to effectively control quality issues in traditional fishery products and uphold their cultural and economic significance.

Chapter 7

Microplastics Issues in Seafood and its Control Measures

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Introduction

Microplastics are defined as plastic materials or fragments of length 0.1µm to 5 mm, are most likely the most numerically abundant plastic debris items in the ocean today, and can be found in various environments, including the oceans, where they can significantly impact marine life. One concern related to microplastics is their association with seafood, which has raised questions about potential health impacts on humans. Microplastics (MPs) are a heterogeneous mixture of plastic polymers with a size of less than 5mm, which have become ubiquitous in the marine environment due to extensive plastic pollution (Thompson *et al*., 2004). The presence of these particles in marine organisms raises increasing concerns over animal welfare and food safety, given that seafood compromises over 17% of animal protein consumption by humans globally (Murray and Cowie,2011; FAO,2018). Therefore, it is extremely important that the risk seafood presents to consumers, regarding exposure and health effects, is accurately quantified. To do this comprehensively, not only should MP contamination be quantified across the breadth of globally consumed organisms, but the MP

Microplastics in the marine environment - Trending global environmental issue

Microplastics can come from various sources, including the breakdown of larger plastic items, microbeads in personal care products, and industrial discharges. They can enter the oceans through wastewater runoff, rivers, and coastal areas. Here are some key points to consider regarding microplastics in seafood and their potential health impacts: Larger plastic particles, which ultimately reach oceans in due course of time, degrade into smaller micro and nano plastics because of photo-oxidation due to sunlight, wave abrasion due to water wave's physical stress. In 2014, the estimated number of floating plastic particles in the world's oceans was 5.25 trillion, of which 4.85 trillion were microplastics (GESAMP, 2019). Microplastics are being classified as primary and secondary microplastics. Primary microplastics are engineered plastic particles manufactured as microbeads, capsules, fibers, or pellets and used in the manufacture of several cosmetic products, paints etc. And plastic

materials that, due to several biological, chemical, and physical processes, disintegrate into smaller plastic fragments in the course of time are known as secondary microplastics.

Bioaccumulation and Biomagnification of microplastics in aquatic organisms and humans

Marine organisms, including fish, shellfish, and plankton, can ingest microplastics. Microplastics are the major cause of concern because their size range mimics the prey size many aquatic organisms ingest. These particles can accumulate in the digestive tracts and tissues of these animals. As larger fish consume smaller fish, there is potential for biomagnification, meaning the concentration of microplastics may increase up the food chain. Most aquatic organisms, including zooplankton, invertebrates, fish, bivalves, birds, cetaceans, and larger mammals, incidentally consume MPs from sediment or the water column, mistaking them as food. These plastics are eaten by lower-tropic-level organisms such as mussels, oysters or copepods, and small fishes, then biomagnified to animals at higher tropic levels that feed on them. Obviously, humans are exposed to microplastics through the consumption of species of commercial importance for fisheries, and aquaculture is significant as this seafood is a recognized source of contaminants in the human diet. About 690 marine species are known to encounter marine litter and microplastics. Plastics contain a variety of chemical additives including fillers, plasticizers, flame retardants, UV and thermal stabilizers, pigments, and antimicrobial agents which are introduced during the manufacturing process to achieve the desired performance and appearance criteria which on disintegration may pose several health impacts as it can alter the hormonal balance of the living organism and the additive PCBs is a carcinogenic compound.

Microplastics' physical and chemical properties facilitate the sorption of contaminants to the particle surface, serving as a vector of contaminants to organisms following ingestion and posing potential health effects. Many recent research studies on Indian beaches, coastal waters, and commercially available finfish and shellfish (especially shrimp and bivalves) revealed the occurrence of microplastics. Recent research reveals that globally on average each human is ingesting 5 grams of plastic every week, the equivalent of a credit card in the form of microplastics. Various studies have confirmed the presence of microplastics in a broad range of marine organisms (Gambardella et al., 2017). The ingestion of these microplastics can be extremely hazardous to organisms, as they can cause blockages in the digestive tract, oxidative and pathological stress, inhibit growth rate, and reproductive disorders. From the available studies, microplastics have been observed in the gastrointestinal tract in 11 out of the 20 most important species and genera of finfish that contribute to global marine fisheries (FAO, 2016)

and in shrimps and lobsters of coastal waters of Europe (Devriese et al., 2015). Among bivalves lowest incidence of microplastics is found in European waters which is 0.5 microplastic items/gram of soft tissue and the highest incidence is observed in Newfoundland, Canada which is 50 microplastic items/gram of soft tissue. But most recent studies conducted in India, the second largest fish-producing country reveal the presence of microplastics in commercially important finfishes, shrimps, and bivalves is many folds higher than that observed in the other parts of the world.

Incidence of microplastics in commercially important seafood

The ubiquitous spread of microplastics in Indian marine waters results in inevitable interaction with a lot of commercially significant organisms like shrimp, bivalves, predatory fishes, etc. Among these organisms, bivalves are excellent filter feeders and are of particular interest because their extensive filter-feeding activity exposes them directly to microplastics present in the water column. The incidence of microplastic accumulation is 27 times higher than in fish and approximately filter 24 liters per day and accumulates these microplastics in their gut and tissues rapidly within hours. Thus they can be used as bio indicators for the plastic pollution status of that region. Commercially important, widely consumed and exported bivalves and gastropods of India like *Perna viridis,* the green mussel (Japanese - Igai, Spanish – Mejillon) *Meritrix casta,* the yellow clam (Japanese - Nimaigai, Spanish- Almeja), *Babylonia spirata* popularly called as baigai, *Katelysia opima* from different coasts are reported to contain microplastics. These clams are exported to different countries like Japan, Taiwan, China, Mexico, Hong Kong, South Africa and Italy. About 80% of Indian clam meat is imported by Japan followed by Spain and China. Yellow clam led the international export, touching 721.88 tons valued at Rs.10.67 lakhs during 2016-’17 (MPEDA, personal communication, 2018). Most of the yellow clam, targets international markets at Japan and Thailand. Commercially consumed bivalves from Chinese waters are estimated to contain 2.1 to 10.5 items/gram, and bivalves from European waters of France, Germany, Belgium, and Norway are estimated to contain an average of 0.5- 1 items/gram. But in a recent study, the most consumed bivalves of the Tamil Nadu coast is 4-6 items/gram and astonishingly that of the Mumbai coast is 52.71 to 77.23 items/gram of bivalve meat and 5- 12 items/gram of gut of shrimp which implies the intense anthropogenic activities of that area. This indicates the present drastic and dangerous situation. If this situation continues, in the near future there is the possibility of quality issues on Indian seafood imports in foreign countries due to its ridiculous microplastic content.

Implications for Human Health, Food Security, and Indian International Trade

Now microplastics have gained increased attention from regulatory bodies of different countries over the past few years as they may cause reduced body growth, intestinal damage, physiological or oxidative stress, and inflammation, effects on the immune system, hormonal dysregulation, aberrant development, cell death, general toxicity and altered lipid metabolism in humans. Some jurisdictions like California have passed laws related to microplastics requiring state regulators to develop standards and incorporate them into regulations. Fish (finfish and shellfish) and its products are major important products among Indian agricultural exports earning US$ 6.3 million (45,106.89 crore INR) in 2017-18. Seafood comprises 10% of the total exports of India and nearly 20% of all agricultural exports and India has approximately 20-22% of the world shrimp trade. As microplastics became a global environmental concern and now as it's under continuous global scrutiny. Considering the deleterious health effects caused by microplastics due to the chemical additives added to them, toxic chemicals adsorbed to their surface, and harmful pathogens attached to them in the near future many countries may categorize it as a food hazard associated with fish/shellfish and may include it in mandatory fish product quality and safety standards. If Indian seafood exports fail to comply with those safety standards it will be a major blow to the economy, and result in the rejection of consignments, and exporters may incur heavy losses.

Consumption of Seafood Contaminated with Microplastics:

When humans consume seafood that contains microplastics, there is the potential for these particles to enter the human digestive system. The extent of human exposure to microplastics through seafood consumption is an area of ongoing research.

Potential Health Impacts:

The health impacts of ingesting microplastics are not yet fully understood, and research is ongoing. Some concerns and potential risks include:

Physical Irritation: Microplastics may cause physical irritation and damage to the digestive tract.

Chemical Exposure: Microplastics can adsorb toxic chemicals from the environment, and there is concern that these chemicals could be released into the body upon ingestion.

Inflammatory Responses: Microplastics may trigger inflammatory responses in the body's tissues.

Reducing Microplastic Exposure:

Many countries and organizations are starting to address the issue of microplastics in seafood. Some have set limits on the amount of allowable microplastics in food products. Additionally, monitoring and research efforts are ongoing to understand the extent of

contamination and potential health risks. To reduce exposure to microplastics through seafood consumption, individuals can take the following steps:

- Choose seafood from sources known for good environmental practices and monitoring.
- Avoid consuming seafood products that are more likely to contain microplastics, such as bivalves (mussels, clams) or bottom-feeding fish.
- Minimize single-use plastic consumption to help reduce the input of microplastics into the environment.
- It's important to note that the long-term health effects of microplastic exposure through seafood consumption are still an active area of research, and the overall risk to human health is not yet fully understood. As such, ongoing research and monitoring are crucial to better assess the potential risks and inform regulatory actions if necessary.

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Chapter 8

Quality Issues and its Control Measures in Thermal Processed Fishery Products

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Processing and preservation of food is an important activates to ensure safe food supply apart from reducing food loss. Fish being highly perishable food commodity, processing and preservation assumes great importance. There are number of reasons for processing fish and shellfish which are given below.

1. To supply safe food
2. To minimize loss/waste of valuable food commodity
3. To meet consumer preference and specified quality standards
4. To extend the shelf life of food for longer duration
5. To make profit by adding value and increasing convenience to the consumer

Thermal sterilization of foods is the most significant part of food processing industry and is one of the most effective means of preserving food supply. Thermal processing, which is commonly referred as heat processing or canning is a means of achieving long-term microbiological stability for non-dried foods without the use of refrigeration, by prolonged heating in hermetically sealed containers, such as cans or retortable pouches, to render the contents of the container sterile. The concept of thermal processing has come a long way since the invention of the process by French confectioner, Nicholas Appert. Later on Bigelow and Ball developed the scientific basis for calculating the sterilization process for producing safe foods. Today, thermal processing forms one of the most widely used method of preserving and extending shelf life of food products including seafood's. Thermal processing involves application of high temperature treatment for sufficient time to destroy all the microorganisms of public health and spoilage concerns. Normally, thermal processing is not designed to destroy all microorganisms in a packaged product, which may result in low quality product which destroys important nutrients. Instead of this, the pathogenic microorganisms in a hermetically sealed container are destroyed by heating and a suitable environment is created inside the container which does not support the growth of spoilage type microorganisms. Several factors must be considered for deciding the extent of heat processing which include,

a) Type and heat resistance of the target microorganism, spore, or enzyme present in the food

b) pH of the food

c) Heating conditions

d) Thermo-physical properties of the food and the container shape and size

e) Storage conditions

Thermal processing is designed to destroy different microorganisms and enzymes present in the food. Normally in thermal processing, exhausting step is carried out to before sealing the containers. In some cases, food is vacuum packed in hermetically sealed containers. In such cases very low levels of oxygen is intentionally achieved. Hence, the prevailing conditions are not favorable for the growth of microorganisms that require oxygen (obligate aerobes) to create food spoilage or public-health problems. Further, the spores of obligate aerobes are less heat resistant than the microbial spores that grow under anaerobic conditions (facultative or obligate anaerobes). The growth and activity of these anaerobic microorganisms are largely pH dependent. From a thermal-processing standpoint, foods are divided into three distinct pH groups which are given below. Changes in the intrinsic properties of food, mainly salt, water activity and pH are known to affect the ability of microorganisms to survive thermal processes in addition to their genotype. Due to health related concerns on the use of salt, there is increased demand to reduce salt levels in foods. The United States Food and Drug Administration (FDA) have classified foods in the federal register (21 CFR Part 114) as follows (Table 2):

1. high-acid foods (pH < 3.7; e.g., apple, apple juice, apple cider, apple sauce, berries, cherry (red sour), cranberry juice, cranberry sauce, fruit jellies, grapefruit juice, grapefruit pulp, lemon juice, lime juice, orange juice, pineapple juice, sour pickles, vinegar)
2. acid or medium-acid foods (pH 3.7 - 4.5; e.g., fruit jams, frit cocktail, grapes, tomato, tomato juice, peaches, piento, pineapple slices, potato salad, prune juice, vegetable juice)
3. low-acid foods (pH > 4.5; e.g., all meats, fish and shellfishes, vegetables, mixed entries, and most soups).

Table 2. Approximate pH range of different food

Food	**pH**	**Food**	**pH**
Lemon juice	2.0 - 2.6	Sweet potato	5.3 – 5.6
Apples	3.1 - 4.0	Onion	5.3 – 5.8
Blueberries	3.1 – 3.3	Spinach	5.5 – 6.8

Sauerkraut	3.3 – 3.6	Beans	5.6 – 6.5
Orange juice	3.3 – 4.2	Soybeans	6.0 – 6.6
Apricot	3.3 – 4.0	Mushroom	6.0 – 6.7
Bananas	4.5 – 5.2	Clams	6.0 – 7.1
Beef	5.1 – 7.0	Salmon	6.1 – 6.3
Carrot	4.9 – 5.2	Coconut milk	6.1 – 7.0
Green pepper	5.2 – 5.9	Milk	6.4 – 6.8
Papaya	5.2 – 6.0	Chicken	6.5 – 6.7
Tuna	5.2 – 6.1	Whole egg	7.1 – 7.9

The acidity of the substrate or medium in which micro-organisms are present is an important factor in determining the extent of heat treatment required. With reference to thermal processing of food products, special attention should be devoted to *Clostridium botulinum* which is a highly heat resistant mesophilic gram positive, rod shaped spore-forming anaerobic pathogen that produces the toxin *botulin*. It has been generally accepted that *C. botulinum* and other spore forming, human pathogens does not grow and produce toxins below a pH of 4.6. The organisms that can grow in such acid conditions are destroyed by relatively mild heat treatments. For food with pH values greater than 4.5, which are known as low-acid products which includes fishery products, it is necessary to apply a time–temperature regime sufficient to inactivate spores of *C. botulinum* which is commonly referred to as a *botulinum cook* in the industry. Thermal processes are calibrated in terms of the equivalent time the thermal centre of the product, i.e. the point of the product in the container most distant from the heat source or cold spot, spends at 121.1°C, and this thermal process lethality time is termed the F_0 value. Although there are other microorganisms, for example *Bacillus stearothermophilus*, *B. thermoacidurans*,and *C. thermosaccolyaticum*, which are *thermophilic* in nature (optimal growth temperature ~ 50–55°C) and are more heat resistant than *C. botulinum a* compromise on the practical impossibility of achieving full sterility in the contents of a hermetically sealed container during commercial heat processing, whereby the initial bacterial load is destroyed through sufficient decimal reductions to reduce the possibility of a single organism surviving to an acceptably low level. This level depends on the organism, usually *Clostridium botulinum*, which the process is designed to destroy. The time required to reduce the number of spores of this organism (or any other micro-organism) by a factor of 10 at a specific reference temperature (121.1°C) is the decimal reduction time, or *D* value, denoted D_0. The D_0 value for

Clostridium botulinum spores can be taken as 0.25 minutes. To achieve a reduction by a factor of 10^{12}, regarded as an acceptably low level, requires 3 minutes at 121.1°C, and is known as the process value, or *F* value, designated F_0 so, in this case, F_0 = 3, which is known as a botulinum cook which is the basis of commercial sterility.

Thermal resistance of microorganisms

For establishing a safe thermal processing, knowledge on the target microorganism or enzyme, its thermal resistance, microbiological history of the product, composition of the product and storage conditions are essential. After identifying the target microorganism, thermal resistance of the microorganism must be determined under conditions similar to the container. Thermal destruction of microorganism generally follow a first-order reaction indicating a logarithmic order of death i.e., the logarithm of the number of microorganisms surviving a given heat treatment at a particular temperature plotted against heating time (survivor curve) will give a straight line (Figure 1). The microbial destruction rate is generally defined in terms of a decimal reduction time (D value) which represents a heating time that results in 90% destruction of the existing microbial population or one decimal reduction in the surviving microbial population. Graphically, this represents the time between which the survival curve passes through one logarithmic cycle (Fig. 1). Mathematically,

$$D = (t_2 - t_1) / (\log a - \log b)$$

where, *a* and *b* are the survivor counts following heating for t_1 and t_2 min, respectively. As the survivor or destruction curve follows the logarithmic nature, the complete destruction of the microorganisms is theoretically not possible.

From the survivor curve, as the graph is known, it can be seen that the time interval required to bring about one decimal reduction, i.e. 90% reduction in the number of survivors is constant. This means that the time to reduce the spore population from 10,000 to 1000 is the same as the time required to reduce the spore population from 1000 to 100. This time interval is known as the decimal reduction time or the 'D' value. The D value for bacterial spores is independent of initial numbers, but it is affected by the temperature of the heating medium. The higher the temperature, faster the rate of thermal destruction and lower the D value. The unit of measurement for D is 'minute'. An important feature of the survivor curve is that no matter how many decimal reductions in spore numbers are brought about by a thermal process, there will always be some probability of spore survival. Different micro-organisms and their spores have different D values as shown in Table–3.

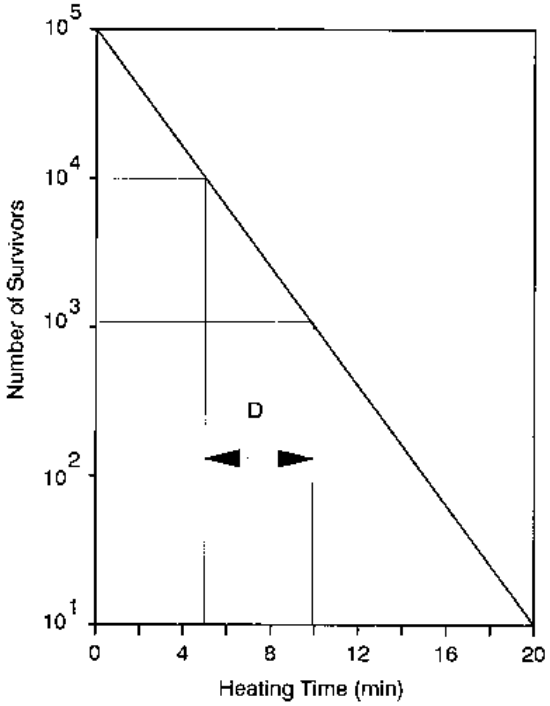

Fig 1. Survivor curve

Table-3. D value (at 121.1°C) of some bacterial spores

Microorganism	Optimum growth temperature (°C)	D value (min)
Bacillus stearothermophilus	55	4 to 5
Clostridium thermosaccharolyticum	55	3 to 4
Clostridium nigrificans	55	2 to 3
Clostridium botulinum types A & B	37	0.1 to 0.25
Clostridium sporogenes (PA 3679)	37	0.1 to 1.5
Bacillus coagulans	37	0.01 to 0.07
Non spore forming mesophilic bacterial yeasts and moulds	30 - 35	0.5 to 1.0

The thermal death time may be defined as the time required at any specified temperature to inactivate an arbitrarily chosen proportion of the spores, the higher the proportion the greater will be the margin of safety. TDT is the heating time required to cause complete destruction of a microbial population. Such data are obtained by subjecting a microbial population to a series of heat treatments at a given temperature and testing for survivors. The thermal death time

curve is obtained by plotting the thermal death time on logarithmic scale against temperature of heating on linear scale on a semilogarithmic graph paper (Fig. 2). Comparing TDT approach with the decimal reduction approach, one can easily recognize that the TDT value depends on the initial microbial load (while D value does not). Further, if TDT is always measured with reference to a standard initial load or load reduction, it simply represents a certain multiple of D value. For example, if TDT represents the time to reduce the population from 10^0 to 10^{-12}, then TDT is a measure of 12 D values. i.e., TDT = nD, where n is the number of decimal reductions. The extent of inactivation in the case of pathogenic microorganisms (*C. botulinum*) is equivalent to a 12 D process. The slope of the TDT curve is defined as 'z' value, which is the number of degrees for the TDT curve to traverse one log cycle. The temperature sensitivity indicator is defined as z, a value which represents a temperature range which results in a ten-fold change in D values or, on a semilog graph, it represents the temperature range between which the D value curve passes through one logarithmic cycle. The 'z' value which is also known as the temperature sensitivity indicator is usually taken as 10°C in the case of *C.botulinum.*

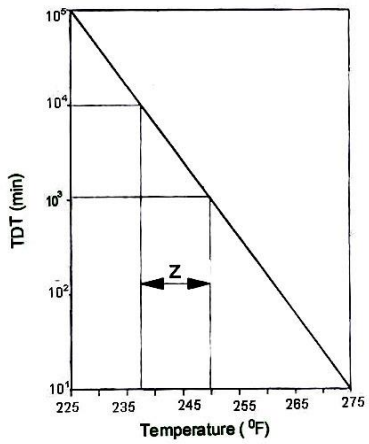

Fig. 2 TDT Curve

For the purpose of heat process determination with respect to their lethality towards specific micro-organisms, the reciprocal of the thermal death time (TDT value) called the lethal rate, L is used. So, instead of temperatures, the corresponding lethal rates are plotted against time, the area enclosed by the graph and the ordinate represent the F value for the process. i.e.,

$$L = \frac{1}{TDT}, \quad \text{and}$$

t

$$F = \int_0 L\, dt$$

Thermal Process Severity or F_0 value

From D value and the initial number of spores inside the sealed container (N_o), an idea of the severity of heat process required to reduce the spore population to a predetermined level, N_t, can be calculated from the equation:

$$t = D (\log No - \log N_t) \text{ or } t = D \log (No/N_t)$$

where, t = time required to achieve commercial sterility

This $\log No/N_t$ is sometimes referred to as the 'order of process', factor 'm' and the value of the product of m and D is called the 'process value' or 'F value'. That is:

$$F_0 = mD_{121.1°C}$$

For example, considering the generally accepted minimum process for prevention of botulism through under processing of canned fishery products preserved by heat alone, assuming that the initial loads are of the order of 1 spore/g and in line with good manufacturing practice guidelines, the final loads shall be no more than $\log 10^{-12}$ spores/g. That is 12 decimal reductions are required. It is also known as 12 D process. The minimum time required to achieve commercial sterility can be calculated from

$$t = 0.25 (\log 1 - \log 10^{-12}),$$

$$\text{i.e., } t = 0.25 \times 12 = 3.00 \text{ min}$$

Thus an F_0 value of 3.00 minutes at 121.1°C at the slowest heating point (SHP) of the container is sufficient for providing safety from pathogenic organism *C. botulinum*.

Commercial sterility

If the thermal process is sufficient to fulfill the criteria of safety and prevention of non-pathogenic spoilage under normal conditions of transport and storage, the product is said to be 'commercially sterile'. In relation to canned foods, the FAO/WHO Codex Alimentarius Commission (1983) defines, commercial sterility as the condition achieved by the application of heat, sufficient alone or in combination with other appropriate treatments, to render the food free from microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during distribution and storage. Apart from this concept there are circumstances where a canner will select a process which is more severe than that required for commercial sterility as in the case of mackerel and sardine where bone softening is considered desirable.

Mechanism of heat transfer

Understanding the mechanism of heat transfer is very important for thermal processing. Normally, there are three different modes of heat transfer: conduction, convection and radiation. Conduction is the transfer of heat by molecular motion in solid bodies. Convection is the transfer of heat by fluid flow, created by density differences and buoyancy effects, in fluid products. Radiation is the transfer of electromagnetic energy between two bodies at different temperatures. In thermal processed foods, the mechanism of heat transfer is either by conduction, convection or by broken heating (combination of conduction and convection). The factors which determine the mode of heat transfer are nature or consistency of a food product, the presence of particles, and the use of thickening agents and sugars. The heating modes in the thermal processing are first by heat transfer to the container or packaging material from heating and cooling media, second through the container wall and third is into the product from container wall. Convective-heat transfer rates depend largely on the velocity of flow of the media over the container, and this is an important factor to be controlled in all processing operations. In conduction heading method, energy transfer takes place when different parts of a solid body are at different temperatures. The slowest heating point or cold point in cylindrical metal containers is at its geometric centre for food products heated by conduction method. Convection heat transfer involves the transfer of heat from one location to the other through the actual movement or flow of a fluid. The slowest heating point for convection heated products in cylindrical metal container is approximately $1/10^{th}$ up from the base of the container. Packaging material forms the most important component of thermal processed foods. It should be able to withstand the severe process conditions and should prevent recontamination of the product.

Containers for thermal processing

Containers used for thermal processing should have special properties like it should withstand high temperature and pressure. Tin cans are commonly used in the canning industry and cans are denoted by trade name. First digit represents diameter of can (in inches) and next two digits represent measurement in sixteenth of inches. Apart from OTS cans, other container used in canning are: aluminium cans, tin free steel (TFS) cans, glass containers, retort pouches and semi-rigid containers.

Glass containers

Glass is a natural solution of suitable silicates formed by heat and fusion followed by immediate cooling to prevent crystallization. It is an amorphous transparent or translucent super cooled liquid. Modern glass container is made of a mixture of oxides viz., silica (S_1O_2), lime (CaO),

Soda (Na_2O), alumina (Al_2O3), magnesia (MgO) and potash in definite proportions. Colouring matter and strength improvers are added to this mixture and fused at 1350 - 1400ºC and cooled sufficiently quick to solidify into a vitreous or non-crystalline condition.

Glass jars for food packing has the advantages of very low interaction with the contents and product visibility. However, they require more careful processing and handling. Glass containers used in canning should be able to withstand heat processing at high temperature and pressure. Breakage occurring due to 'thermal shock' is of greater significance in caning than other reasons of breakage. Thermal shock is due to the difference in the temperature between the inside and the outside walls of the container giving rise to different rates of expansion in the glass wall producing an internal stress. This stress can open up microscopic cracks or 'clucks' leading to large cracks and container failure. Thermal shock will be greater if the wall thickness is high. Therefore, glass container in canning should have relatively thin and uniform walls. Similarly the bottom and the wall should have thickness as uniform as possible. More failures occur at sharp containers and flat surface and hence these should be avoided. Chemical surface coatings are often applied to make the glass more resistant to 'bruising' and to resist thermal shock. Various types of seals are available, including venting and nonventing types, in sizes from 30 to 110 mm in diameter, and made of either tin or tin-free steel. It is essential to use the correct overpressure during retorting to prevent the lid being distorted. It is also essential to preheat the jars prior to processing to prevent shock breakage.

Metal containers

Metal cans are most widely used containers for thermal processed products. Metal containers are normally made of tin, aluminium or tin-free steel.

Tin plate cans

Tinplate is low metalloid steel plate of can making quality (CMQ) coated on both sides with tin giving a final composition of 98% steel and 2% tin. Thickness varies from0.19 to 0.3 mm depending on the size of the can. Specifications with respect to content of other elements are: Carbon (0.04 - 0.12%), manganese (0.25 - 0.6%), sulphur (0.05 % max), phosphorus (0.02 % max), silicon (0.01% max) and copper (0.08% max). Corrosive nature of tin plate depends principally on the contents of copper and phosphorous. The higher the contents of these metals, greater the corrosiveness of steel. However, higher phosphorous content imparts greater stiffness to steel plate which is advantageous in certain applications where higher pressure develops in the container, eg; beer can.

Base plate for can making is manufactured using the cold reduction (CR) process. CR plates are more advantageous over hot reduced plates because of the following characteristics.

1. Superior mechanical properties – possible to use thinner plates without loss of strength
2. More uniform gauge thickness
3. Better resistance to corrosion
4. Better appearance

Aluminium cans

Pure aluminium of 99.5 to 99.7% purity is alloyed with one or more elements like magnesium, manganese, zinc, copper etc. to obtain the desired composition. Aluminium alloyed with mangnesium is the most commonly used material. Alloyed aluminium is first given an anticorrosive treatment; usually anodising in dilute sulphuric acid. The thin layer of oxides formed provides corrosion resistance. To enhance this, the sheet is further coated with a suitable lacquer.

Advantages of aluminium cans

- Light weight, slightly more than 1/3 of the weight of a similar tinplate can
- Nonreactive to many food products
- Clear, bright and aesthetic image
- Not stained by sulphur bearing compounds
- Nontoxic, does not impart metallic taste or smell to the produce
- Easy to fabricate; easy to open
- Excellent printability
- Recyclability of the metal

However, aluminium cans are not free from some disadvantages

- Thick gauge sheet needed for strength
- Not highly resistant to corrosion, acid fruits and vegetables need protection by lacquering or other means
- Special protection needed during heat processing to avoid permanent distortion
- Aluminium has great tendency to bleach some pigmented products
- Service life is less than that of tinplate for most aqueous products

Tin free steel containers

Tin free steel (TFS) apart from aluminium, is a tested and proven alternate to tinplate in food can making. It has the same steel substitute as the tinplate. It is provided with a preventive coating of chromium, chromium oxide, chromate-phosphate etc. TFS is manufactured by electroplating cold-rolled base plate with chromium in chromic acid. This

process does not leave toxin substrate such as chromates or dichromates on the steel and it can be formed or drawn in the same way as tinplate.

Advantages:

- The base chromium layer provides corrosion barrier
- The superimposed layer of chromium oxide prevents rusting and pick up of iron taste
- Provides an excellent base for lacquer adhesion
- Good chemical and thermal resistance
- Tolerance to high processing temperature and greater internal pressure
- Improved and more reliable double seam

Disadvantages:

- Low abrasion resistance; hence compulsory lacquering
- Difficulty in machine soldering
- The oxide layer needs removal even for welding
- Limitations in use for acid foods

An important problem associated with TFS can ends is scuffing of lacquer on the double seam. This may occur at the seamer or downstream at different stages of lacquering. TFS cans have been found quite suitable for canning different fish in various media. Thus it holds good scope as an important alternate to tinplate cans.

Rigid plastic containers

The rigid plastic material used for thermal processing of food should withstand the rigors of the heating and cooling process. It is also necessary to control the overpressure correctly to maintain a balance between the internal pressure developed during processing and the pressure of the heating system. The main plastic materials used for heat-processed foods are polypropylene and polyethylene tetraphthalate. These are usually fabricated with an oxygen barrier layer such as ethylvinylalcohol, polyvinylidene chloride, and polyamide. These multilayer materials are used to manufacture flexible pouches and semi-rigid containers. The rigid containers have the advantage for packing microwavable products.

Retortable pouches

Retort pouch can be defined as a container produced using 2,3 or 4-ply material that, when fully sealed, will serve as a hermetically sealed container that can be sterilized in steam at pressure and temperature similar to those used for metal containers in food canning. Retort pouch has the advantages of metal can and boil-in plastic bag. Configuration of some typical pouches are:

- 2 ply 12µ nylon or polyester/70µ polyolefin
- 3 ply 12µpolyester/9-12µ aluminium foil/70µ polyolefin
- 4 ply 12µ polyester/9-12µaluminium foil/12µpolyester/70µ polyolefin

3-ply pouch is most commonly used in commercial canning operations. This is a three-layer structure where a thin aluminium foil is sandwiched between two thermoplastic films. The outer polyester layer provides barrier properties as well as mechanical strength. The middle aluminium foil provides protection from gas, light and water. This also ensures adequate shelf life of the product contained within. The inner film which is generally polyproplyline, provides the best heat sealing medium.

The normal design of a pouch is a flat rectangle with rounded corners with four fin seals around 1 cm wide. A tear notch in the fin allows easy opening of the pouch. The rounded corners allow safe handling and help to avoid damage to the adjacent packs. The size of the pouch is determined by the thickness that can be tolerated at the normal fill weight. The size ranges (mm) available are:

A_1	130 x 160
A_2	130 x 200
A_3	130 x 240
B_1	150 x 160
B_2	150 x 250
B_3	150 x 240
C_1	170 x 160
C_2	170 x 200
C_3	170 x 240
D_1	250 x 320 (Catering pack)
D_2	250 x 1100
D_3	250 x 480

Advantages

- Thin cross- sectional profile – hence rapid heat transfer – 30-40% saving in processing times – no overheating of the product near the walls
- Better retention of colour, flavour and nutrients
- Shelf life equal to that of the same product in metal can
- Very little storage space for empty pouches – 15% of that for cans

- Easy to open

Disadvantages

- Pouches, seals more vulnerable to damage, can be easily damaged by any sharp material, hence necessitates individual coverage
- With an over wrap cost may go up above that of cans
- Slow rate of production, 30 pouches in place of 300-400 cans per minute
- Needs special equipment
- Higher packaging cost and low output push up the cost of production

Fig.: Containers used for thermal processing

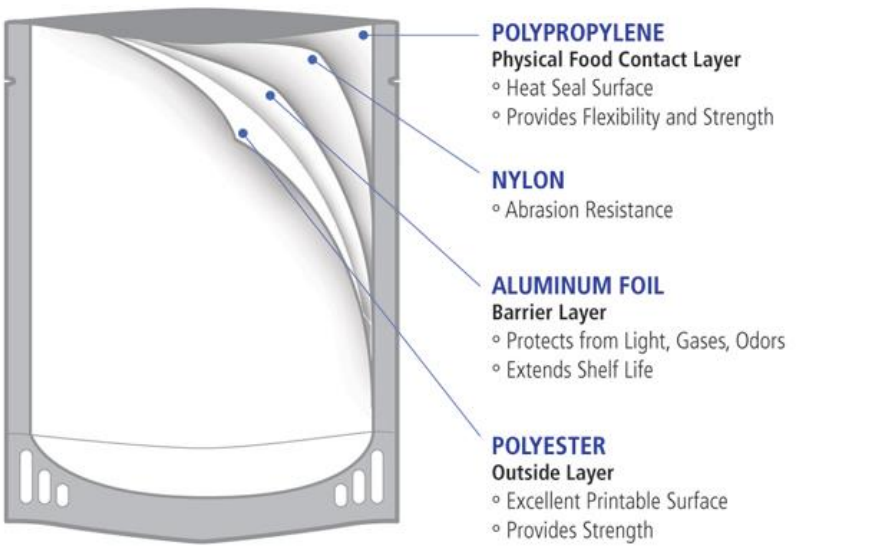

Fig.: Composition of Retortable pouch

Ideally, the container used for thermal processing should fulfill following characteristics:

- Should withstand the sterilisation pressure and temperature
- Should be impervious to air, moisture, dust and disease germs once the can is sealed air tight
- Internal lacquer should not impart toxicity to the contents
- Strong enough to protect the contents during transportation and handling
- Inexpensive, preferably cheap enough to discard after use
- Capable of sealing at high speed
- Pleasing and sanitary appearance

Thermal Processing of Fishery Products

The thermal processing is carried out for achieving two objectives; the first is consumer safety from botulism and the second is non-pathogenic spoilage which is deemed commercially acceptable to a certain extent. If heat processing is inadequate the possibility of spoilage due to C. *botulinum* is more and will endanger the health of the consumer. Safety from botulism is made possible by making the probability of *C. botulinum* spores surviving the heat process sufficiently remote and presents no significant health risk to the consumer. An acceptable low level in the context of this dangerously pathogenic organism means less than one in a billion (10^{-12}) chance of survival. Such a low probability of spore survival is commercially acceptable as it does not represent a significant health risk. The excellent safety record of the canning industry with respect to the incidence of botulism through under processing, confirms the validity of this judgment. An acceptable low level in the case of thermophilic non-pathogenic organisms should be arrived at judiciously considering the factors like very high D value, risk of flat sour spoilage, commercial viability and profitability etc. Since non-pathogenic organisms do not endanger the health of the consumer process adequacy is generally assessed in terms of the probability of spore survival which is judged commercially acceptable. Considering all these facts, it is generally found acceptable if thermophilic spore levels are reduced to around 10^{-2} to 10^{-3} per g. Another reason for this acceptance is that the survivors will not germinate if the storage temperature is kept below the thermophilic optimum growth temperature i.e. below 35°C.

Fishery products, being categorized as low acid foods require heat processing severity with respect to *C botulinum* and F_0 value recommended is 5-20 min. Thermal processing of fishery products include various steps. These steps include, preparations like washing, beheading, gutting, removing scales / fins, cutting into required size, blanching (hot / cold),

pre-cooking, filling fish pieces into containers, filling content or medium, exhausting to remove air, sealing, loading into the retort or autoclave, sterilization, washing and storing. Various packaging materials have been used from historically starting from glass container to metal container, flexible retortable pouches and rigid plastic containers. The sterilization process in the canned product can be subdivided into three phases. First one is heating phase, in which the product temperature is increased from ambient to the required sterilization temperature by means of a heating medium (water or steam). This temperature is maintained for a defined time (phase 2 = holding phasing). In (phase 3 = cooling phase) the temperature in the container is decreased by introduction of cold water into the autoclave. In order to reach temperatures above 100°C (sterilization), the thermal treatment has to be performed under pressure in pressure cookers, also called autoclaves or retorts. Simple autoclaves are generally vertical ones with the lid on top. Through the opened lid, the goods to be sterilized are loaded into the autoclave. The cans are normally placed in metal baskets. The autoclave and lid are designed to withstand higher pressures up to 5.0 bar. These types of autoclaves are best suited for smaller operations as they do not require complicated supply lines and should be available at affordable prices. Larger autoclaves are usually horizontal and loaded through a front lid. Horizontal autoclaves can be built as single or double vessel system. The double vessel systems have the advantage that the water is heated up in the upper vessel to the sterilization temperature and released into the lower (processing) vessel, when it is loaded and hermetically closed. Using the two–vessel system, the heat treatment can begin immediately without lengthy heating up of the processing vessel and the hot water can be recycled afterwards for immediate use in the following sterilization cycle. In rotary autoclaves, the basket containing the cans rotates during sterilization which enhances the heat penetration resulting in reduced process time. This technique is useful for cans with liquid or semi-liquid content as it achieves a mixing effect of the liquid/semi-liquid goods. Water immersion retorts are also used in the industry for thermal processing which is advantageous over steam retorts due to its uniform temperature distribution as there is no possibility of forming air pockets in the retort which limits the heat transfer in steam retorts. At the final stage of the sterilization process the products must be cooled as quickly as possible by introducing cold water. The contact of cold water with steam causes the latter to condense with a rapid pressure drop in the retort. However, the overpressure built up during thermal treatment within the cans, jars or pouches remain for a certain period. During this phase, when the outside pressure is low but the pressure inside the containers is still high due to high temperatures there, the pressure difference may induce permanent deformation of the containers. Therefore, high pressure difference between the autoclave and the thermal

pressure in the containers must be avoided. This is generally achieved by a blast of compressed air into the autoclave at the initial phase of the cooling. Sufficient hydrostatic pressure of the introduced cooling water can also build up counter pressure so that in specific cases, in particular where strong resistant metallic cans are used, the water pressure can be sufficient and compressed air may not be needed unlike in flexible retortable pouches. After thermal processing, the containers are washed with chlorinated potable water and stored for conditioning for 2 – 4 weeks. Conditioning helps in proper mixing of the ingredients with the fish products and helps in assessing the extent of thermal process severity. If the containers do not show any deformation, it indicates the effectiveness of the thermal processing.

The important steps in canning process are:

1. Raw material preparation
2. Blanching/ Precooking
3. Filling into containers
4. Addition of fill (brine/ oil/ gravy)
5. Exhausting
6. Seaming/ sealing
7. Retorting (heat processing)
8. Cooling
9. Drying
10. Labelling and storage

Fig.: Steam retort and water immersion retort

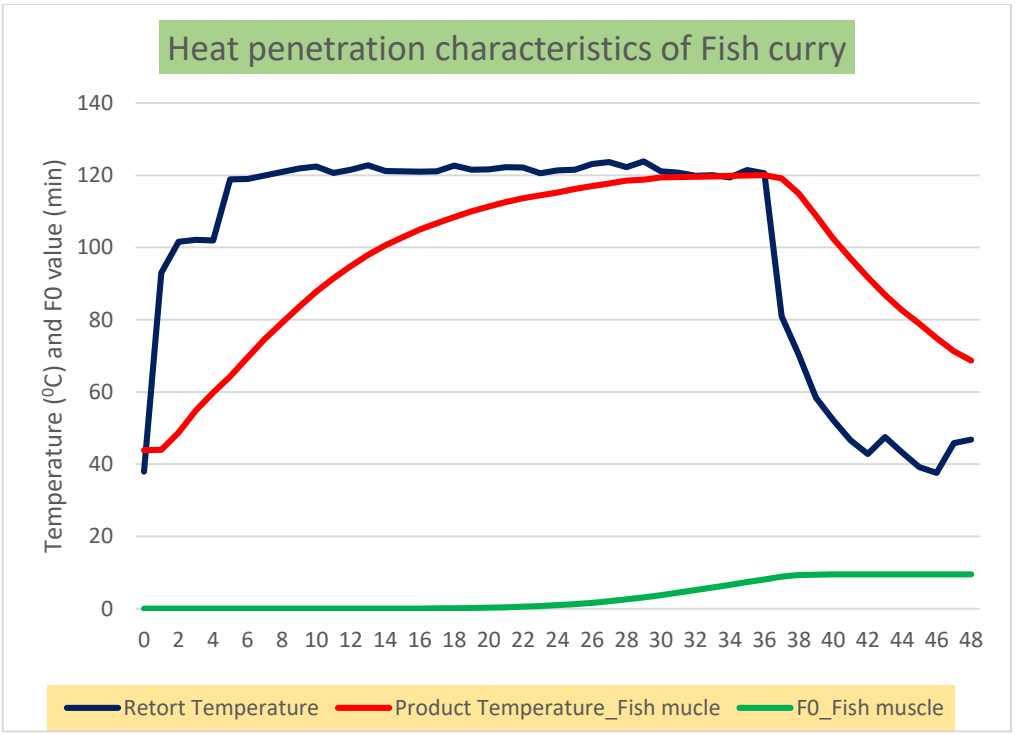

Fig.: Typical heat penetration curve of fish curry in retortable pouches

Chapter 9

Physical Hazards in Seafood

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A physical hazard is any potential material not commonly found in food which causes illness/injury to consumer on consumption. Hazard Analysis and Critical Control Point (HACCP) is a system which identifies, evaluates, and controls hazards which are significant for food safety. In HACCP, hazard is defined as a biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. Accordingly mere contamination or undesirable conditions such as insects, hair, filth, spoilage, economic fraud, and violations of regulations/standards of food, cannot be considered as a hazard.

Physical hazards are potentially harmful extraneous matter, that are not normally found in food; but mistakenly consumed foreign material or object, which is likely to cause choking, injury or other adverse health effects to the consumer. These hazards can enter into food product at any stage of production.

Category of physical hazards:

In general, the physical hazards can be categorized into three

1. Objects naturally present in the foods

Naturally, different kinds of extraneous matter can be found in food, like bone fragments, broken pieces of shells in molluscs and broken pieces of chelate & carapace in shrimp and crab *etc.*,

2. Objects added during production:

Some extraneous materials may get introduced into the food system during the production process. For example, stone particles, rocks, and mud in the case of vegetables and fruits. These kinds of things can be categorized as ‘physical hazards added during production’.

3. Objects added during processing:

During processing/preparation step, due to poor handling practices, anything that comes into direct contact with food can introduce, some physical hazards into the food. Some examples are jewelry, glass pieces, plastics, small concrete pieces, metal fragments, *etc.*

Glass is a very common physical hazard, that can be introduced into the food system from the lightening facilities and glass containers used in the processing plant. Metal is another physical hazard that can be introduced from metallic equipment's, from worn utensils, broken needles, stapler *etc.*, Packaging materials, gloves, cleaning equipment's and all can introduce plastic into the food system. Stones from concrete structures and floors in food processing facilities; broken pieces of wood from wooden structures and wooden pallets used to store or transport ingredients or food products, fields, boxes, buildings, *etc*. are also contribute towards the physical hazards.

These extraneous materials can be again categorized into 2- avoidable and unavoidable. Unavoidable extraneous materials can be a by-product of the processing or something inherent to the raw material such as minute insect fragments in fig, microscopic airborne debris, dirt on potatoes *etc*., But avoidable extraneous materials are preventable and are having zero tolerance in the food system. These may be introduced as a result of poor hygienic/handling practices.

Health issues associated with the physical hazards:

Generally, physical hazards do not cause a disease, but it can result in an injury like laceration (a deep cut or tear in skin or flesh), perforation (piercing) of tissue in the mouth, throat, stomach or intestines, broken teeth, damage to gums, and choking. The severity will vary with infants, elderly, medically compromised and healthy people. Hence control of this physical hazard is important in food processing.

Control measures of physical hazards:

Preventative approach is the best way to control physical hazards in food system and this approach includes

- ✓ Good Manufacturing Practices (GMP)
- ✓ Standard operating procedures (SOP)
- ✓ Pest control measures
- ✓ Ingredient specifications
- ✓ Supplier certification
- ✓ Use of equipment to screen for physical hazards
- ✓ Using appropriate design of equipment
- ✓ Employee training
- ✓ Personnel precautions (hair cover, gloves, mask, etc.)
- ✓ End product screening

Chapter 10

Orientation to Chemical hazards and other contaminants in Fishery Products

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Introduction:

Global population is depending upon seafood as a healthy diet choice because of its richness in high value proteins, health beneficial vitamins, minerals and poly unsaturated fatty acids. Fish is also a primary protein source in most parts of the world. Even though fish supplies many health benefits, seafood can be compromised by different chemical contaminants which are harmful to consumers. Fishes are harvested from waters that are contaminated by varying amounts of industrial chemicals, heavy metals, pesticides and antibiotics. These contaminants may accumulate in fish at levels that can cause human health problems (e.g. carcinogenic and mutagenic effects). Food can become contaminated at any point during production, distribution and preparation. Everyone along the production chain, from producer to consumer, has a role to ensure the safety of seafood.

The number of chemical contaminants is increasing day by day, hence threats associated with chemical contamination of seafood is also increasing. Environmental contaminants mainly include ubiquitous pollutants such as heavy metals and dioxins. Even though they are naturally present in the environment their level can be increased due to anthropogenic influences. Contaminants can also come as toxins produced by fungi (Eg. aflatoxins) and algae (Eg. ciguatoxin). The different chemical contaminants in seafood can also include food additives that are intentionally added like preservatives, colour retention agents etc. The contaminants can also generate during processing or cooking which include acrylamide and heterocyclic amines. Residue of agricultural chemicals resulting from previous application of pesticides, and veterinary drugs during production and storage of food crops and animals, have been considered as human health hazards. But these types of contaminants have a great potential in control by proper conditions of usage and their presence. Also some natural components of food can also act as contaminant like allergic substances and phyto haemagglutinin.

Basically the chemical contaminants are classified into three main groups such as:

(i) **Naturally occurring** – allergens, Mycotoxins, Scomberotoxin (Histamine), Ciguatera poison, Puffer fish poison, Shellfish toxins (PSP, DSP, NSP, ASP)

(ii) **Unintentionally or incidentally added chemicals** – Pesticides, Fungicides, Fertilizers, Toxic compounds, Toxic metals

(iii) **Intentionally added chemicals and food additives** - Food preservatives, Food additives, Vitamins, Minerals, Antibiotics used in aquaculture, Sulfites used in shrimp to prevent melanosis, Nitrites as preservatives, Colouring agents, Detergents

Biotoxins

Marine biotoxins are responsible for many seafood borne diseases. It includes both shellfish toxins and ichthyotoxins (fish toxins). Shellfish toxins include Paralytic shellfish toxins, Diarrhetic shellfish toxins, Azaspriacid shellfish toxins, Neurotoxic shellfish toxin and Amnesic shellfish toxins. Ichthyotoxins include Ciguatera toxin and Tetradotoxin. Fish poisoning is caused by consuming fish containing poisonous tissues and shellfish poisoning results from ingestion of shellfish that have accumulated toxins from the plankton they have consumed.

(i)Tetradotoxin (Puffer fish poison): It is the most lethal of all fish poisons. Toxin production is due to the activity of symbiotic bacteria. Toxin will be accumulated in liver, ovaries and intestine as a defence mechanism. But the muscle is free of toxin. It is also called as Tetradon poisoning or Fugu poisoning. It is 275 times more toxic than cyanide. On an average a dose of 1-2 mg of purified tetrodotoxin can be lethal to humans.

(ii) Ciguatera - Ciguatera is a clinical syndrome caused by eating the flesh of toxic fish caught in tropical reef and island waters. Most common fish poisoning and the fish becomes toxic due to feeding of toxic algae – dinoflagellates, *Gambeirdiscus toxicus*. Red snapper *(Lutjanus bohar),* Grouper *(Variola louti) and* Moray eel are recorded as ciguateric. More than 400 species have been implicated in ciguatera poisoning.

(iii)Paralytic shell fish poisoning (PSP) –This is associated with dinoflagellate blooms (*Alexandrium catenella, Gonyaulax tamerensis*). Heat stable saxitoxin will be accumulated inmussels, clams, oysters, scallops etc. grown in algal bloom areas. Greater number of human deaths is reported due to consumption of contaminated shellfish. The current regulatory level for fresh bivalve molluscs in most countries is 80 µg/100 g.

(iv)Diarrhetic shellfish poisoning (DSP) - Dinoflagellate *Dinophysis forti* is the algae which producesokadoic acid, the causative of DSP. Primary symptom is acute diarrhoea. Regulatory level in fresh bivalve molluscs in most countries is 0-60 µg /100 g.

Mouse bioassay and analysis by HPLC are the important methods for monitoring biotoxins. Reliable sampling plans are required for effective monitoring.

Heavy metals

Heavy metals are toxic metals and above a normal level can affect the quality, safety and marketability of seafood. They are "Cumulative poisons" which can irreversibly accumulate in the body. They have atomic weight higher than 40.04 and specific density > 5g/ cm. The main threats are Arsenic, Cadmium, Mercury and Lead. These metals have no beneficial effects in human and they have no homeostasis mechanism. These contaminants are highly depend upon geographic location, species and fish size, feeding pattern, solubility of chemical and their persistence in the environment.

Lead is mostly deposited in bones and not in soft tissues. But, from food safety point of view lead accumulation in edible parts is important. Compared to fish lead content is higher in shellfishes as it is getting accumulated in hepatopancreas. The organic form of lead, tetra alkyl lead is mostly found in fish. In fishes Cd is mostly deposited in kidney and liver and in muscles the level is quite low. In invertebrates like Cephalopods it can go as high as 30 ppm in digestive glands. Hence the digestive gland must be removed immediately after catch. Both Cd and Pb are carcinogenic in nature. Mercury is one of the most toxic heavy metal in the environment. Among metal contaminants methyl mercury ha s elicited the most concern among consumers. It is toxic to the nervous system especially the developing brain. Arsenic is a widely distributed metalloid and major contaminant in case of ground water. IARC has classified inorganic arsenic as a human carcinogen.

The most widely used techniques for detection and quantification of heavy metals are Atomic Absorption Spectrometry, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

Histamine in fish

Though all types of biogenic amines can be formed in fish, the most toxic amine detected in fish is histamine. Histamine poisoning is the most common form of toxicity caused by ingestion of fish and is generally due to the ingestion of foods containing unusually high levels of histamine. The commonly implicated incidents of histamine poisoning are associated with the fish families Scombridae and Scomberesocidae. It is also known as Scombroid poisoning. Histamine is a powerful biologically active chemical present in the mast cells and basophils in larger amounts. Histamine poisoning is often manifested by a wide variety of symptoms. Major symptoms affecting the cutaneous system include rashes, urticaria, edema and localized inflammation etc. gastrointestinal effects include nausea, vomiting, diarrhoea and abdominal

cramps. Also include symptoms like hypotension, headache, palpitation, tingling and flushing. Severe suffocation and respiratory distress have been reported in severe cases of histamine poisoning. The onset of histamine poisoning can extend from 10 minutes to 1 hour following consumption of contaminated fish and can last from 12 hour to a few days. Histamine concentration required to produce poisoning varies with respect to the susceptibility of each individual. In case of susceptible individuals concentration between 5 and 10 mg/100g can cause symptoms. Many foods contain small amounts of histamine which can be tolerated easily.

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.

A wide variety of procedure for the determination of histamine and biogenic amines is available. Include both semi quantitative and quantitative methods. Methods based on colorimetry, fluorometry and enzyme-linked immunosorbent assay (ELISA) are available. Mostly biogenic amines including histamine is analysed by High Performance Liquid Chromatography (HPLC) methods with pre and post column derivatisation and UV–visible or fluorescence detection. LC with tandem mass spectrometry (MS/MS) can also be a useful approach for an unequivocal confirmation of the studied analytes.

Antibiotics

Illegal use of antibiotics for veterinary purposes has become a matter of public concern. Antibiotics are used in aquaculture as prophylactics, as growth promoters and for treatment of diseases. They are usually administered in feeds and most commercial shrimp feeds contain antibiotics.The feeding of antibiotics as growth promoters is associated with decrease in animal gut mass, increased intestinal absorption of nutrients and energy sparing. But inappropriate and frequently abusive, use of antibiotics can affect human health. The two major concerns are the presence of antimicrobial residues in edible tissues and the emergence of antimicrobial resistance, which represents a huge threat to public health worldwide.

The greatest potential risk to public health associated with antimicrobial use in aquaculture is the development of a reservoir of transferable resistance genes in bacteria of aquatic environments. The antibiotics lose their efficacy over time because of the emergence and dissemination of resistance among bacterial pathogens.

EU implemented "zero tolerance policy" regarding antibiotic residue. Using LCMSMS method EU laboratories are equipped to detect traces of prohibited carcinogenic antibiotics like chloramphenicol up to 0.3 ppb and nitrofuran up to 1 ppb levels. Many of the antibiotics are listed as prohibited substance in fish and fishery products. In India the tolerance limit has been set only for the following antibiotics

Antibiotic	MRL (ppm)
Tetracycline	0.1
Oxytetracycline	0.1
Trimethoprim	0.05
Oxolinic Acid	0.3

The monitoring of antimicrobial residues in fish tissues requires sensitive and selective analytical methodologies to verify the accomplishment of the legal framework and reach the desirable high standards of quality and food safety. The methods can be microbiological, immunochemical or physico chemical. European council directive 96/23/EC, 1996 gives direction on measures of monitoring residues in live and animal products. It specifies spectrometric detection, GC, HPLC, ELISA and LC-MS/MS methods.

Pesticides

Pesticides are substances used for preventing, destroying or controlling any pest. The major chemical types of pesticides include (i) Organochlorine pesticides – mostly banned because of its lipophilic nature. Have properties of bioaccumulation and high persistence (Eg: DDT and its derivatives, BHC, Endosulfan, aldrin, dieldrin etc). (ii) Carbamates – widely used insecticides (Eg: carbaryl, carbofuran, carbosulfan). (iii) Organophosphates – have rapid aciton at lower concentration, easy biodegradable in nature (Eg: malathon, Moncrotophos). (iv) Pyrethroids – have low mammalian toxicity and knock down effect against insects (Eg: Deltamethrin, Cypermethrin, Cyhalothrin, Fenvalerate etc.). Pesticide contamination in fish mainly comes though agricultural runoff and municipal sewage effluent.

Persistent organic pollutants (POPs) – they are organic chemicals that remain intact in the environment for long periods, become widely distributed, bio accumulate in food chain and are toxic to humans, wild life and environment. The POPs to which seafood consumers are most likely exposed are dioxins and PCBs. The Stockhome convention on POPs initially identified twelve POPs, called as 'dirty dozen' include 9 pesticides, 2 industrial chemicals and 1 un intentional by product. They are aldrin, chlordane, DDT, dieldrin, endrin, heptachlor,

hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls (PCBs), dioxins and furans. Later nine new chemicals were again added to Stockhome convention.

The chromatographic techniques mainly Gas chromatography (GC), Gas chromatography-tandem mass spectrometry (GC-MS/MS) and Liquid chromatography-tandem mass spectrometry (LC-MS/MS) are used for the analysis of pesticide residues.

Food additives

Food additives means substances that normally are not used independently as food or its ingredient and which, after being added to the food during its production, processing packaging, transportation or storage, remain included in the food, even in changed state. In simpler terms, food additives are the substances which are added to food by the manufacturers to facilitate processing or to improve appearance, texture, flavour and keeping quality. Functions of food additives are

- To maintain product consistency – Eg: emulsifiers, stabilizers, thickners etc
- To improve nutritional quality – Eg: vitamins, minerals
- To improve product safety and quality – Eg: preservatives, antioxidants
- To aid in process or preparations – Eg: leavening agents
- To enhance sensory characteristics of the product

Classification of food additives

Food additives are classified based on their function in food, i.e. the purpose for which the additive has been incorporated in the food.

- antioxidants
- preservatives
- food colours
- food flavours
- emulsifiers and stabilizers
- anti-caking agents
- sequestrants
- acid, bases and buffers
- anti-foaming agents
- sweeteners
- enzymes, and leavening agents.

Chapter 11

Orientation to Biological hazards

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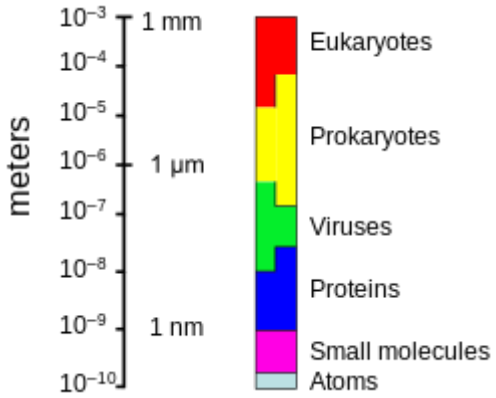
A microorganism, or microbe, is an organism of microscopic size, which may exist in its single-celled form or as a colony of cells. Technically a microorganism or microbe is an organism that is microscopic. The scientific study of microorganisms began with their observation under the microscope in the 1670s by Anton van Leeuwenhoek. The microorganisms are classified into Bacteria, Fungi, Archaea, Protists, Microscopic plants (green algae), Microscopic animals (plankton) and Virus. Microorganisms can be found almost anywhere on Earth. Bacteria and archaea are almost always microscopic, while a number of eukaryotes are also microscopic, including most protists, some fungi, as well as some micro-animals and plants. Bacteria like archaea are prokaryotic - unicellular, and having no cell nucleus or other membrane-bound organelle.

Bacteria function and reproduce as individual cells, but they can often aggregate in multicellular colonies. Some species such as myxobacteria can aggregate into complex swarming structures, operating as multicellular groups as part of their life cycle, or form clusters in bacterial colonies such as *E. coli*. Their genome is usually a circular bacterial chromosome – a single loop of DNA, although they can also harbor small pieces of DNA called plasmids. These plasmids can be transferred between cells through bacterial conjugation. Bacteria have an enclosing cell wall, which provides strength and rigidity to their cells. In general, bacteria are between 0.2 and 2.0 um - the average size of most bacteria. Research studies have shown their size to play an important role in survival over time. Due to their small size, bacteria are able to exploit and thrive in various microenvironments. The small size of bacteria is also beneficial for parasitism and oligotrophy.



The following are the major categories of bacteria based on their shapes:

a) Cocci: Cocci bacteria appear spherical or oval in shape. For the most part, the shape is determined by the cell wall of the organism and therefore varies from one type of cocci bacteria to another. Cocci bacteria may exist as single cells or remain attached to each other. Attached Cocci bacteria include: **Diplococci** bacteria - Diplococci bacteria are the type of cocci bacteria that occur as a pair (two joined cells). Some examples of Diplococci bacteria include: *Streptococcus pneumonia, Moraxella catarrhalis, Enterococcus* spp, *Neisseria gonorrhoea*. While some of these cells may be truly round shaped, others may appear elongated (ovoid) or bean-shaped/kidney shaped. For instance, some Neisseria cells may appear round while others are bean-shaped when viewed under the microscope. **Tetrad bacteria** - Tetrad bacteria are arranged in groups of four cells. Following division, the cells remain attached and grow in this attachment. Common examples of Tetrad bacteria include: *Pediococcus, Tetragenococcus.*

Sarcinae sarcina/Bacteria - Sarcina bacteria occur in groups of 8 cells. Unlike tetrads that divide into two planes, Sarcinae is produced through the perpendicular plane division. Some of the characteristics associated with these bacteria include being strict anaerobes, Gram-positive bacteria and that measure between 1.5 and 3.0 um. Examples of Sarcinae bacteria include: *Sarcina aurantiaca, Sarcina lutea, Sarcina ventriculi*. **Streptococci Bacteria**- Streptococci bacteria are a type of bacteria that arrange in a chain form (resembling chains). A majority of these bacterial cells are also ovoid in shape and may form paired chains. As members of the family Streptococcaceae, this group of bacteria is characterized by being non-motile, Gram-positive organisms. Examples of Streptococcus bacteria include: *Streptococcus pyogenes, Streptococcus pneumonia, S. mutans*. **Staphylococci Bacteria**- Staphylococci Bacteria are a type of bacteria that form grape-like clusters. This type of arrangement is the result of division that occurs in two planes. Two of the main characteristics of these organisms

are that they are immobile, Gram-positive bacteria. Examples of Staphylococci bacteria include: *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus aureus* *Staphylococcus capitis*.

b) Bacillus Bacteria (Rod-Shaped): Bacillus bacteria have the following traits: Are all rod-shaped, form endospores and are facultative anaerobes. bacillus bacteria are also arranged differently. While some exist as single, unattached cells (e.g. *Salmonella enterica* subsp, *Bacillus cereus*, and *Salmonella choleraesuis*), others are attached. The following are the different types of bacillus arrangements: *Diplobacilli bacteria* - Like Diplococci bacteria, Diplobacilli occur in pairs. Following cell division, the two cells do not separate and continue existing as a pair. Examples of Diplobacilli bacteria include: *Coxiella burnetii*, *Klebsiella rhinoscleromatis*, *Moraxella bovis*. **Coccibacilli bacteria** - Compared to other bacilli, Coccibacilli bacteria are shorter in length and thus appear stumpy. Examples of Coccibacilli include: *Chlamydia trachomatis*, *Haemophilus influenza*. Unlike cocci and bacilli bacteria, some types of bacteria appear curved when viewed under the microscope. However, they vary in shape making it possible to differentiate them from each other. These include: Vibrio bacteria - Generally, vibrio bacteria are comma-shaped and thus not fully twisted (curved rods). Examples of Vibrio bacteria include: *Vibrio mytili*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Vibrio cholerae*. **Spirochete** - Spirochetes are characterized by a helical shape. Spirochetes are also flexible and have been shown to produce mycelium. The movement involves the use of axial filaments, which is one of the distinguishing features between the bacteria and other types of bacteria. Examples of Spirochetes include: Leptospira, Spirochaeta, Treponema. **Spirilla** bacteria - Like Spirochetes, Spirilla bacteria possess a helical shape. However, they are more rigid and have the typical flagella found in other types of bacteria. Some examples of Spirilla bacteria include: *Aquaspirillum*, *Campylobacter jejuni*, *Spirillum winogradskyi*.

In microbiology and bacteriology, Gram stain or Gram staining, also called Gram's method, is a method of staining used to classify bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria. The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique in 1884. Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet. Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out on addition of ethanol. They are stained pink or red by the counterstain, commonly safranin or fuchsine. Lugol's iodine solution is always added after addition of crystal violet to strengthen

the bonds of the stain with the cell membrane. Gram staining is almost always the first step in the preliminary identification of a bacterial organism. While Gram staining is a valuable diagnostic tool in both clinical and research settings, not all bacteria can be definitively classified by this technique. Acid-fast staining is the differential staining techniques which was first developed by Ziehl and later on modified by Neelsen. So this method is also called Ziehl-Neelsen staining techniques. Neelsen in 1883 used Ziehl's carbol-fuchsin and heat then decolorized with an acid alcohol, and counter stained with methylene blue. Thus Ziehl-Neelsen staining techniques was developed. The main aim of this staining is to differentiate bacteria into acid fast group and non-acid fast groups. This method is used for those microorganisms which are not staining by simple or Gram staining method, particularly the member of genus Mycobacterium, are resistant and can only be visualized by acid-fast staining.

Growth Curve

In a closed system with enough nutrients, a bacteria shows a predictable growth pattern that is the bacterial growth curve. It consists of four different phases. Read on to learn about the phases in detail. Phases of the Bacterial Growth Curve: Upon inoculation into a new nutrient medium, the bacteria shows four distinct phases of growth. Let us dive into each of the phases in detail.

Lag Phase: The bacteria upon introduction into the nutrient medium take some time to adapt to the new environment. In this phase, the bacteria does not reproduce but prepares itself for reproduction. The cells are active metabolically and keep increasing in size. The cells synthesise RNA, growth factors and other molecules required for cell division.

Log Phase: Soon after the lag phase, i.e., the preparation phase, the bacterial cells enter the log phase. The log phase is also known as the exponential phase. This phase is marked by the doubling of the bacterial cells. The cell number increases in a logarithmic fashion such that the cell constituent is maintained. The log phase continues until there is depletion of nutrients in the setup. The stage also comes to a stop if toxic substances start to accumulate, resulting in a slower growth rate. The cells are the healthiest at this stage and researchers prefer to use bacteria from this stage for their experimental processes. Plotting this phase on the bacterial growth curve gives a straight line. Upon calculation of the slope of this line, the specific growth rate of the organism is obtained. It is the measure of divisions per cell per unit of time.

Stationary Phase: In the stationary phase, the rate of growth of the cells becomes equal to its rate of death. The rate of growth of the bacterial cells is limited by the accumulation of toxic compounds and also depletion of nutrients in the media. The cell population remains constant at this stage. Plotting this phase on the graph gives a smooth horizontal linear line.

Death Phase: This is the last phase of the bacterial growth. At this stage, the rate of death is greater than the rate of formation of new cells. Lack of nutrients, physical conditions or other injuries to the cell leads to death of the cells.

Physical factors that affect microbial growth

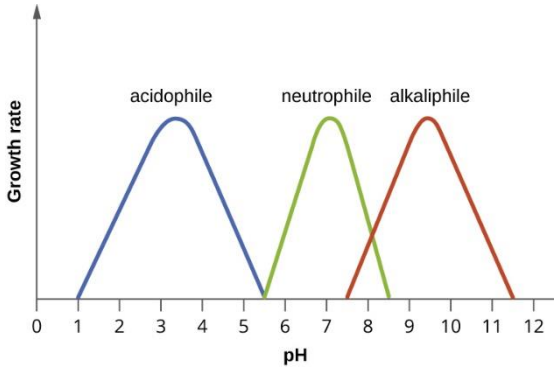
a) Temperature: Generally,an increase in temperature will increase enzyme activity. But if temperatures get too high, enzyme activity will diminish and the protein (the enzyme) will denature. On the other hand, lowering temperature will decrease enzyme activity. At freezing temperatures enzyme activity can stop. Repeated cycles of freezing and thawing can denature proteins. In addition, freezing causes water to expand and also forms ice crystals, hence cells begin to rupture. Every bacterial species has specific growth temperature requirements which is largely determined by the temperature requirements of its enzymes. PSYCHROPHILES grow best between -5^{o}C and 20^{o}C, MESOPHILES grow best between 20^{o}C and 45^{o}C and THERMOPHILES grow best at temperatures above 45^{o}C. THERMODURIC organisms can survive high temperatures but don't grow well at such temperatures. Organisms which form endospores would be considered thermoduric. Some organisms have exotic temperature requirements. *Thermus aquaticus* is a bright orange gram negative rod isolated from hot water and steam vents at Yellowstone Park. This organism grows best at temperatures between 70-75^{o}C (158-167^{o}F). Some of its unique enzymes are in demand for molecular biological and industrial applications.

b) **Oxygen**: Microbes display a great diversity in their ability to use and to tolerate oxygen. In part this is because of the paradoxical nature of oxygen which can be both toxic and essential to life. OBLIGATE AEROBES rely on aerobic respiration for ATP and they therefore use oxygen as the terminal electron acceptor in the electron transport chain. Pseudomonas is an example of this group of organisms. MICROAEROPHILES require O_2 for growth but they are damaged by normal atmospheric levels of oxygen and they don't have efficient ways to neutralize the toxic forms of oxygen such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2). The Streptococci are examples of this group. OBLIGATE ANAEROBES will die in the presence of oxygen because they lack enzymes like superoxide dismutase and catalase. Organisms like Clostridium, metabolize through fermentation and / or anaerobic respiration. AEROTOLERANT organisms like Lactobacillus ferment and therefore do not use oxygen, however they do tolerate it. FACULTATIVE ANAEROBES are the most adaptable. They are capable of both fermentation and aerobic respiration. *Escherichia coli* is an example of this class of organisms. ANAEROBIC PATHOGENS: *Clostridium tetani* - agent of tetanus, puncture wounds, produces a toxin which enters the spinal column and blocks the inhibitory

spinal motor neurons. This produces generalized muscle spasms or spastic paralysis. *Clostridium botulinum* - this soil organism is the causative agent of botulism which typically occurs after eating home canned alkaline vegetables which were not heated enough during canning. The neurotoxin blocks transmission across neuromuscular junctions and this results in flaccid paralysis. *Clostridium perfringes* and *Clostridium sporogenes* - these organisms are associated with invasive infections known as GAS GANGRENE. *Clostridium difficile* - the causative agent of pseudomembranous colitis, a side effect of antibiotic treatment which eliminates the normal flora. MICROAEROPHILES: These organisms are all catalase negative, therefore the catalase test is useful in identification. They also have distinctive colonial morphology on blood agar which is differential for them. It is important to note if the colonies are alpha, beta, or gamma hemolytic. Group A Streptococcus - *Streptococcus pyogenes*, This beta hemolytic organism is also bacitracin sensitive. It is the cause of strep throat, rheumatic fever, glomerulonephritis and scarlet fever. Group D Streptococcus - Enterococcus - *Streptococcus faecalis*, This organism is a normal inhabitant of the large intestine. It is also a frequent cause of bladder infections. *Streptococcus pneumonia*, This organism is a normal inhabitant of the respiratory tract. It is a frequent cause of pneumonia in people who have been compromised by other illness.

Based on the nutritional requirements, bacteria are classified as follows:

Energy source:	light:	phototrophic
	chemical:	chemotrophic
Electron source:	inorganic compounds:	lithotrophic
	organic compounds:	organotrophic
Carbon source:	CO_2:	autotrophic
	organic:	heterotrophic



Based on pH bacterial requirements are classified as follows:

Most bacteria are neutrophiles, meaning they grow optimally at a pH within one or two pH units of the neutral pH of 7. Most familiar bacteria, like *Escherichia coli*, *Staphylococci*, and *Salmonella* spp. are neutrophiles and do not fare well in the acidic pH of the stomach. However, there are pathogenic strains of *E. coli*, *S. typhi*, and other species of intestinal pathogens that are much more resistant to stomach acid. In comparison, fungi thrive at slightly acidic pH values of 5.0-6.0.Microorganisms that grow optimally at pH less than 5.55 are called acidophiles. Eg. *Lactobacillus* bacteria. Acidophilic microorganisms display a number of

adaptations to survive in strong acidic environments. For example, proteins show increased negative surface charge that stabilizes them at low pH. Pumps actively eject H^+ ions out of the cells. At the other end of the spectrum are alkaliphiles, microorganisms that grow best at pH between 8.0 and 10.5. *Vibrio cholerae*, the pathogenic agent of cholera, grows best at the slightly basic pH of 8.0; it can survive pH values of 11.0.

Foodborne bacterial pathogens

Foodborne pathogens are mainly bacteria, viruses, or even parasites that are present in the food and are the cause of major diseases such as food poisoning. Foodborne pathogens are categorized according to the specific foods that are consumed. Foodborne illness occurs when a pathogen is ingested with food and establishes itself (and usually multiplies) in the human host, or when a toxigenic pathogens establishes itself in a food product and produces a toxin, which is then ingested by the human host. Thus, foodborne illness is generally classified into: (a) foodborne infection and (b) foodborne intoxication. In foodborne infections, since an incubation period is usually involved, the time from ingestion until symptoms occur is much longer than that of foodborne intoxications. More than 200 different food-borne diseases have been identified. Aomng them, the common pathogenic bacteria associated with the fish and fishery products includes: *Aeromonas hydrophilia*, *Bacillus anthracis*, *Bacillus cereus/subtilis/lichniformis*, *Brucella/abortus/melitensis/suis*, *Campylobacter jejuni/coli*, *Clostridium botulinum/perfringens*, *Escherichia coli*, *Enterobacter sakazakii*, *Listeria monocytogenes*, *Mycobacterium paratuberculosis*, *Salmonella enterica*, *Shigella spp.*, *Staphylococcus aureus, Vibrio cholera, V. cholerae* non-01, *V. parahemolyticus, V. vulnificus, V. fluvialis and Yersinia enterocolitica*. *Campylobacter* sp. (mostly associated with raw or undercooked poultry) is the major foodborne pathogen, causing more than two million infections per year, while *Salmonella*, mostly found in meat, poultry, and eggs, is responsible for more than one million cases of food poisoning. *Shigella*, *Escherichia coli* (mostly found in meat and unpasteurized milk), *Clostridium botulinum* (often found in improperly home-canned foods), *Clostridium perfringens*, *Yersinia*, *Vibrio cholerae*, *V. vulnificus*, *V. parahaemolyticus*, *Staphylococcus aureus*, *Bacillus* spp., and *Listeria* (in uncooked meats, vegetables, unpasteurized milk, and soft cheese) also cause foodborne disease.

The specific bacterial pathogens, isolation and identification protocols are mentioned below:

a) *Clostridium botulinum*

- **Bacteria:** Anaerobic, spore-forming, motile GPR

- **Source:** Soils, sediments, intestinal tracts of fish/mammals, gills and viscera of crabs and other shellfish
- **Illness:** Intoxication (heat-labile neurotoxin)
- **Symptoms:** Weakness, vertigo, double vision, difficulty in speaking, swallowing and breathing, respiratory paralysis
- **Foods:** Semi-preserved seafood, improperly canned foods
- **Transmission:** Spores present in raw foods
- **Control:** Proper canning, a_w <0.93, pH <4.7
- **Isolation:** Inoculate the sample into cooked meat medium and incubate for 48-72 h. Streak onto blood agar medium supplemented with gentamycin and metronidazole ans incubate the plates under anaerobic conditions in anerobic jar for 48 h at 37^{o}C. After incubation observe for the growth.
- **Toxin testing:** The toxins produced by Clostridium botulinum is tested using mouse bio assay and also by other methods such as PCR, ELISA, endopeptidase assay, lateral flow tets

b) *Clostridium perfringens*

- **Bacteria:** Anaerobic, spore-forming, nonmotile GPR
- **Source:** Soil, dust, intestinal tract of animals and humans
- **Illness:** Infection (toxin released on sporulation)
- **Symptoms:** Intense abdominal cramps and diarrhea
- **Foods:** Temperature abuse of prepared foods such as meats, meat products, and gravy
- **Transmission:** Spores present in raw foods
- **Control:** Proper time/temperature control; preventing cross-contamination of cooked foods
- **Identification:** The bacterium is mainly identified by performing biochemical tests such as Grams staining, Litmus milk test, haemolysis (double zone), CAMP test
- **Toxin testing:** Nagler test

c) *Bacillus cereus*

- **Bacteria:** Facultatively aerobic, spore-forming, motile GPR
- **Source:** Soil, dust, raw foods
- **Illness:** 1) diarrheal type (infection, heat-labile toxin); 2) emetic type (intoxication, heat-stable toxin)
- **Symptoms:** 1) profuse watery diarrhea, abdominal pain; 2) vomiting, nausea

- **Foods:** 1) vegetables, salads, meats, casseroles; 2) rice **Transmission:** Spores present in raw foods
- **Control:** time/temperature; reheat cooked foods to >165^{o} F
- **Isolation:** The bacterium is isolated on commonly used microbiological media such as nutrient agar.

C) *Campylobacter jejuni*

- **Bacteria:** Microaerophilic, motile GNR
- **Source:** Intestines of poultry, livestock, domestic animals; streams and ponds
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Diarrhea, abdominal pain, headache, weakness
- **Foods:** undercooked chicken & hamburger, raw milk & clams
- **Transmission:** Contaminated foods & water; cross-contamination; person to person
- **Control:** Proper cooking, proper hand and equipment washing, sanitary food handling practices
- **Isolation:** The bacterium is isolated from the samples by using Bolton broth incubated at 42 ^{o}C for 24 h followed by streaking on chromogenic media incubated under micro-aerophilic conditions. The intense red colored colonies on a translucent agar facilitates the reading compared to charcoal based agar.
- **Identification:** PCR

d) Pathogenic *Escherichia coli* O157:H7

- **Bacteria:** Facultative anaerobic, motile or nonmotile GNR
- **Source:** Intestines of animals and poultry
- **Illness:** Hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP)
- **Symptoms:** HC) diarrhea & vomiting, HUS) diarrhea & acute renal failure, TTP) diarrhea, GI hemorrhage, Brain blood clots
- **Foods:** Meat, poultry, potatoes, raw milk
- **Transmission:** Cross-contamination, sewage pollution
- **Control:** Proper cooking, temp. control, preventing cross-contamination, proper personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using *E. coli* broth incubated initially at 25 ^{o}C for 2 h and at 42 ^{o}C for 8 h followed by streaking on chromogenic media incubated under aerophilic conditions (37 ^{o}C for 18-24 h). *E. coli* produces blue colour colonies.

- **Identification:** Biochemical tests and PCR

e) *Listeria monocytogenes*

- **Bacteria:** Microaerophilic, motile, GPR
- **Source:** Widespread in the environment
- **Illness:** Infection
- **Symptoms:** Mild flu-like symptoms to meningitis, abortions, septicemia, and death
- **Foods:** Coleslaw, raw milk, Mexican style soft cheese, smoked mussels
- **Transmission:** Cross-contamination, from raw to cooked food, contaminated raw foods
- **Control:** Proper cooking, preventing, cross-contamination, pasteurizing milk
- **Isolation:** The bacterium is isolated from the samples by using half-Fraser broth incubated at 30 ^{o}C for 24 h and later 0.1 ml of enriched broth (0.1 ml) was transferred to Fraser broth incubated at 37 ^{o}C for 24 h followed by streaking on selective media (Ottoviani and Agosti) or secondary selective media (PALCOM, OXFORD) and incubate under aerophilic conditions (37 ^{o}C for 18-24 h). β-D-glucosidase activity, common to the Listeria genus, is detected using a chromogenic substrate (X-glucoside). Its hydrolysis induces the formation of a blue to blue-green color in all Listeria colonies. PI-PLC is an enzyme only detected in pathogenic Listeria species: *L. monocytogenes* and *L. ivanovii*. AL medium contains phosphatidylinositol which, when it breaks down, produces an opaque halo around the colonies of these two bacterial species. The halo is visible after 24 hr for *L. monocytogenes* and 48 hr for *L. ivanovii*.
- **Identification:** Biochemical tests and PCR

f) ***Salmonella spp.***

- **Bacteria:** Facultative anaerobic, motile, GNR
- **Source:** Intestine of mammals, birds, amphibians and reptiles
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Nausea, vomiting, abdominal cramps, fever
- **Foods:** Poultry, poultry salads, meats, dairy products, egg products
- **Transmission:** Cross-contamination, human contamination, sewage pollution of coastal waters
- **Control:** Proper cooking, temperature control, preventing cross-contamination, personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using Buffered peptone water incubated at 37 ^{o}C for 24 h followed by enrichment in Rappaport and Vassiliadis broth

(incubation at 41.5 ^{o}C for 24 h), Muller-Kauffman Tetrathionate Novobiocin broth (incubation at 37 ^{o}C for 24 h) and later streaking on XLD agar incubated at 37 ^{o}C for 24 h under aerophilic conditions. On XLD agar it produces red colour colonies with black centre.

- **Identification:** Biochemical, serological and PCR

g) *Shigella spp.*

- **Bacteria:** Facultative anaerobic, motile, GNR
- **Source:** Intestine of mammals, birds, amphibians and reptiles
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Nausea, vomiting, abdominal cramps, fever
- **Foods:** Poultry, poultry salads, meats, dairy & egg products
- **Transmission:** Cross-contamination, human contamination, sewage pollution of coastal waters
- **Control:** Proper cooking, temperature control, preventing cross-contamination, personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using *Shigella* broth supplemented with Novobiocin incubated initially at 44 ^{o}C for 24 h under anerobic conditions followed by streaking on MacConkey agar incubated under aerophilic conditions (35 ^{o}C for 20 h). Colonies are non-lactose fermenting (except S. sonnei) large, circular, convex, smooth, and translucent.
- **Identification:** Biochemical tests and Serological

h) *Pathogenic Staphylococcus aureus*

- **Bacteria:** Facultative anaerobic, motile, GNR
- **Source:** Intestine of mammals, birds, amphibians and reptiles
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Nausea, vomiting, abdominal cramps, fever
- **Foods:** Poultry, poultry salads, meats, dairy products, egg products
- **Transmission:** Cross-contamination, human contamination, sewage pollution of coastal waters
- **Control:** Proper cooking, temperature control, preventing cross-contamination personal hygiene
- **Isolation:** The bacterium is isolated from the samples by using Baird parker agar supplemented with egg yolk and potassium telurite incubated initially at 35 ^{o}C for 24 h under anerobic conditions. *Staphylococcus aureus* is characterized by the formation of

black, shiny, convex colonies surrounded by a lightening halo of the egg yolk. Coagulase negative staphylococci are almost completely inhibited and if, however, a culture does appear, areas of thinning would be absent.

- **Identification:** Mannitol fermentation, genotypic characterisation (pvl, spa typing, SCCmec typing) and phenotypic characterization (growth on ORSAB agar)

i) ***Vibrio cholerae***

- **Bacteria:** Facultative aerobic, motile, curved GNR
- **Source:** Naturally occurring in estuaries, bays and coastal water
- **Illness:** Infection (cholera or gastroenteritis)
- **Symptoms:** 01: watery diarrhea, vomiting, abdominal cramps; non-01: Diarrhea, abdominal cramps, fever
- **Foods:** Molluscan shellfish
- **Transmission:** Contaminated water, cross-contamination from raw to cooked seafood, contaminated raw seafood
- **Control:** Proper cooking, preventing cross-contamination, harvesting from approved waters
- **Isolation:** The bacterium is isolated from the samples by using alkaline peptone water incubated initially at 37 ^{o}C for 6-18 h under anerobic conditions followed by streaking on TCBS agar incubated under aerophilic conditions (37 ^{o}C for 18-20 h). *Vibrio cholera* produces flat yellow colonies with 2-3 mm in diameter
- **Identification:** Biochemical tests, Serological and PCR

j) ***Vibrio parahaemolyticus***

- **Bacteria:** Facultative aerobic, motile, curved GNR
- **Source:** Naturally occurring in estuaries and other coastal areas throughout the world
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Diarrhea, abdominal cramps, nausea, vomiting, headache
- **Foods:** Raw, improperly cooked, or cooked and contaminated fish and shellfish
- **Transmission:** Cross-contamination from raw to cooked seafood, consumption of raw seafood
- **Control:** Proper cooking, preventing cross-contamination
- **Isolation:** The bacterium is isolated from the samples by using alkaline salt peptone water incubated initially at 37 ^{o}C for 6-18 h under anerobic conditions followed by streaking on TCBS agar incubated under aerophilic conditions (37 ^{o}C for 18-20 h). *Vibrio parahaemolyticus* produces colorless colonies with a green center.

- **Identification:** Biochemical tests, pathotyping and PCR

k) *Yersinia enterocolitica*

- **Bacteria:** Facultative aerobic, motile, GNR
- **Source:** Soil, water, domesticated and wild animals
- **Illness:** Infection (gastroenteritis)
- **Symptoms:** Diarrhea, vomiting, abdominal pain, fever
- **Foods:** Meats, oysters, fish, raw milk
- **Transmission:** Cross-contamination from raw to cooked food, poor sanitation, time/temperature abuse
- **Control:** Preventing cross-contamination, proper sanitation and food handling practices
- **Isolation:** The bacterium is isolated from the samples by using buffered peptone water incubated initially at 4 ^{o}C for 1-3 weeks under anerobic conditions or treat the samples with alkali and later streaking on CIN or mVYE agar incubated under aerophilic conditions (30 ^{o}C for 24 h). *Vibrio parahaemolyticus* produces red (red bulls eye) colonies.

Identification: Biochemical tests (Urea, TSI, LIM), PYZ and AA tests, Biotyping and Serotyping, Real time PCR

Chapter 12

Prerequisite Programs (GMP & SSOP) Implementation in Seafood Industry

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Prerequisite programs (PRPs) are those procedures that address environmental and operational conditions which provide the foundation for the HACCP system. Prerequisite programs provide the basic conditions that are necessary for the production of safe and wholesome food. Some of these programs are required by regulations such as Good Manufacturing Practices (GMPs) and Sanitation Control Procedures (SCP) and others are recommended *viz*., Environmental Monitoring, Shipping Controls, Recall and Traceability Programs, Supplier controls, Preventive maintenance. Based on the existing Seafood HACCP Regulation and FSMS, the following prerequisite programs are required to have in place in order to support the Seafood HACCP program

1. Employee training and training records
2. Good Manufacturing Practices
3. Sanitation Control Procedures

Employee training and training records

Employees who supervise or manufacture, process, pack or hold food must be qualified, trained and/or experienced enough to perform their assigned duties to produce safe food. To meet the training requirements employees must receive training in the principles of food hygiene and food safety, as well as the importance of employee health and personal hygiene. The training may be provided by facility personnel, a third-party source, or a combination of both. Although there is no frequency interval specified in the HACCP regulation for training; it is expected that appropriate training should be conducted prior to employees independently performing their duties. It is also anticipated that refresher training will be provided when needed.

The processors must provide adequate facilities, required to keep records that document the training on the principles of food hygiene and food safety for those who supervise or perform manufacturing, processing, packing, or holding activities for food. Processors must maintain records of this training for at least 2 years.

Good Manufacturing Practices (GMP)

Good Manufacturing Practices (GMPs) provides the basis for determining whether the facility, processing methods, practices and controls used to process food products are suitable to allow for the production of safe and wholesome food and whether the products have been processed under sanitary conditions.

GMPs outline the minimum standards that a food processing facility needs to meet including, but not limited to, personnel, buildings and facilities, equipment, production and process controls, raw materials, and manufacturing operations. GMPs were first released in 1969 as 21 CFR Part 110, and revised in 1986 and again in 2015 (21 CFR Part 117). The 2015 updated version of GMPs explicitly address the allergen cross contact. "Cross-contact" differs from "cross-contamination". Allergen cross-contact is the unintentional incorporation of undeclared food allergens into food while cross-contamination is the contamination of food with bacterial, chemical or physical hazards.

21 CFR Part 117 - Subpart B - Current Good Manufacturing Practices

The 21 CFR part 117 – Good Manufacturing Practices covers various aspects such as

- Personnel
- Plant and grounds
- Sanitary operations
- Sanitary facilities and controls
- Equipment and utensils
- General processes and controls
- Raw materials and other ingredients
- Manufacturing operations
- Warehousing and distribution
- Holding and distribution of human food byproducts for use as animal food
- Defect action levels

Subpart A - General Provisions	
§117.1	Applicability and status
§117.3	Definitions
§117.4	Qualifications of individuals who manufacture, process, pack or hold food
§117.9	Records required for this Subpart
Subpart B - Current Good Manufacturing Practices	
§117.10	Personnel
§117.20	Plant and grounds
§117.35	Sanitary operations
§117.37	Sanitary facilities and controls
§117.40	Equipment and utensils
§117.80(a)	General processes and controls
§117.80(b)	Raw materials and other ingredients
§117.80(c)	Manufacturing operations
§117.93	Warehousing and distribution
§117.95	Holding and distribution of human food by-products for use as animal food
§117.110	Defect action levels
Subpart F - Requirements applying to records that must be established and maintained	
§117.305	General requirements applying to records

Sanitation Control Procedures (SCPs)

Sanitation Control Procedures are the necessary procedures to meet specified GMPs requirements which, in the absence of control, could impact food safety. When SCPs are in place, HACCP plans can more effectively focus on the hazards associated with the product or process and rather than the processing plant environment or employee practices.

The Seafood HACCP Regulation SCPs (21 CFR part 123.11) include one recommendation and three requirements. It is recommended that processors create a written sanitation standard operating procedure (SSOP) that describes how sanitation procedures will be performed. Written SSOPs would outline the goals, methods and activities that are needed to be performed in order to meet the SCP requirements. Well-designed, written SSOPs that are

properly implemented are an effective means to prevent insanitary conditions associated with the processing environment and employee practices that may contribute to food safety hazards.

It is required that processors should monitor the facility sanitation conditions and provisions related to eight key sanitation areas, correct deficiencies noted during monitoring and maintain sanitation control records which document sanitation monitoring and corrections. This monitoring must occur with sufficient frequency to show compliance with current GMP requirements. The regulation also requires that processors correct problems that are identified during monitoring, and keep records of their monitoring results and the corrections that were made.

Eight Key Sanitation Areas

1) *Safety of water*: Water (and ice) that contacts food or food-contact surfaces shall be of safe and of sanitary quality

2) *Condition and cleanliness of food contact surfaces*: Food contact surfaces shall be of a proper design and maintained in a clean and sanitary manner to prevent food contamination

3) *Prevention of cross contamination*: Employee hygiene, personnel practices and the design of the facility must prevent cross-contamination and allergen cross-contact

4) *Maintenance of hand washing, hand sanitizing and toilet facilities*: Sanitary facilities must be accessible, properly maintained, and adequately supplied. An adequate sewage disposal system must be in place

5) *Protection from adulterants*: Food, food contact surfaces, and food packaging material must be protected from microbiological, chemical and physical contaminants and allergen cross-contact

6) *Labeling, storage and use of toxic compounds:* Toxic cleaning compounds, sanitizing agents and pesticides must be properly labeled, used and stored in a manner that protects food, food contact surfaces and packaging material from contamination. Toxic compounds must be stored in a secured area with limited access separated from food processing and areas where food and packaging materials are stored

7) *Employee health*: Controls are necessary to ensure that employee health conditions do not cause food contamination.

8) *Exclusion of pests*: Processors must ensure that pests, such as rodents, birds, domestic animals and insects are not allowed in any area of a food processing and/or storage facility

These eight key areas of sanitation should be monitored at a frequency sufficient to ensure conformance. In addition to that the monitoring results and corrections made for any deficiencies must be recorded. The frequency or time for monitoring will vary according to

various types of products and the schedule of operations. The SCP monitoring forms or records must include the name and location of the processor, the date and time the monitoring was performed, corrections made and the signature or initials of the person conducting the monitoring. The sanitation monitoring, corrections, and sanitation control recordkeeping may be performed as part of a firm's HACCP Plan controls, or separately.

Sanitation controls are not typically included in the HACCP plan. Sanitation controls address the overall processing plant environment and employee practices. If sanitation controls are established as a prerequisite program, HACCP controls can then focus on the control of species-related and process-related hazards for a given finished product.

Chapter 13

Orientation of HACCP Implementation in Seafood Industry

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Introduction

Safety of food remains a major concern in the seafood industry. The production and consumption of safe food are important to any society. The seafood safety is of more concerns in international fish trade due to its vast expansion recent decades. The export value of seafood had increased from US$8 billion in 1976 to US$ 160.5 billion in 2020 (FAO, 2021). The advent of emerging pathogens and the impacts of climate change on seafood safety major concern in fish processing industries. Each year, millions of illnesses can be attributed to contaminated food. Hence, a food safety system aimed at ensuring all food is as safe as possible is required. In this connection, the Hazard Analysis and Critical Control Points (HACCP) system is a single system that has been adopted by national and international bodies for ensuring seafood safety. However, HACCP system is not a standalone programme as it requires prerequisite programmes to work effectively. In present decade, the International Organization for Standardization (ISO) has developed the ISO 22000 family of standards on food safety management systems (FSMS) by taking approach of ISO 9001 as a management system, and incorporates the hygiene measures of prerequisite programmes and the HACCP principles and criteria.

The behaviour of consumers has been gradually changing. The consumer's awareness and demand of safe food is increasing every year. They currently require not only much higher dietary quality, hygiene and health standards in the products they purchase, but they also look for certification and reassurance of products' origins (national or geographical) and production methods. These change in customer's approach had led to adoption of HACCP system by the food processors in various countries to protect their customer's health. HACCP is a scientific and systematic approach to identify, assess and control hazards in the food production process. With the HACCP system, food safety control is integrated into the design of the process rather than relied on end-product testing. Therefore, HACCP system provides a preventive and thus cost-effective approach in food safety.

The HACCP system

HACCP system identifies, evaluates and controls hazards that are significant for food safety. HACCP system requires a team work. It requires firm commitment from top management level for effective

implementation. HACCP does not assure zero risk. It is a systematic tool to minimize risk of food safety hazards. HACCP plan once developed doesn't mean it is the ultimate plan. It needs to be modified whenever required. HACCP is a continuous process and is mainly risk based. HACCP need to be implemented from farm to fork. HACCP programme is a sum total of all pre- prerequisite programmes. The emphasis is on forecast rather than reaction, on getting the process right initially rather than correcting it after problems have occurred. It emphasized on identifying potential food safety problems and determining how and where these can be controlled or prevented. Describing what to do and training the personnel, implementation, recording and assurance throughout the food chain are taken care under HACCP system.

Pre-requisite programmes (PRPs)

PRPs such as standard operating procedures (SOP), sanitation standard operating procedures (SSOP), good manufacturing practises (GMP), etc. are implemented prior to HACCP plans. PRPs focus on employees, facilities and equipment and deals with illness policy, cleaning and sanitizing procedures, garbage removal, pest control, equipment selection, employee hygiene. It also deals with control of harvest operation and the overall plant environment which are not directly related to food (e.g. water quality, transportation and storage, plant sanitation, employee training, etc.).

Objectives of HACCP system

- Prevention of foodborne illness
- Reduction of economic losses due to product recall
- Protection of reputation
- Reduction of production costs
- To compete effectively in the international market

Benefits of HACCP system

- Increase food safety standards
- Increase food quality standards
- Ensures compliance with the regulatory guidelines and laws
- Promote teamwork
- Increase staff efficiency
- Due diligence defense in court

HACCP plan

It is a document prepared in accordance with the principles of HACCP to ensure control of hazards that are significant for food safety in the segment of the food chain under consideration. It is implemented following pre-requisite programmes. Prior to the application of HACCP to a fish or seafood establishment, that establishment should be operating proper prerequisite programmes

according to the Recommended International Code of Practice –General Principles of Food Hygiene (CAC/RCP 1-1969, Revision 2008/2020). Management awareness and commitment are necessary for the implementation of an effective HACCP system. The effectiveness will also rely upon management and employees having the appropriate HACCP knowledge and skills. Therefore, ongoing training is necessary for all levels of employees and managers, as appropriate. If the necessary expertise is not available on-site for the development and implementation of an effective HACCP plan, expert advice should be obtained from other sources, such as trade and industry associations, independent experts and regulatory authorities. Two steps are involved in HACCP plan preparation.

1. Conducts five preliminary steps
2. Applies the seven HACCP principles

Preliminary steps

- Step 1. Assemble the HACCP team.
- Step 2. Describe product.
- Step 3. Identify intended use.
- Step 4. Construct flow diagram.
- Step 5. Confirm flow diagram.

HACCP principles

- Principle 1. Conduct a hazard analysis and identify control measures
- Principle 2. Determine CCPs
- Principle 3. Establish validated critical limits
- Principle 4. Establish a system to monitor control of CCPs
- Principle 5. Establish the corrective actions to be taken when monitoring indicates a deviation from a critical limit at a CCP has occurred
- Principle 6. Validate the HACCP plan and then establish procedures for verification to confirm that the HACCP system is working as intended
- Principle 7. Establish documentation concerning all procedures and records appropriate to these principles and their application

HACCP plan is a final document that describes how a fish or seafood operation will manage the identified CCPs for each product under its particular environment and working conditions. The following are the details on how to apply the above sequence for the preparation of a specific HACCP plan.

1. ***Assemble the HACCP Team***

HACCP Team consists of one HACCP coordinator with HACCP skills and other supporting members from various background. Larger companies – seven or eight people while small companies – two or three people. The HACCP coordinator should have responsibility for the whole HACCP program and be the Team leader.

The HACCP team should have access to all relevant and necessary information. The HACCP team should have expertise in the fields of management, production, quality assurance, maintenance, marketing and sales. The team should represent diverse personnel from the above fields.

2. ***Describe the product:***

A full description of the product should be drawn up, including relevant safety information such as: harvesting area and technique; raw materials and ingredients used including commercial and Latin name of the fish; factors that influence safety such as composition, physical/chemical parameters, such as water activity (aw), pH, salt content; processing such as heating, freezing, brining or smoking; packaging type; storage conditions and methods of distribution; shelf-life under specified condition should also be recorded.

3. Identify the intended use:

The intended use should be based on the expected uses by the end user or consumer. The use and preparation before use greatly influence the safety of the product. Certain products may carry harmful organisms as part of the natural flora. If the processing does not include a killing step, the only possibility to render the product safe is adequate heat treatment (e.g. cooking) during preparation. It is important to identify whether the product is to be used in a way that increases the risk of harm to the consumer, or whether the product is particularly used by consumers who are especially susceptible to a hazard. In specific cases, e.g. institutional feeding, vulnerable groups of the population, such as elderly and infants, must be considered.

4. Construct a process flow diagram:

A flow diagram should be constructed by the HACCP team to provide a clear and simple description of all steps involved in the operation. When applying HACCP to a given operation, consideration should be given to steps preceding and following the specific operation. Receiving and storage steps for raw materials and ingredients should be included. Time and temperature conditions during processing should be mentioned

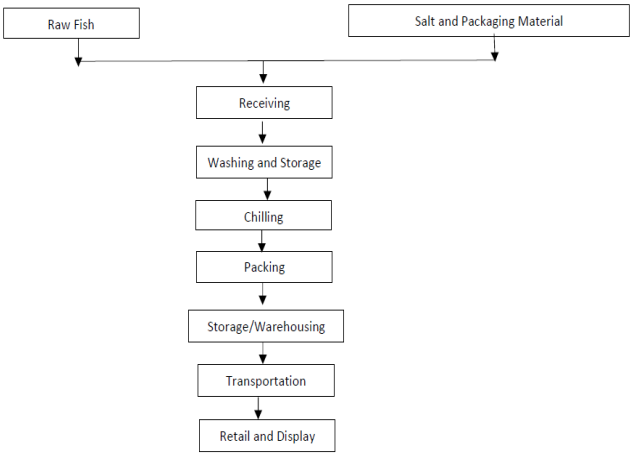

Fig 1. Example of flowchart of chilled fish

whenever there is a holding step, e.g. in holding vats, buffer tanks or other areas, where there could be a potential delay or temperature abuse.

5. On site verification of the process flow diagram:

The HACCP team should confirm on-site the production operations against the flow diagram and amend it with information, such as correct durations, temperatures, and salt concentration, where appropriate. The site should be inspected during all hours (including night shifts and weekends) of operation to check for correctness and ensure that nothing crucial has been overlooked.

Principles of HACCP

1. Conduct a hazard analysis and identify control measures

A hazard is defined as a biological, chemical or physical agent in, or condition of, food (e.g. temperature abuse, insufficient thermal process), with the potential to cause an adverse health effect and harm. The HACCP team should list all hazards that may reasonably be expected to occur during production, processing, transportation and distribution until the point of fish consumption. Hazard analysis is the first HACCP principle and the science-based component of HACCP. An inaccurate hazard analysis would inevitably lead to the development of an inadequate HACCP plan. The HACCP team should identify which hazards are of such a nature that their elimination or reduction to acceptable levels is essential for the production of a safe product. A decision tree with a number of questions can be used to determine whether potential hazards are “real”, as demonstrated below:

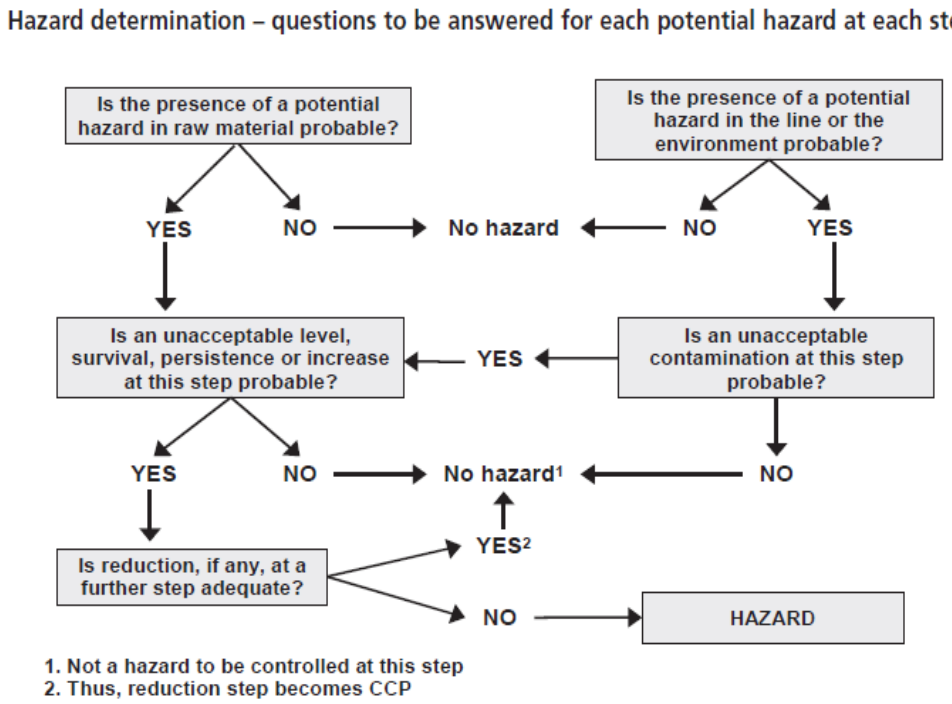

Fig 2. Hazard determination decision tree

Upon completion of the hazard analysis, the HACCP team must consider what control measures, if any, exist that can be applied for each hazard. More than one control measure may be required to control a specific hazard (or hazards) and more than one hazard may be controlled by a specific control measure. Control measures are activities that prevent, eliminate or reduce hazard to an acceptable level.

USFDA suggested following control measure for seafood-borne hazards:

Pathogenic bacteria:

- Time/temp control, heating/cooking, freezing, fermentation, salt/preservatives.

Pathogenic viruses:

- Cooking, source control from acceptable region

Parasites:

- Cooking, freezing.

Chemical hazard:

- Source control (Biotoxins, contaminants), time-temp (histamine), labelling (allergens)

Physical hazard:

- Source control (metal/glass), metal detector (metal pieces), PRPs

2. Determine CCPs

A CCP is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. CCPs are product and process specific. There may be more than one CCP at which control is applied to address the same hazard. Likewise, several hazards can be controlled at a single CCP. Complete and accurate identification of all the CCPs is fundamental for controlling food safety hazards. The determination of a CCP in the HACCP system can be facilitated by the application of a decision tree.

The application of the decision tree should be flexible depending upon the type of operation under consideration. Other approaches than the decision tree may be used for the determination of CCPs. If a hazard has been identified at a step where control is necessary for safety, and if no control measure exists at that step or at any other, then the product or the process should be modified at that step, or at an earlier or later stage, to include a control measure. This exercise should be conducted at each step and for each hazard to identify CCPs.

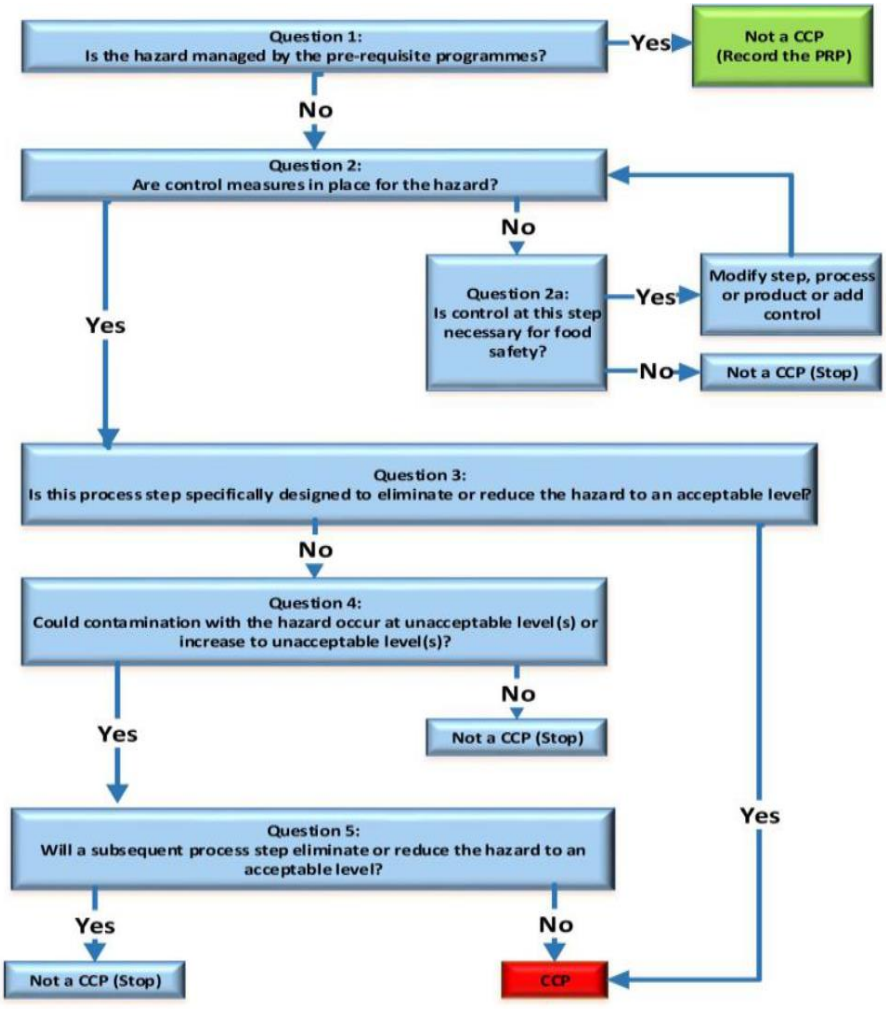

Fig 3. CCP decision tree

3. Establish validated critical limits

Critical limits are defined as criteria that separate acceptability from unacceptability. Critical limits represent the boundaries that are used to judge whether an operation is producing safe products as a result of proper application of the control measures. Critical limits should be scientifically based and refer to easily measurable factors such as temperature, time, chlorine levels, water activity (aw), pH, titratable acidity, salt concentration, available chlorine, preservatives, and sensory quality. Microbiological limits, which often require days for their measurement, should be avoided by all means. However, when microbiological limits are necessary, reliable rapid microbiological techniques should be used. The critical limits should meet the requirements of government regulations and/or company standards and/or be supported by other scientific data. It is essential that the persons responsible for establishing critical limits have knowledge of the process and of the legal and commercial standards required for the products. Example: There is a cooking (80°C for 2.5 min) step in the process line to control biological hazard. Here predefined time and temperature is the CL.

4. Establish a system to monitor control of CCPs

Monitoring is defined as the act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control. The monitoring procedures will determine whether the control measures are being implemented properly and ensure that critical limits are not exceeded. The monitoring procedures must be able to detect loss of control at the CCP. It can be qualitative or quantitative. It can be continuous or non-continuous. It can be of sensory evaluation, physical measurement (pH, a_w, humidity), chemical testing (chlorine level in water), microbiological examination (raw material and end product.

Components:

- What will be monitored?
- How the critical limit and control measures will be monitored?
- When (frequency)? And
- Who will monitor?

5. Establish the corrective actions to be taken when monitoring indicates a deviation from a critical limit at a CCP has occurred

As the main reason for implementing HACCP is to prevent problems from occurring, corrective actions should be predefined and taken when the results of monitoring at the CCP indicate a loss of control. Loss of control can cause a deviation from a critical limit for a CCP. All deviations must be controlled by taking predetermined actions to control the non-compliant product and to correct the

cause of non-compliance. Product control includes proper identification, control and disposition of the affected product. The establishment should have effective procedures in place to identify, isolate (separate), mark clearly and control all products produced during the deviation period. Corrective action procedures are necessary to determine the cause of the problem, take action to prevent recurrence and follow up with monitoring and reassessment to ensure that the action taken is effective. Reassessment of the hazard analysis or modification of the HACCP plan may be necessary to eliminate further recurrence. The control and disposition of the affected product and the corrective actions taken must be recorded and filed. Records should be available to demonstrate the control of products affected by the deviation and the corrective action taken. Adequate records permit verification that the establishment has deviations under control and has taken corrective action.

6. Validate the HACCP plan and then establish procedures for verification to confirm that the HACCP system is working as intended

Verification is the application of methods, procedures and tests, including random sampling and analysis and other evaluations, in addition to monitoring, to determine compliance with the HACCP plan. The objective of verification procedures is to determine whether the HACCP system is working effectively. Careful preparation and implementation of the HACCP plan does not guarantee the plan's effectiveness. Verification procedures are necessary to assess the effectiveness of the plan and to confirm that the HACCP system adheres to the plan. Verification should be undertaken by an appropriately qualified individual (or individuals) capable of detecting deficiencies in the plan or its implementation. Verification activities should be documented in the HACCP plan. Records should be made of the results of all verification activities. Records should include methods, date, individuals and/or organizations responsible, results or findings and actions taken. Apart from the initial validation, subsequent validation as well as verification must take place whenever there is a change in raw materials, product formulation, processing procedures, consumer and handling practices, new information on hazards and their control, consumer complaints, recurring deviations or any other indication, that the system is not working.

7. Establish documentation concerning all procedures and records appropriate to these principles and their application

Records and documentation are essential for reviewing the adequacy of and adherence to the HACCP plan. Several types of records should be considered among those relevant in an HACCP programme:

- Support documentation, including validation records, for developing the HACCP plan;
- Records generated by the HACCP system: monitoring records of all CCPs;

- Deviation and corrective action records, verification/validation records;
- Documentation on methods and procedures used;
- Records of employee training programmes.

Records may be in different forms, e.g. processing charts, written procedures or records, and tables. They can be stored in paper or electronic forms, provided that assurance of record integrity is provided. It is imperative to maintain complete, current, properly filed and accurate records. Failure to document the control of a CCP or implementation of a corrective action would be a critical departure from the HACCP plan.

Example of HACCP implementation in battered and breaded fishery product

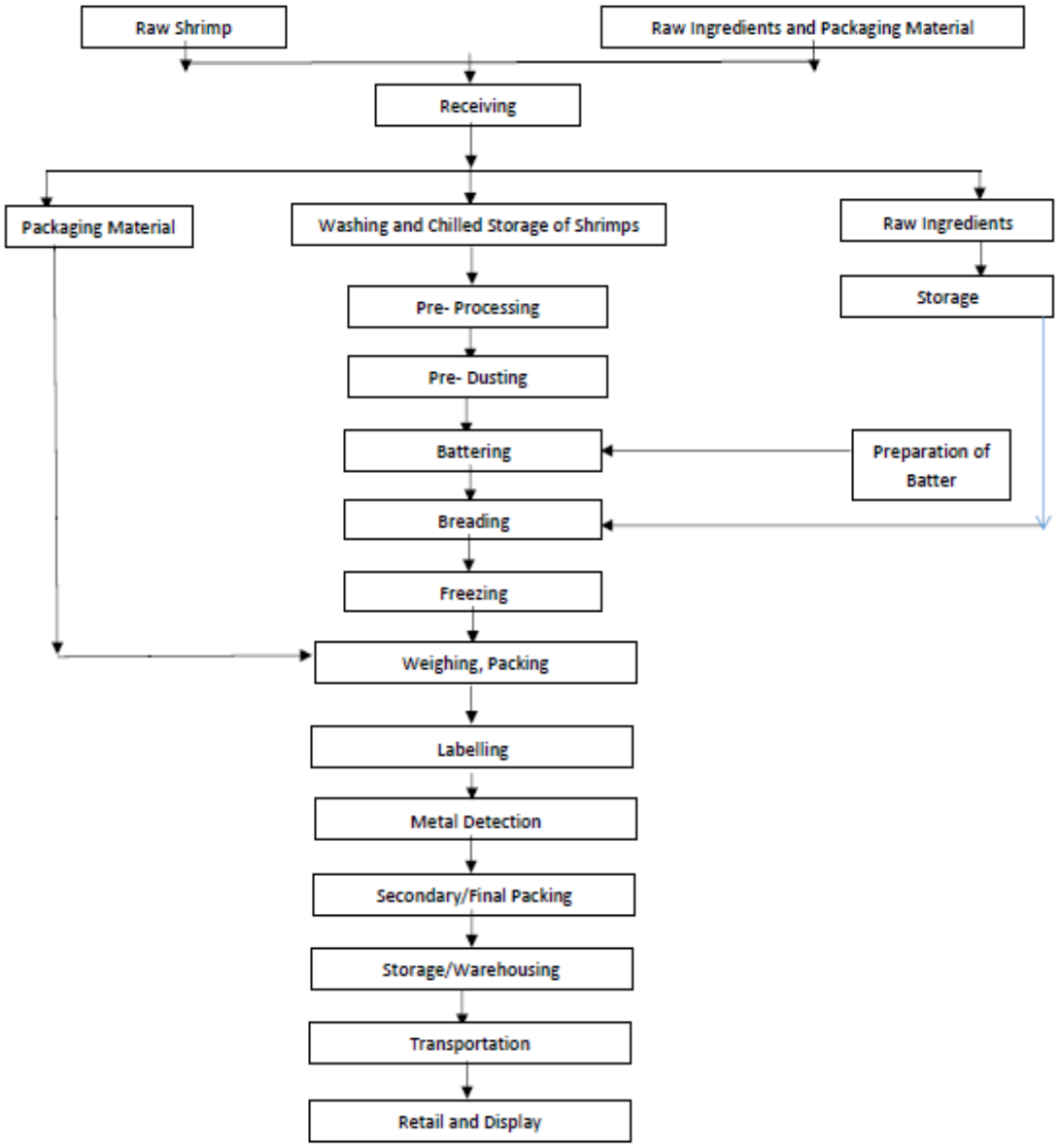

Fig 4. Example: IQF breaded shrimp

Note: This is only a reference model for Risk Assessment & CCP determination example. These may vary from manufacturing plant to plant depending on risk assessment and process control

Sl No.	Process Step	Hazard Type	Potential hazard	Likelihood	Severity	Risk	Preventive Measure	Q1	Q2	Q2A	Q3	Q4	Q5	CCP Y/N	Reason for decision
1.	Receiving Of Shrimp	Biological	Microbial pathogens	M	L	ML	Controlled in further processing steps	Y	-	-	-	-	-	N	Reduced to acceptable level in the subsequent freezing step.
		Chemical	Sulphite Pesticide Antibiotic in case of Aquaculture	M	L	ML	Adherence to raw material specifications Supplier's guarantee that sulphiting agents are not used and the raw product is free from pesticide residues. Supplier's gurantee taking into account withdrawal period	Y						N	Supplier's declaration Adherence to specifications.
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
1.b.	Receiving of other raw material	Biological	None	-	-	-	-	-	-	-	-	-	-	-	-
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	Presence of foreign material	M	L	ML	Taken care by PRPs a	Y	-	-	-	-	-	N	Visual Inspection to detect presence of foreign material
1.c.	Receiving and storage of Packaging material	Biological	Contamination due to poor storage conditions	L	M	LM	Taken care by PRP's	Y	-	-	-	-	-	N	Maintain good air quality, cleanliness and humidity of the storage room
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	Low quality packaging material	L	M	LM	Taken care by PRP's	Y	-	-	-	-	-	N	Purchase specifications and visual inspection of all lots of packaging material. Packaging material used must be food grade.
2.	Washing	Biological	Microbial Pathogens	M	L	ML	Taken care by PRPs and eliminated during retorting stage Use only potable water for washing	Y	-	-	-	-	-	N	Microbial pathogens are reduced or eliminated in the subsequent pre-cooking and retorting stage. Testing of potable water done against IS10500 standard requirements.
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
3.	Storage	Biological	Microbial pathogens	M	L	ML	Time – Temperature control	Y	-	-	-	-	-	N	Adherence to PRP's control microbial multiplication.
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
4.	Pre-processing	Biological	Microbial pathogens	M	L	ML	Taken care by GHP	Y	-	-	-	-	-	N	Adherence to GHP prevents microbial contamination
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	Metal Fragments	M	L	ML	Controlled in the following steps	N	Y	-	N	Y	Y	N	Controlled during the metal detection step.
5	Pre-dusting	Biological	Microbial pathogens	M	L	ML	Controlled by GHP	Y	-	-	-	-	-	N	Adherence to GHP
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	Metal fragments	M	L	ML	Final Product is passed through metal detector	Y	-	-	-	-	-	N	There are chances of metal contamination from the conveyor belts and equipment. Metal detection step eliminate the hazard.
6.	Battering	Biological	Microbial Pathogens	M	L	ML	Taken care by PRPs and GHP	Y						N	Adherence to GHP controls bacterial multiplication.
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	Metal fragments	M	L	ML	Final Product is passed through metal detector	Y	-	-	-	-	-	N	There are chances of metal

															contamination from the conveyor belts and equipment. Metal detection step eliminate the hazard.
7.	Breading	Biological	Microbial pathogens	M	L	ML	Taken care by PRPs	Y	-	-	-	-	-	N	Adherence to GHP controls bacterial multiplication
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	Metal fragments	M	L	ML	Final Product is passed through metal detector	Y	-	-	-	-	-	N	There are chances of metal contamination from the conveyor belts and equipment. Metal detection step eliminate the hazard.
8.	Freezing	Biological	Microbial pathogens	M	H	MH	Proper and adequate freezing	N	Y	-	Y	-	N	Y CCP - 1	Improper freezing may lead to pathogen growth and multiplication
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
9.	Weighing/ Packing	Biological	None	-	-	-	-	-	-	-	-	-	-	-	-
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
10.	Labelling	Biological	None	-	-	-	-	-	-	-	-	-	-	-	-
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
11.	Metal Detection	Biological	None	-	-	-	-	-	-	-	-	-	-	-	-
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	Metal fragments	M	H	MH	Reject or reprocess the pouch containing metal pieces	N	Y	-	Y	-	-		Metal fragments entering into the product from the processing

														Y; CCP-2	machinery are detected at this step. Product containing metal fragments are rejected or reprocessed.
12.	Secondary /Final Packing	None	-	-	-	-	-	-	-	-	-	-	-	-	None
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
13.	Storage/ Warehousing	Biological	Microbial pathogens	M	L	ML	Temperature to be maintained	N	Y	-	Y	-	-	N	Finished Product Storage done makes hazard unlikely to occur.
		Chemical		-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
14.	Transportation	Biological	Microbial pathogens	M	L	ML	Cleaning of vehicles Time-temperature control	Y	-	-	-	-	-	N	Controlled by sanitation programmes and PRP's
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-
15.	Retail & Display	Biological	Microbial pathogens	M	L	ML	Adherence to GHP	Y	-	-	-	-	-	N	SOP for finished product storage during retail and display makes hazard unlikely to occur
		Chemical	None	-	-	-	-	-	-	-	-	-	-	-	-
		Physical	None	-	-	-	-	-	-	-	-	-	-	-	-

Note: This is only a reference model for Risk Assessment & CCP determination example. These may vary from manufacturing plant to plant depending on risk assessment and process control

Sl.No.	CCP			Critical limit	Monitoring	Corrective Action		Verification	HACCP Record
						Immediate	Long Term		
1.	CCP No. 1	**Process Step-** Freezing	**Hazard Addressed** Microbial Pathogens	**Critical limit (CL)-** Freezing Time – 10 – 20minutes Temperature- -25°C Core temperature at or below -18°C (Documentation of Validation of Critical Limit to be made available)	**What -** Freezing Time & Temperature Frozen Product Temperature **How –** Monitoring of gauges/display Thermometer Probes **When-** Every half an hour **Where -** Freezer hall **Who –** Operator	Reprocess the lot if a process deviation occurs. Ensure the core temperature is ≥ -18°C	Proper maintenance of freezer	**What -**Product core temperature **How –** Using probe type thermometer **When-** Once in a shift **Who –** QA/QC Supervisor/Manager	1.Hazard Analysis records with justification for CCPs. 2. CL Validation Records 3.Freezing time and temperature monitoring records 4. Fish temperature monitoring record 5. Correction Record 6. Corrective Action Records 7. Daily Verification Records 8. Audit Records 9. Calibration Records of Probes 10. Microbiological Analysis Record. 11. Online QC Record
2	CCP No. 2	**Process Step-** Metal Detection	**Hazard Addressed-** Physical (Metal Particles)	**Critical Limits-** Metal detector should able to detect test stripes of 1.5 mm Ferrous, 2.5 mm SS & 2.0 mm Nonferrous (Documentation of Validation of Critical Limit to be made available)	**What:** Metal Detector sensitivity **How:** by passing all three test stripes from the metal detector **When:** before start of each shift and every hour **Where:** Metal Detector Point **Who:** Production Supervisor/Manager	Supervisor to hold previous production back to last "passed" calibration check. Re pass the product after proper calibration.	Periodic Maintenance of metal detector	**What:** Metal detector operation **How:** by passing test stripes **When:** At least two times per shift **Responsibility:** QC/QA Supervisor/Manager	1. Hazard Analysis Records 2. CL validation record. 3. Monitoring Records 4. Daily Verification Records. 5. Internal Audit Records 6. Correction Records 7. Corrective Action Records 8. Calibration Records of Probes

Conclusion

The safety of seafood products varies considerably and is influenced by a number of factors such as origin of the fish, microbiological ecology of the product, handling and processing practices and preparations before consumption. However, the food safety hazards and risk in seafood products cannot be made nil through any approach, it can only be minimized or reduced to an acceptable level. A large number of hazards are related to the pre-harvest situation or raw-material handling and must be under control by implementation of HACCP when the raw material is received at the processing factory.

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Chapter 14
Implementation of ISO 22000:2018 Food Safety Management System

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ISO 22000:2018 is the latest global food safety management system (FSMS). This standard replaces the old ISO 22000:2005. ISO 22000:2018 was published in 19 June 2018. The aim of the standard is to harmonize the requirements for food safety management on a global level. The ISO 22000:2018 international standard enables organizations to control food safety hazards along the food chain in order to ensure that food is safe at the time of consumption. ISO 22000:2018 applies to all organizations participating in the food chain, regardless of type, size and complexity. The standard contributes to ensure food safety throughout the whole food chain farm-to-table.

ISO (International Organization for Standardization) is a non-governmental organization (NGO) established in 1947. The head quarter is in Geneva, Switzerland. It has a membership of around 165 national standards institutes from countries in all regions of the world. ISO 22000 was developed by a working group (WG) under ISO Technical Committee 34 (Food Products). This working group evolved into ISO sub-committee (SC 17). This subcommittee is responsible for the management of the ISO 22000 family of standards.

Due to the ever growing global population and raising demand for food to meet the requirements, made food safety a very important aspect. In the manufacturing process it is vital to ensure that the products delivered to consumers do not interfere with the consumers' health adversely. If the production system fails to comply with the food safety regulations, that will lead to the transmission of foodborne illness.

ISO 22000

ISO 22000 is a global standard for Food Safety Management Systems (FSMS). It is designed to enable organizations to control food safety hazards along the food chain. The standard applies to all types and sizes of organizations participating in the food supply chain. ISO 22000 (Food safety management systems -- Requirements for any organization in the food chain) describes the requirements for a food safety management system. The standard is utilized with ISO 22002-1 (Prerequisite programmes on food safety - Part 1: Food manufacturing) to form the technical basis for a Global Food Safety Initiative (GFSI) recognized audit scheme known as FSSC 22000 ISO 22000:2018 is having high level structure

with a different approach to understand risk i.e. It is having a risk based approach. As a result of the high-level structure, the clauses of the standard are largely changed compared to the previous version – ISO 22000:2005.

Food Supply chain:

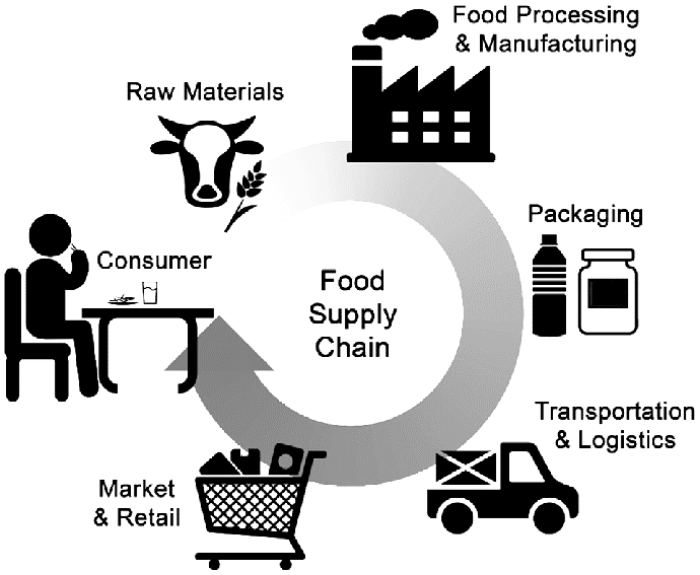

Fig. 1. Food Supply Chain

Food supply chain or food system refers to the processes that describe how food from a farm ends up on our tables. The processes include production, processing, distribution, consumption and disposal. Every step of the supply chain requires human and/or natural resources. In the food supply chain, food moves from producer to consumer via the processes of production, processing, distribution, retailing and consumption; At the same time, money that consumers pay for food moves from consumers to producers in the reverse process.

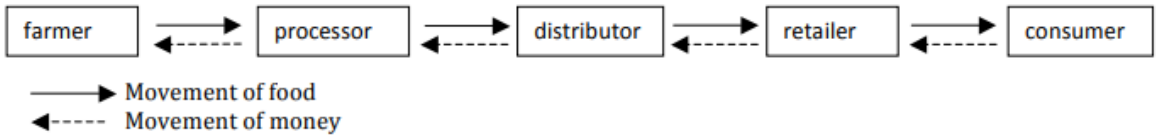

Fig.2.Movements of food and money in a simple food supply chain

According to World Health Organization reports, about 2 million deaths occur every year from contaminated food or drinking water. Around 600 million cases are caused by 22 different enteric diseases (disease caused by intestinal infection) and among that about 52000 deaths are caused by enteric disease caused by *Salmonella typhi.* Over 40% people suffering from enteric diseases caused by consumption of contaminated food were children under the age of 5 years.

ISO 22000:2018 Food Safety Management System (FSMS)

The adoption of a food safety management system (FSMS) is a strategic decision for an organization that can help to improve its overall performance in food safety. The potential benefits to an organization of implementing a FSMS based on this document are:

a) the ability to consistently provide safe foods and products and services that meet customer and applicable statutory and regulatory requirements;

b) addressing risks associated with its objectives;

c) the ability to demonstrate conformity to specified FSMS requirements.

ISO 22000:2018 employs the process approach which incorporates the Plan-Do-Check-Act (PDCA) cycle and risk-based thinking. This process approach enables an organization to plan its processes and their interactions. The PDCA cycle enables an organization to ensure that its processes are adequately resourced and managed, and that opportunities for improvement are determined and acted on. Risk-based thinking enables an organization to determine the factors that could cause its processes and its FSMS to deviate from the planned results, and to put in place controls to prevent or minimize adverse effects.

ISO 22000:2018, the following verbal forms are used:

- "shall" indicates a requirement;
- "should" indicates a recommendation;
- "may" indicates a permission;
- "can" indicates a possibility or a capability.

"NOTES" provide guidance in understanding or clarifying the requirements in this document.

FSMS principles

Food safety is related to the presence of food safety hazards at the time of consumption (intake by the consumer). Food safety hazards can occur at any stage of the food chain. Therefore, adequate control throughout the food chain is essential. Food safety is ensured through the combined efforts of all the parties in the food chain. This document specifies the requirements for a FSMS that combines the following generally recognized key elements:

- interactive communication;
- system management;
- prerequisite programmes;
- hazard analysis and critical control point (HACCP) principles.

In addition, ISO 22000:2018 is based on the principles that are common to ISO management system standards. The management principles are:

- customer focus;
- leadership;
- engagement of people;
- process approach;
- improvement;
- evidence-based decision making;
- relationship management.

Process approach

ISO 22000:2018 adopts a process approach when developing and implementing a FSMS and improving its effectiveness to enhance production of safe products and services while meeting applicable requirements. Understanding and managing interrelated processes as a system contributes to the organization's effectiveness and efficiency in achieving its intended results. The process approach involves the systematic definition and management of processes, and their interactions, so as to achieve the intended results in accordance with the food safety policy and strategic direction of the organization. Management of the processes and the system as a whole can be achieved using the PDCA cycle, with an overall focus on risk-based thinking aimed at taking advantage of opportunities and preventing undesirable results. The recognition of the organization's role and position within the food chain is essential to ensure effective interactive communication throughout the food chain.

Plan-Do-Check-Act cycle

The PDCA cycle can be described briefly as follows:

Plan: establish the objectives of the system and its processes, provide the resources needed to deliver the results, and identify and address risks and opportunities;

Do: implement what was planned;

Check: monitor and (where relevant) measure processes and the resulting products and services, analyse and evaluate information and data from monitoring, measuring and verification activities, and report the results;

Act: take actions to improve performance, as necessary.

The process approach uses the concept of the PDCA cycle at two levels. The first covers the overall frame of the FSMS (Clause 4 -7 and Clause 9 - 10). The other level (operational planning and control) covers the operational processes within the food safety system as

described in Clause 8. Communication between the two levels is therefore essential. So, the Plan-Do- Check- Act (PDCA) cycle of ISO 22000:2018 is having 2 separate cycles working together to handle management system and principles of Hazard Analysis and Critical Control Point (HACCP) respectively. The operation process, clearly deals with key points -Critical Control Points(CCPs), Operational Pre-requisite Programmes (OPRPs) and Pre-requisite Programmes (PRPs).

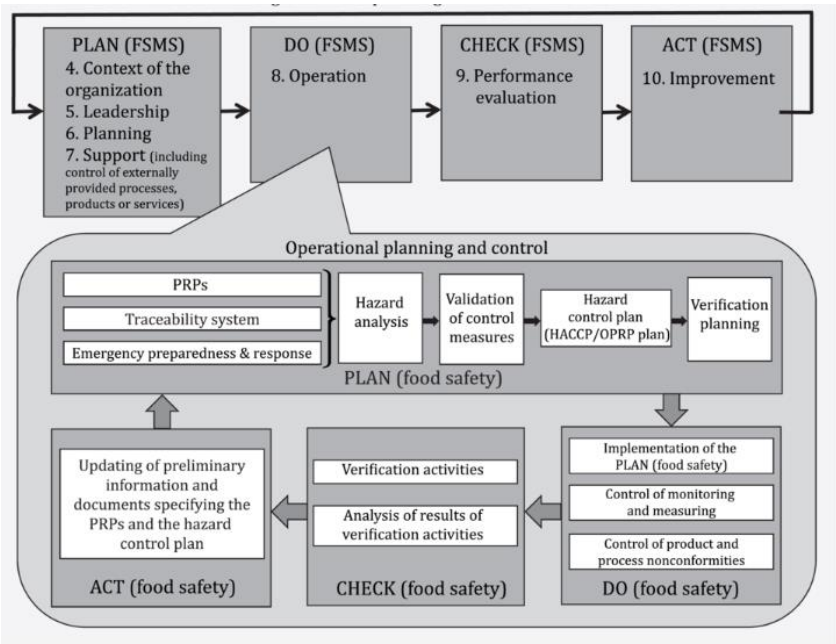

Fig Organizational planning and control of ISO 22000:2018

(*Source: ISO 22000:2018- Food safety management systems)*

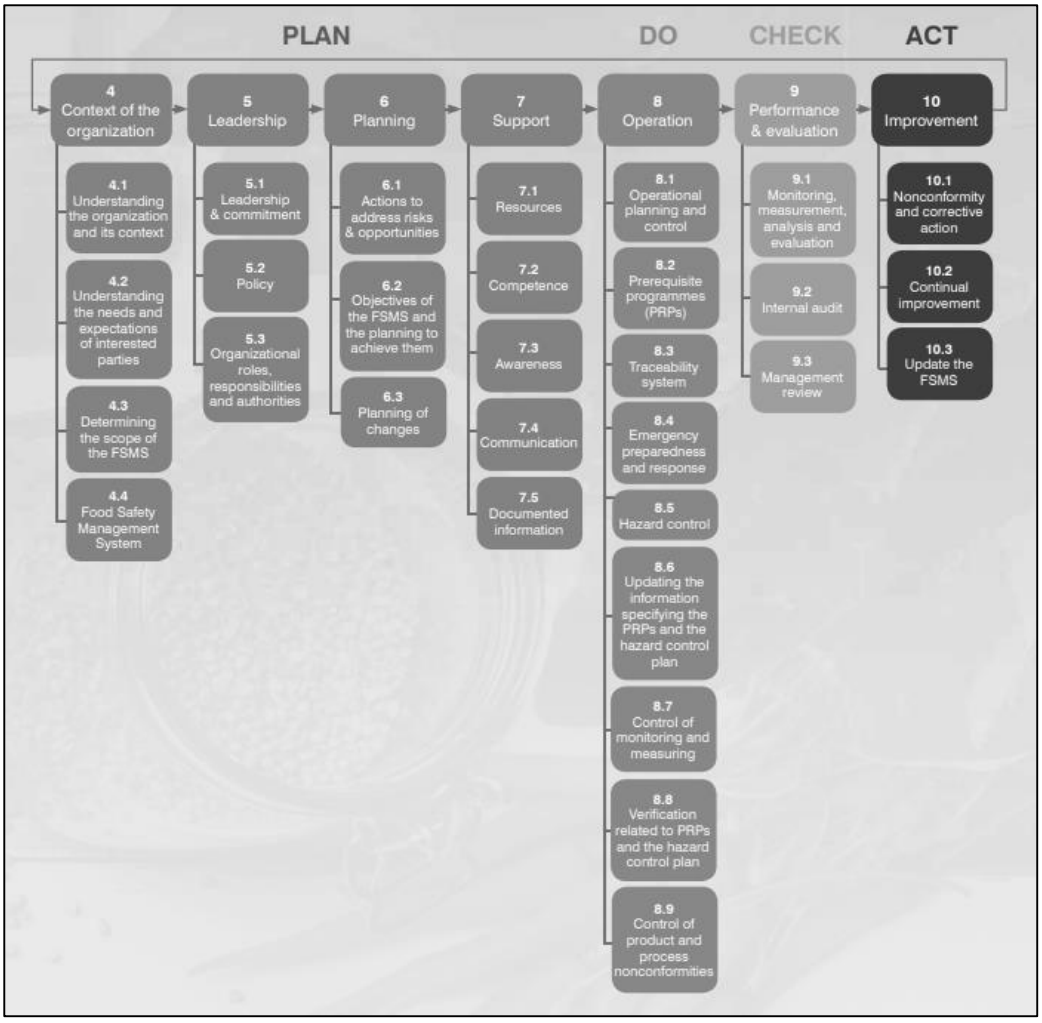

Fig.3. PDCA cycle of ISO22000:2018 (*source: NQA-ISO-22000-Implementation-Guide)*

Risk-based thinking

Risk-based thinking is essential for achieving an effective FSMS. In ISO 22000:2018, risk-based thinking is addressed on two levels, organizational and operational, which is consistent with the process approach.

Organizational risk management:

Risk is the effect of uncertainty, and any such uncertainty can have positive or negative effects. In the context of organizational risk management, a positive deviation arising from a risk can provide an opportunity, but not all positive effects of risk result in opportunities. Addressing risks establishes a basis for increasing the effectiveness of the FSMS, achieving improved results and preventing negative effects.

Hazard analysis — Operational processes:

The concept of risk-based thinking based on the HACCP principles at the operational level is implicit in ISO22000:2018. The subsequent steps in HACCP can be considered as the

necessary measures to prevent hazards or reduce hazards to acceptable levels to ensure food is safe at the time of consumption. Decisions taken in the application of HACCP should be based on science, free from bias and documented. The documentation should include any key assumptions in the decision-making process.

Relationship with other management system standards:

ISO 22000:2018 has been developed within the ISO high level structure (HLS). The objective of the HLS is to improve alignment between ISO management system standards. It enables an organization to use the process approach, coupled with the PDCA cycle and risk-based thinking, to align or integrate its FSMS approach with the requirements of other management systems and supporting standards. ISO 22000:2018 is the core principle and framework for FSMSs and sets out the specific FSMS requirements for organizations throughout the food chain. Other guidance related to food safety, specifications and/or requirements specific to food sectors can be used together with this framework.

In addition, ISO has developed a family of associated documents. These include documents for:

- prerequisite programmes (ISO/TS 22002 series) for specific sectors of the food chain;
- requirements for auditing and certification bodies;
- traceability.

Key changes in ISO 22000:2018

These are some of the key changes to consider:

Changes due to the adoption of HLS

Clause no. 4- Business Context and interested parties.

4.1 - for systematic determination and monitoring of the business context

4.2 - introduces demands to identify and understand factors that can (potentially) affect the ability of Management System to reach the intended results.

Clause no. 5 - Strengthened emphasis on leadership and management commitment:

5.1- new demands to actively engage and take accountability for the effectiveness of the management system.

Clause no. 6 - Risk management

6.1 - companies to determine, consider and, where necessary, take action to address any risks that may impact (either positively or negatively) the ability of the management system to deliver its intended results.

6.2 - Strengthened focus on objectives as drivers for improvements

Clause no. 7 - Extended requirements related to communications

7.4 - "mechanics" of communication, including determination of what, when and how to communicate

7.5 - Documented information shall be controlled to ensure it is adequately protected (ref. 7.5.3). The explicit requirement to have a documented procedure has been removed.

Clause no. 9 - Performance evaluation

Other changes that are specific to ISO 22000 and food safety management

- The PDCA cycle: the standard clarifies the Plan-Do-Check-Act cycle, by having two separate cycles in the standard working together: one covering the management system and the other, covering the principles of HACCP.
- The scope now specifically includes animal food: food for animals not producing food for human consumption. Feed is intended to be fed to food producing animals.
- Some important changes in the definitions: 'Harm' is replaced by 'adverse health effect' to ensure consistency with definition of food safety hazard. The use of 'assurance' highlights the relationship between the consumer and the food product, based on the assurance of food safety.
- Communicating the food safety policy – Clause no. 5.2.2: Explicitly requires the management to facilitate understanding of the food safety policies by employees.
- Food Safety Management System Objectives: Establishing objectives for the food safety management system is further specified in Clause no. 6.2.1 and includes items as *e.g.*, 'consistent with customer requirements', 'monitored' and 'verified'.
- Control of externally-provided processes, products or services – Clause no. 7.1.6- introduces the need to control the suppliers of products, processes and services (including outsourced processes) and to ensure adequate communication of relevant requirements, to meet the food safety management system requirements.

ISO 22000:2018 FSMS - Food safety management systems — Requirements for any organization in the food chain

The main clauses of ISO 22000:2018 FSMS with high level structure are as follows:

1. Scope
2. Normative references
3. Terms and Definitions
4. Context of the organization
5. Leadership
6. Planning
7. Support
8. Operation
9. Performance evaluation
10. Improvement

Annex A: cross references between the CODEX HACCP and this document

Annex B: cross references between this document and ISO 22000:2005

Scope of the standard:

ISO 22000:2018 FSMS specifies requirements to enable an organization that is directly or indirectly involved in the food chain:

a) to plan, implement, operate, maintain and update a FSMS providing products and services that are safe, in accordance with their intended use;

b) to demonstrate compliance with applicable statutory and regulatory food safety requirements;

c) to evaluate and assess mutually agreed customer food safety requirements and to demonstrate conformity with them;

d) to effectively communicate food safety issues to interested parties within the food chain

e) to ensure that the organization conforms to its stated food safety policy

f) to demonstrate conformity to relevant interested parties

g) to seek certification or registration of its FSMS by an external organization, or make a self-assessment or self-declaration of conformity to this document

Terms and Definitions:

Some of the important terms and definitions used in the standard are as follows:

- **Acceptable level** - level of a food safety hazard not to be exceeded in the end product provided by the organization

- **Action criterion** - measurable or observable specification for the monitoring of an OPRP
- **Audit**- systematic, independent and documented process for obtaining audit evidence and evaluating it objectively to determine the extent to which the audit criteria are fulfilled
- **Competence**- ability to apply knowledge and skills to achieve intended results
- **Conformity** - fulfilment of a requirement
- **Contamination** - introduction or occurrence of a contaminant including a food safety hazard in a product or processing environment
- **Continual improvement** - recurring activity to enhance performance
- **Control measure** - action or activity that is essential to prevent a significant food safety hazard or reduce it to an acceptable level
- **Correction**-action to eliminate a detected nonconformity
- **Corrective action** -action to eliminate the cause of a nonconformity and to prevent recurrence
- **Critical Control Point (CCP)** -step in the process at which control measure(s) is (are) applied to prevent or reduce a significant food safety hazard to an acceptable level, and defined critical limit(s) and measurement enable the application of corrections
- **Critical limit** - measurable value which separates acceptability from unacceptability
- **Effectiveness** - extent to which planned activities are realized and planned results achieved
- **End product** -product that will undergo no further processing or transformation by the organization
- **Flow diagram** -schematic and systematic presentation of the sequence and interactions of steps in the process
- **Food chain**- sequence of the stages in the production, processing, distribution, storage and handling of a food and its ingredients, from primary production to consumption
- **Food safety**- assurance that food will not cause an adverse health effect for the consumer when it is prepared and/or consumed in accordance with its intended use
- **Management system** - set of interrelated or interacting elements of an organization to establish policies and objectives and processes to achieve those objectives
- **Measurement** -process to determine a value
- **Monitoring** - determining the status of a system, a process or an activity

- **Nonconformity**-non-fulfilment of a requirement
- **Objective**-result to be achieved
- **Operational Prerequisite Programme (OPRP)-**control measure or combination of control measures applied to prevent or reduce a significant food safety hazard to an acceptable level, and where action criterion and measurement or observation enable effective control of the process and/or product
- **Pre-Requisite Programme (PRP)-** basic conditions and activities that are necessary within the organization and throughout the food chain to maintain food safety
- **Organization**-person or group of people that has its own functions with responsibilities, authorities and relationships to achieve its objectives
- **Policy**-intentions and direction of an organization as formally expressed by its top management
- **Top management-** person or group of people who directs and controls an organization at the highest level
- **Process** -set of interrelated or interacting activities which transforms inputs to outputs
- **Product** -output that is a result of a process
- **Risk** -effect of uncertainty
- **Traceability** -ability to follow the history, application, movement and location of an object through specified stage(s) of production, processing and distribution
- **Update** -immediate and/or planned activity to ensure application of the most recent information
- **Validation** - obtaining evidence that a control measure (or combination of control measures) will be capable of effectively controlling the significant food safety hazard
- **Verification** -confirmation, through the provision of objective evidence, that specified requirements have been fulfilled

Reference:

1. ISO 22000:2018- Food Safety Management Systems — Requirements for any organization in the food chain

Chapter 15

Advanced Microbiological Detection Techniques

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Microbiology has always been very traditional and very labour intensive with the view that automation was for other disciplines but not suited for microbiology. Over the last few years, however, new and improved automated technologies have provided solutions to the challenges facing today's microbiology lab. The first stand-alone automation for the micro lab was introduced in the 1950s, with the initial systems primarily designed for studying human specimen samples such as blood cultures, tissue samples, urine samples antibiotic susceptibility, and biochemical based identification. It wasn't until 2006 that the first true bacteriology automation was introduced with barcoding of dishes, inoculation, moving tracks systems, automated incubation, and digital imaging. Like many other industry advancements, laboratory automation is designed to increase efficiency, streamline processes and deliver high-quality, consistent results in less time.

Today, automation is a complex integration of computers, robotics, liquid handling/processing, and other combined technologies. Automation of routine procedures such as dedicated workstations and software to program instruments has already impacted laboratories worldwide. With repetitive tasks such as pipetting, transporting plates, and various types of assay being the first to be automated. In last decade, automation has steadily spread throughout the analytical chemistry and clinical areas of medical diagnostic laboratories, microbiology laboratories have been excluded from this trend. In general automated microbial identification systems, and automated antimicrobial susceptibility testing systems are widely utilized in microbiology laboratories. In conventional microbiology, microbiology samples are collected and transported by utilizing a wide variety of devices and are processed by maceration, digestion, sonication prior to being plated, or plated directly, and analysis can be quantitative, semi-quantitative, or non-quantitative.

In most inoculation and streaking systems that are fully automated, the samples first need to be in a liquid format The common perception is that digital imaging can be used to make a determination. In fact, it is used to sort the plates, which may be of interest to do further work or sensitivity testing. The others can be sent to discard without being handled by a

biomedical scientist. There will always be some plates that may require a visual check by the laboratorian prior to doing any further work being performed. With automation, a majority of manual processing of bacteriology is removed and reading using digital imaging is different and takes some getting used to by biomedical scientists. Automation changes the workflow of the lab by allowing continuous flow processing as opposed to batch processing. This is a move from the traditional approach of reading plates in the morning and setting up plates in the afternoon and is more compatible with a 24/7 operation. The centralized processing and reading gets away from the traditional specialized benches or areas, staff can easily access all the data from a particular sample and compare on one screen. It also frees trained, experienced staff from doing dull repetitive tasks they can be usefully employed in using their skills and knowledge where it is most needed - in the unusual results rather than the routine ones.

Prerequisites for automation in microbiology laboratory

The main factors for automation in microbiology laboratory are the continued pressure on reducing costs whilst increasing productivity, turnaround time, and result reliability. The current trend is towards merging smaller labs into large super labs, which are considered to be the most cost-effective and efficient way to process samples, and these have the advantage of creating centers of excellence in terms of expertise. Automated systems are ideally suited to meet accreditation requirements by automatically monitoring each step of the analysis, retaining the data for later access. Recruiting and retaining qualified, experienced staff, especially with a trend towards 24/7 working, is also an issue for many labs, so again automation can step in. For automation in microbiology laboratory to be successful, it need to be flexible in design, embrace the human element, and adapt to the challenges of analysing diverse samples. Flexibility acknowledges that one size will not fit all and incorporates an open, expandable architecture that can be adapted to a laboratory's available space and potential future growth. Moreover, flexibility will also require that automation systems embrace diversity of equipment manufacturers. Microbiology must move as much as is practical to liquid-based transport devices to facilitate automated plating. The automated solutions must be able to accommodate the introduction of manually inoculated media into their systems.

Advantages of Lab Automation:

- Increased productivity, more samples processed per person
- A move away from batch processing to continuous, even 24/7 processing
- The ability to handle surge demands
- Remote reading and access to images of plates and organisms

- Assurance that the sample is processed correctly with the right plates and incubation conditions
- Ability to view the whole patient's plate set and historical plate sets
- Reduction in technical and transcription errors
- Improvement in traceability and fully audit trails including the reading process
- Images available for retrospective and training purposes

Process to be automated in microbiology laboratory

In microbiology laboratory several process are required for processing and analysis of samples. In this process automation is possible in many stages

a) Media Preparation: Perhaps the most well established and long-standing area that can be automated is media preparation, labs will not see this as a core activity with all the associated validations and Quality Control protocols and will buy in ready to use media.

b) Specimen Preparation (Plating/Inoculation/Streaking): Plates Most fully automated inoculation and streaking systems require liquid transport swabs or liquid samples. Specimens can be loaded into racks and then loaded onto the instrument; alternatively, samples can be added to a turntable for continuous loading. The sample is scanned, and the system will know how to process the specimen and what plates are required. After vortexing the required plates arrive ready barcoded so that they can be tracked and traced throughout the process. Plates are then planted/inoculated or streaked depending on what was specified for that particular specimen. A HEPA environment ensures no cross-contamination. Specific streaking patterns can be pre-programmed and achieved by robotic loop. This results in a consistent, reproducible inoculation and streaking pattern and produces single colonies more often than by a manual process. Systems will include a monitoring step to ensure that some sample has indeed been taken up by the pipette or loop. Inoculated plates can then be sorted according to required atmospheric conditions and temperature and transported by conveyor belt to the appropriate incubators. Any non-liquid or other specialized samples can be done in a semi-automated fashion whereby the technician prepares the plate, which then goes back into the system with the bulk of samples.

C. Incubation: As each plate is barcoded, on the way to the incubator, it's scanned so incubation start time is registered and how long that plate will need to be incubated before going to the plate reader.

D. Plate Reading and Interpretation: After incubation plates are automatically moved to the image analyzer for reading and may subsequently be returned to the incubator if necessary, this

means plates get exactly the correct incubation time even if due for reading during the night if the lab is 24/7. The barcode on the plate contains information on which camera and lighting settings are required to take images for that particular plate. Even chromogenic plates, can be automatically read and interpreted. The whole plate set from a patient can be put together on one screen for viewing together in one place, so secondary plates such as antibiotic sensitivities can be seen with the primary plates, or the image from day 1 can be viewed with day 2. Images can be saved for later reference or auditing purposes.Looking at plates on a screen is probably one of the most significant changes that automation brings for the biomedical staff who are used to holding a plate, seeing it in 3D, and maybe quickly doing some basic biochemical tests. But plates can always be called up to the workbench for examination by eye, and as staff gain more confidence in the digitized system they will most likely need to only call up those plates that are necessary, leaving the bulk routine plates to be handled by the instrument.

E. Antibiotic Sensitivity Testing: The inoculation and streaking modules are able to produce seeded plates for sensitivities. However, the relevant antibiotic sensitivity discs need to be added using traditional disc dispensers. These plates can be returned to a workbench for the discs to be added.

F. Artificial Intelligence: Artificial Intelligence can be applied to screening and interpretation of plates following incubation; algorithms can be adjusted to meet a particular lab's requirements to enable the automated screening of non-critical plates, depending on visual appearance, sample or patient histories, etc. This results in the vast majority of plates being automatically read and recorded without the need for any technician intervention.

Systems Available

Larger automated systems are modular and can be configured to fit into the available laboratory space. Quite often, the systems must be built to specific design specifications. However, the inoculation and streaking modules have a fixed footprint and are available off-the-shelf. Additional modules can be added on, which include the fully automated transport of plates to fully-automated incubators. Many of these systems will have a lead in time, however this allows time for the lab to prepare for the change and complete any enabling works. The following automated systems are widely used for identification of bacteria in microbiology laboratory.

A) API (Analytical Profile Index) KIT

API identification products are test kits for identification of Gram positive and Gram negative bacteria and yeast. API strips give accurate identifications based on extensive databases and are standardized, easy-to-use test systems. The kits include strips that contain up to 20

miniature biochemical tests which are all quick, safe and easy to perform.API (Analytical Profile Index) 20E is a biochemical panel for identification and differentiation of members of the family Enterobacteriaceae. It is hence a well-established method for manual microorganism identification to the species level. The API range provides a standardized, miniaturized version of existing identification techniques, which up until now were complicated to perform and difficult to read. In the API 20E, the plastic strip holds twenty mini-test chambers containing dehydrated media having chemically-defined compositions for each test. They usually detect enzymatic activity, mostly related to fermentation of carbohydrate or catabolism of proteins or amino acids by the inoculated organisms. A bacterial suspension is used to rehydrate each of the wells and the strips are incubated. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. All positive and negative test results are compiled to obtain a profile number, which is then compared with profile numbers in a commercial codebook (or online) to determine the identification of the bacterial species.

The test kit enables the following tests:

ONPG: test for β-galactosidase enzyme by hydrolysis of the substrate o-nitrophenyl-b-D-galactopyranoside

ADH: decarboxylation of the amino acid arginine by arginine dihydrolase

LDC: decarboxylation of the amino acid lysine by lysine decarboxylase

ODC: decarboxylation of the amino acid ornithine by ornithine decarboxylase

CIT: utilization of citrate as only carbon source

H2S: production of hydrogen sulfide

URE: test for the enzyme urease

TDA (Tryptophan deaminase): detection of the enzyme tryptophan deaminase: Reagent- Ferric Chloride.

IND: Indole Test-production of indole from tryptophan by the enzyme tryptophanase . Reagent- Indole is detected by addition of Kovac's reagent.

VP: the Voges-Proskauer test for the detection of acetoin (acetyl methylcarbinol) produced by fermentation of glucose by bacteria utilizing the butylene glycol pathway

GEL: test for the production of the enzyme gelatinase which liquefies gelatin

GLU: fermentation of glucose (hexose sugar)

MAN: fermentation of mannose (hexose sugar)

INO: fermentation of inositol (cyclic polyalcohol)

SOR: fermentation of sorbitol (alcohol sugar)

RHA: fermentation of rhamnose (methyl pentose sugar)

SAC: fermentation of sucrose (disaccharide)

MEL: fermentation of melibiose (disaccharide)

AMY: fermentation of amygdalin (glycoside)

ARA: fermentation of arabinose (pentose sugar)

Method

Confirm the culture is of an Enterobacteriaceae. To test this, a quick oxidase test for cytochrome c oxidase may be performed. Pick a single isolated colony (from a pure culture) and make a suspension of it in sterile distilled water. Take the API20E Biochemical Test Strip which contains dehydrated bacterial media/bio-chemical reagents in 20 separate compartments. Using a pasteur pipette, fill up (up to the brim) the compartments with the bacterial suspension. Add sterile oil into the ADH, LDC, ODC, H_2S and URE compartments. Put some drops of water in the tray and put the API Test strip and close the tray. Mark the tray with identification number (Patient ID or Organism ID), date and your initials. Incubate the tray at 37oC for 18 to 24 hours.

Result interpretation

For some of the compartments, the color change can be read straightway after 24 hours but for some reagents must be added to them before interpretation.

Add following reagents to these specific compartments:

TDA: Put one drop of Ferric Chloride

IND: Put one drop of Kovacs reagent

VP: Put one drop of 40 % KOH (VP reagent 1) & One drop of VP Reagent 2 (α-Naphthol)

Get the API Reading Scale (color chart) by marking each test as positive or negative on the lid of the tray. The wells are marked off into triplets by black triangles, for which scores are allocated. Add up the scores for the positive wells only in each triplet. Three test reactions are added together at a time to give a 7-digit number, which can then be looked up in the codebook. The highest score possible for a triplet is 7 (the sum of 1, 2 and 4) and the lowest is 0. Identify the organism by using API catalog or apiweb (online).

B. VITEK® 2 COMPACT

The VITEK® 2 Compact system offers quality control testing solutions for fast and accurate microbial identification. The efficiency of the VITEK® 2 COMPACT instrument and VITEK® 2 PC software have the capacity to help improve therapeutic success and patient outcomes through reliable microbial identification (ID) and antibiotic susceptibility testing (AST). The instrument also lets you enhance laboratory efficiencies with reduced hands-on

time and rapid reporting capabilities. All this, in a cost-effective, space-saving design.With technology that includes an extensive and robust identification database, rapid results, and minimal training time, it will streamline laboratory workflow for increased productivity. The system identifies the majority of microorganisms that contaminate production areas and finished products in a minimal amount of time. Identification cards presently available for product safety include: Gram-negative bacilli (time to result: 2 – 10 h); Gram-positive cocci (time to result: 2 – 8 hours); Yeast-like organisms (time to result: 18 hours); Anaerobic bacteria (time to result: 6 hours); Gram-positive spore forming bacilli (time to result: 14 hours) Coryneform bacteria (Time to result: 8 hours).

Testing using VITEK 2 can be performed as follows:

a. Select the appropriate card based on the Gram stain reaction and the organism's microscopic appearance. Allow the card to come to room temperature before opening the package liner.

b. Aseptically transfer at least 3 mL of sterile saline into a clear polystyrene 12×75 mm test tube. Using sterile cotton swabs, prepare a homogenous organism suspension by transferring several isolated colonies from the plates to the saline tube. Adjust the suspension to the McFarland standard required by the ID reagent. The required inoculum concentrations card McF range for differnent bacteria are as follows: GN 0.5-0.63; GP 0.5-0.63; ANC 2.7-3.3; BCL 1.8-2.2.

c. Place the prepared suspensions in the cassette

d. Insert the straw. The age of the suspension must not exceed 30 minutes before inoculating the cards.

e. Proceed to data entry. Enter the card data by scanning the car code on the card. The Cursor must be in the Bar Code space to be entered.

f. Filling the Cards: lace the cassette in the Filler box on the left side of the V2C unit and hit Start Fill button on the instrument. Filling the cards takes approximately 70 seconds for a cassette regardless of the number of cards in the cassette holder. The cassette must be placed inside the Loader Door within 10 minutes from the end of the filling cycle to avoid the cards being rejected. When the cards are finished filling, the Load Door is automatically unlocked.

g. Place the cassette in the Load Door. The V2C Instrument will verify the scanned barcodes against the Virtual Cassette (the information scanned in by the analyst). Cards are sealed, straws are cut and the cards are loaded automatically into the carousel. The V2C will beep once all cards are loaded into the cassette.

h. When the cards are loaded, remove the cassette and dispose of the tubes and straws in a biohazard container.

i. The V2C automatically processes the cards once all the cards are loaded.

j. When the cards are processed and results obtained, cards will be automatically ejected into the waste collection bin

k. Results are concurrently printed and the data sent to the Results View folder on the left side of the screen also called the Navigation Tree where the information is archived.

l. The VITEK system analyses the data results and determines the identity of the test microbes/QC organism based on colorimetric tests (biochemical reactions).

C. VIDAS

VIDAS® is a multiparameter, automated immunoanalyser. It includes an analytical module, a computer and a printer. The analytical module automatically performs all stages of the analysis. The VIDAS® system contains five independant compartments, each accepting up to 6 tests. The computer module is used to manage and print out the results. The VIDAS® system can manage up to two analytical modules simultaneously, giving the system a capacity of 60 tests per hour and is based on Enzyme Linked Fluorescent Assay (ELFA) based technology. VIDAS® reagents are optimized, ready-to-use and stem from an integration of antibody engineering, immuno-concentration, and phage recombinant protein technology. VIDAS® offers a wide range of next-day, simple protocols to answer the need of detecting *Salmonella*, *Listeria* spp., *Listeria monocytogenes*, *Escherichia coli* O157, *Campylobacter* and *Staphylococcal* enterotoxins.

The detection protocol can be broken down as follows:

a. Enrichment

b. Enzyme immunoassay

c. Cultural confirmation

D. ASSURANCE® Gene detection system

The Assurance® GDS genetic detection system combines the latest advancements in molecular detection technology and food microbiology to provide faster results with the increased accuracy required to meet today's food and environmental testing challenges. The Assurance® GDS system comprises three simple steps: Sample enrichment, Sample preparation assays utilizing our innovative GDS PickPen® immunomagnetic separation (IMS) device, and PCR analysis with the GDS Rotor-Gene® thermal cycler. GDS uses proprietary magnetic particles to capture the target organism from the enriched sample. The innovative GDS PickPen® concentration device quickly and easily collects and transfers the concentrated target – 8 samples at a time. It utilizes probes and primers which are highly conserved target gene sequences and ensures greater specificity with fewer indeterminate or false positive reactions.

Also accompanied with multiplex platform allows for the simultaneous detection of multiple targets within each amplification tube.

It works on the combination of two different technologies such as immunomagnetic separation (IMS) and polymerase chain reaction (PCR) to create a single method. IMS is the use of paramagnetic particles coated with specific antibodies to capture and separate cells containing the target antigen from the surrounding environment (sample). This technique has been widely used by microbiologists to aide in the isolation and recovery of low levels of pathogenic organisms from problematic sample matrices and high background microflora environments. It can provide additional advantages when utilized in preparation of samples for PCR-based pathogen detection. Assurance GDS™ utilizes a novel intrasolution IMS method to prepare samples for analysis via PCR. In this method, the sample aliquot and particles are combined in a deep well plate. The magnetic tips of the Assurance GDS PickPen™ device are inserted directly into the wells to collect the particles and transfer them through a wash solution into a resuspension buffer. Once deposited in the buffer, the particles and the associated captured organisms are ready for analysis with the Assurance GDS system.

E. MALDI-TOF

Identification of microorganisms is typically performed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). It works on the principle of protein based spectral identification of bacteria. One of the great advances in microbiology in recent years due to its speed of result together with a low cost per test it easily outperforms biochemical based approaches. Most MALDI-TOF will sit near or immediately next to an automated system, and some systems can use a loop to seed the MALDI-TOF target plate automatically. The technology touts accurate, rapid, and inexpensive identification of microorganisms isolated from samples. MALDI-TOF procedures are highly amenable to automation because they are technically relatively simple and reproducible. Additionally, spotting of target plates and extraction of proteins can be standardized for most organisms and, when combined with automation, can be performed with minimal staffing.

The identification protocol includes

The sample for analysis by MALDI/MS is prepared by mixing or coating with solution of an energy-absorbent, organic compound called matrix. When the matrix crystallizes on drying, the sample entrapped within the matrix also co-crystallizes. The sample within the matrix is ionized in an automated mode with a laser beam. Desorption and ionization with the laser beam generates singly protonated ions from analytes in the sample. The protonated ions are then accelerated at a fixed potential, where these separate from each other on the basis of their mass-

to-charge ratio (m/z). The charged analytes are then detected and measured using different types of mass analyzers like quadrupole mass analyzers, ion trap analyzers, time of flight (TOF) analyzers. For microbiological applications mainly TOF mass analyzers are used. During MALDI-TOF analysis, the m/z ratio of an ion is measured by determining the time required for it to travel the length of the flight tube. A few TOF analyzers incorporate an ion mirror at the rear end of the flight tube, which serves to reflect back ions through the flight tube to a detector. Thus, the ion mirror not only increases the length of the flight tube, it also corrects small differences in energy among ions. Based on the TOF information, a characteristic spectrum called peptide mass fingerprint (PMF) is generated for analytes in the sample. Identification of microbes by MALDI-TOF MS is done by either comparing the PMF of unknown organism with the PMFs contained in the database, or by matching the masses of biomarkers of unknown organism with the proteome database.

F. Polymerase Chain Reaction (PCR)

One of the most commonly used molecular-based method for the detection of foodborne bacterial pathogens is polymerase chain reaction (PCR). PCR was invented about 30 years ago and it allows the detection of a single bacterial pathogen that present in food by detecting a specific target DNA sequence. PCR operates by amplifying a specific target DNA sequence in a cyclic three steps process. Firstly, the target double-stranded DNA is denatured into single-stranded DNA at high temperature. Then, two single-stranded synthetic oligonucleotides or specific primers which are the forward and reverse primer will anneal to the DNA strands. This is followed by the polymerization process whereby the primers complementary to the single-stranded DNA are extended with the presence of deoxyribonucleotides and a thermostable DNA polymerase. The PCR amplification products are visualized on electrophoresis gel as bands by staining with ethidium bromide. PCR have been used in the detection of numerous foodborne pathogens like *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Campylobacter jejuni*, *Salmonella* spp. and *Shigella* spp.

G. Multiplex PCR (mPCR)

Multiplex PCR offers a more rapid detection as compared to simple PCR through the simultaneous amplification of multiple gene targets. The basic principle of mPCR is similar to conventional PCR. However, several sets of specific primers are used in mPCR assay whereas only one set of specific primers are used in conventional PCR assay. Primer design is very important for the development of mPCR, as the primer sets should have similar annealing temperature in order to produce a successful mPCR assay. Besides, the concentration of primers is also important in mPCR. This is because interaction may occur between the multiple

primer sets in mPCR that results in primer dimers, thus, the concentration of primers may need to be adjusted to ensure the production of reliable PCR products. Other important factors for a successful mPCR assay include the PCR buffer concentrations, the balance between magnesium chloride and deoxynucleotide concentrations, the quantities of DNA template, cycling temperatures and Taq DNA polymerase

H. Real-Time or Quantitative PCR (qPCR)

Real-time PCR or quantitative PCR is different from simple PCR whereby it does not require agarose gel electrophoresis for the detection of PCR products. This method is able to monitor the PCR products formation continuously in the entire reaction by measuring the fluorescent signal produced by specific dual labelled probes or intercalating dyes. The fluorescence intensity is proportional to the amount of PCR amplicons. Several fluorescent systems have been developed for qPCR and the most commonly used fluorescent systems include SYBR green, TaqMan probes and molecular beacons. SYBR green is a double-stranded DNA (dsDNA)-binding fluorescent dye. This non-sequence-specific intercalating dye emits little fluorescence and the fluorescence signal is enhanced when bound to the minor groove of the DNA double helix. TaqMan probes and molecular beacons are the common alternatives to SYBR green. TaqMan probes, also known as double-dye probes, are oligonucleotides that contain a fluorophore as the reporter dye at the 5′-end and the quenching dye at the 3′-end. The reporter dye and the quenching dye are close to each other and this prevent the emitted fluorescence of the reporter. TaqMan probe is complementary to a specific nucleotide sequence in one of the strands of amplicon internal to both primers and the system depends on the 5′-3′ exonuclease activity of Taq DNA polymerase that cleaves the probe and separates both dyes in order to generate the fluorophore signal.

I. Loop-Mediated Isothermal Amplification (LAMP)

LAMP is a novel nucleic acid amplification method developed by Notomi et al. which provides a rapid, sensitivity and specific detection of foodborne pathogens. LAMP is based on auto-cycling strand displacement DNA synthesis carried out by Bst DNA polymerase large fragment under isothermal conditions between 59°C and 65°C for 60 min. In LAMP, four primers comprising two inner primers and two outer primers are used to target six specific regions of target DNA. Cauliflower-like DNA structures bearing multiple loops as well as stem-loop DNAs of different sizes are the final products of LAMP. Large amount of amplicons can be produced by LAMP within 60 min which is usually 103-fold or higher as compared to simple PCR. The LAMP amplicons can be detected by agarose gel electrophoresis or SYBR Green I dye.

Problems/draw-backs with automated systems

Several factors have contributed to the current dearth of automation in microbiology labs. These include the ideas that microbiology is too complex to automate, no machine can replace a human in the microbiology laboratory, automation is too expensive for microbiology laboratories, and microbiology laboratories are too small to automate. Microbiology samples are more complex for analysis by conventional methods. Humans are generally considered capable of performing tasks faster than machines and that machines cannot think. The perception that machines cannot exercise the critical decision-making skills required to process microbiology specimens has persisted. Specifically, human observation of organism growth on agar plates is still considered essential by many. Automation has historically been considered too expensive for microbiology. It simply has not been viewed as cost-effective. Although automation is justified for chemistry, the relative test volumes for microbiology are much smaller, making automation seemingly less attractive. Most microbiology laboratories have been considered to be too small for automation. Automation may have a place in the very largest microbiology labs, it does not have a place in the average-sized laboratory as these labs are small, automation would be underutilized. At last shortage of well trained personnel for operation of automated instruments also play an important role in automation of microbiology laboratory.

Chapter 16

Quality Issues in Powdered Fishery Products and Its Control Measures

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What is Edible Fish Powder

Edible fish powder describes a food grade powder product designated primarily for human consumption applications. It differs significantly from fish meal products which are designated for animal feed applications. Fish protein powders have various sanitary processing, purity and functional characteristics which establish them as human food ingredients.

Why Edible Fish Powder?

- Fish is a potential source of protein food. The quality of fish protein is high and the lysine level is particularly good.
- The use of surplus fish can well be used for production of fish powders of edible quality.
- Edible fish powder contains all the nutritional ingredients like protein, vitamins and minerals, and has the organoleptic qualities, like taste and flavor of dry fish
- Its use even in small quantities would serve to boost the protein quality of cereal-based diets wherever feasible.
- Can use for the formulation of convalescent and formulated foods
- Edible fish powder is prepared for human consumption by a hygienic process that does not involve solvent extraction. It is completely free from toxic organic solvents and added chemicals.

Method for the preparation of edible fish powder (IS:10059-1981)

Edible fish powder means the product prepared from non-oily white fish like sprats, either from a single species or their mixture. Whole cleaned fish was thoroughly minced in a meat mincer and the minced meat dried in a tunnel dryer to a moisture level of below 10%. The dried meat was powdered and sieved to give a fine powder.

Raw material

Fresh fish of edible quality which is normally consumed whole should be used for the Preparation.

Edible fish powder can be prepared form lean white fish of pelagic type such as sprats

- Poisonous fish like marine snakes, elasmobranch fish with a high quantity of urea, oily fish and fish with black viscera are not considered suitable for preparation of edible fish powder.
- The fish need not be dressed but should be washed and cooked well for the preparation of the powder.

Preparation of pressed cake

- Raw miscellaneous fish received from the boat shall undergo preliminary sorting and fatty fish like sardine, shark, cat fish and non-edible varieties are removed by hand picking.
- The material shall then be washed well, in a concrete washing tank lined inside with glazed tiles and fitted with false bottom and an outlet pipe, using potable water several times to remove sand, dirt, slime and other extraneous matter.
- The washed mass is transferred as such without dressing to a steam jacketed stainless steel hemispherical kettle having a tilting arrangement, using sufficient quantity (1:1) of potable water to completely immerse the fish.
- The fish is then cooked at 100^{o}C and boiled for 30 minutes under frequent agitation using a hand ladle till the whole mass is completely disintegrated.
- After cooking, the slurry is cooled and allowed to stand for some time to settle, so that the oil floats up.
- The oil-water mixture is decanted off by tilting the vessel.
- The operation is repeated once more.
- The solid mass is then taken in a nylon bag and pressed in a screw hydraulic press at a pressure of 5 kg/cm^{2}to remove the maximum amount of water.
- The pressed cake so obtained is manually broken into small lumps.

PRODUCTION OF DRY POWDER

- The pressed mass is then dried on aluminium trays in a hot air tunnel drier at a temperature of 67-70^{o}C to a final moisture level of 5 percent and below.
- The dried cake while hot is pulverized in a beater type puveriser to a fine powder.
- The powder is sieved in a mechanical gravity-type sieving machine to 150-micron size and the oversized produce Is pulverized once again sieved and the final oversize which contains mainly bones, scales etc. is discarded.
- The sieving machine shall have all its contact parts made of stainless steel and shall be fitted with two sieves (80 and 150 micron) in two decks, with an arrangement for

continuous charging and for receiving products and oversize products continuously without stopping the machine.

- The product is tested chemically and bacteriological. The edible fish powder is then packed.

Packing

- ✓ The edible fish powder shall be packed in clean, sound containers made of tinplate, Post-Consumer Recycled Content (PCRC) sheets, cardboard paper or other material agreed to between the purchaser and vendor in such a way as to protect it form spillage, contamination, migration of moisture or air from the atmosphere, and seepage of fat into the material through the packing material.
- ✓ When packed in flexible material the packaging material should be capable of withstanding handling during transportation.
- ✓ The edible fish powder shall not come in direct contact with packaging material other than grease-proof or sulphate paper, cellulose paper or any other non-toxic packing material which may be covered with moisture-proof laminate or coated paper.
- ✓ When packed in metallic containers, the containers shall be airtight and completely filled to have minimum air or the space shall be filled with inert gas, or the contents held in vacuum.

Marking on the container

The following details shall be clearly marked on the container:

- Name, type and grade of the material;
- Name and address of manufacturer;
- Batch/Code number;
- Minimum net quantity and gross quantity;
- Date of manufacture; and
- Any other requirements under the Legal Metrology (Packaged Commodities)2011 and the Food Safety Standards (Packaging and Labelling) regulation,2011 .
- Each container may also be marked with the ISI Certification Mark

Sampling

Representative samples of material for test and criteria for conformity shall be drawn according to the method prescribed in IS:5315-1978

Requirements IS:10059-1981

*Specification for fish protein concentrate

Sr.No.	ic	Requirement
1	Moisture present% by weight, Max	10
2	Crude protein content (NX 6.25) on dry basis percent by weight, Min	65
3	Total available lysine g/100g of Protein, Min	6
4	Fat content on dry basis % by Weight, Max	6
5	Ash on dry basis % by weight, Max	18
6	Acid insoluble ash on dry basis % by weight, Max	0.5
7	Fluoride (as F), mg/kg, Max	250
8	Mercury, mg/kg, Max	0.5
9	Lead, mg/kg, Max	2.5

BACTERIOLOGICAL REQUIREMENTS OF EDIBLE FISH POWDER *(Clause 3.5)*

CHARACTERISTIC	REQUIREMENT	METHOD OR TEST, REF TO
2	3	4
Total bacterial count, Max.	15000 per g	IS:5402-1969*
E.Coli and pathogenic organisms including *salmonella*	Nil	IS:5887-1976 (Parts I and III)
* Method for plate count of bacteria in foodstuffs. Methods for detection of bacteria responsible for food poisoning: Part I Isolation and identification of enteropathogenic *Escherichia coli* and the enumeration of *Escherichia coli* Part III Isolation and identification of *salmonella and shigella.*		

FSSAI has notified the final Food Safety and Standards (Food Products Standards and Food Additives) Eleventh Amendment Regulations, 2017. This notification prescribes the standards for Edible Fish Powder, along with other standards of F &F products. The regulation is

effective from the date of its publication in the Official Gazette. 15th September, 2017. The specifications of Edible Fish Powder as per FSSAI is as follows

(a) Edible fish powder means the product prepared from non-oily white fish like sprats, either from a single species or their mixture. Fresh fish of edible quality which is normally consumed whole should be used for the preparation. Poisonous fish like marine snakes, elasmobranch fish with a high quantity of urea, oily fish and ish with black viscera are not considered suitable for preparation of edible fish powder.

(b) The fish need not be dressed but should be washed and cooked well for the preparation of the powder.

(c) Requirements.-

(i)Edible fish powder shall be a fine powder free from needle-like bones. It shall blend easily with cereal flours. It shall have a faint yellow colour and the characteristic flavour and taste of dry fish. It shall be free from rancidity and off-flavours.

(ii)No organic solvent or chemicals shall be used in its preparation.

(iii)Particle Size – Unless otherwise specified, the edible fish powder shall be of such fineness that it passes completely through a 100-mesh sieve.

Characteristic	Requirement
Moisture % by weight, Max	10
Crude protein content (NX 6.25) on dry basis percent by weight, Min	65
Total available lysine g/100g of Protein, Min	6
Fat content on dry basis % by Weight, Max	6
Ash on dry basis % by weight, Max	18
Acid insoluble as on dry basis % by weight, Max	0.5

(v) The Protein Efficiency Ratio (PER) shall not be less than 2.5 (IS : 7481).

(d) Food Additives.- Only the food additives permitted under these regulations shall be used.

(e) Hygiene.-

The product shall be prepared and handled in accordance with the guidelines specified in part-II of Schedule 4 ofthe Food Safety and Standards (Licensing and Regulation of Food Businesses) Regulations, 2011 and such guidelines as provided from time to time under the provisions of the Food Safety and Standard Act, 2006.

Chapter 17

Packaging and Labelling Requirements Fish Products as per International Regulations

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Packaging is crucial to our modern food distribution and marketing systems. Without protective packaging, food spoilage and wastage would increase tremendously. The advent of modern packaging technologies and new methods of packaging materials made possible the era of convenience products. In the past packaging emphasized the expectations of the producers and distributors but now it has shifted towards the consumer since they are becoming more demanding and aware of different choices to choose from. A food package usually provides a number of functions in addition to protection.

Fish is one of the most perishable of all foods. The best package material cannot improve the quality of the contents and so the fish must be of high quality prior to processing and packaging. Different products have different packaging requirements and it is important to choose suitable packaging material accordingly. The intended storage conditions of the product, i.e., temperature, relative humidity and expected shelf life have to be known. Multilayered plastics are very popular since properties of different films can be effectively used to pack different products. The basic function of food packaging is to protect the product from physical damage and contaminants, to delay microbial spoilage, to allow greater handling and to improve presentation.

Types of Packaging Material

Glass

Glass containers have been used for many centuries and still one of the important food packaging material. Glass has its unique place in food packaging since it is strong, rigid and chemically inert. It does not appreciably deteriorate with age and offers excellent barrier to solids, liquids and gases. It also gives excellent protection against odour and flavor and product visibility. Glass can also be moulded to variety of shapes and sizes. But it has disadvantages like fragility, photo oxidation and heavier in weight.

Cans

Most frequently used container for packing food for canning is tin plate can. Tin plate containers made their appearance in 1810. The tin can is made of about 98% steel and 2% tin coating on either side. The base steel used for making cans is referred as CMQ or can making quality steel. Corrosion behavior, strength and durability of the tin plate depend upon the chemical composition of the steel base. The active elements are principally copper and phosphorous. The more of these elements present the greater the corrosiveness of steel. Cans are traditionally used for heat sterilized products and different types are standard tin plates, tin free steel and vacuum deposited aluminium on steel and aluminium cans. For food products packing they are coated inside to get desirable properties like acid resistance and sulphur resistance. But care has to be taken to avoid tainting of the lacquer.

Polymer coated two-piece cans of 6 oz capacity (307 x 109) with a universal polymer coating can be widely used for a variety of products. The can is made of Electrochemically chromium coated steel (ECCS) plate with clear polyethylene terephtahalate (PET) coating on either side The finished plate has a thickness of 0.19mm (0.15 mm of base steel + 20 µ PET coating on either side). The cans are made out of the steel plate by draw and redraw (DRD) process. The chromium coating along with the PET coating provides the can with a smooth, greyish, glistening appearance in addition to act as a barrier between the product and the base steel. The bottom of the can is designed for better stackability so that it can be stacked vertically without risk of toppling on the shelf. This also helps to reduce the storage space requirement for the cans. These cans are found to be suitable for thermal processing of fish and fish products. These cans are having easy open ends. Metal cans are advantageous as packages because of superior strength, high speed manufacturing and easy filling and dosing. Disadvantages of metal cans are weight, difficulty in reclosing and disposal.

Paper

A very considerable portion of packaged foods is stored and distributed in packages made out of paper or paper based materials. Because of its low cost, easy availability and versatility, paper is likely to retain its predominant position in packaging industries. Paper is highly permeable to gases, vapour and moisture and loses its strength when wet. Ordinary paper is not grease and oil resistant, but can be made resistant by mechanical processes during manufacturing.

Paper board

Thicker paper is called as paper board. There is not a clear cut dividing line between the heaviest grade of paper and the lightest board. Moreover the lightest standard board is 0.19

mm thick and heavy papers are of 0.125 mm thickness. Paper boards are used for making corrugated fibre board cartons.

Polymer Packaging

Plastics offer several advantages over other packaging materials since they are light in weight, flexible and offers resistant to cracking. Plastics have the advantage that most of them possess excellent physical properties such as strength and toughness. The requirements with a particular food may not be met with in a single packaging material, as it may not possess all the desired properties. In such cases copolymers or laminates consisting of two or more layers of different polymers having different properties can also be used.

Low Density Polyethylene (LDPE)

Most commonly used as it possesses qualities such as transparency, water vapour impermeability, heat sealability, chemical inertness and low cost of production. Organic vapours, oxygen and carbon dioxide permeabilities are high and has poor grease barrier property. Resists temperature between – 40°C to 85°C. Polyethylene (polythene, PE) is the material consumed in the largest quantity by the packaging industry.

High Density Polyethylene (HDPE)

HDPE resins are produced by low-pressure process. HDPE posses a much more linear structure than LDPE and has up to 90% crystallinity, compared with LDPE which exhibits crystallinities as low as 50%. It is stronger, thicker, less flexible and more brittle than LDPE and has lower permeability to gases and moisture. It has a higher softening temperature (121°C) and can therefore be heat sterilized. High molecular weight high density polythene (HM-HDPE) has very good mechanical strength, less creep and better environmental stress crack resistance property.

Linear Low Density Polythene (LLDPE)

Linear low density polythene is low density polythene produced by a low pressure process. Normal low density polythene has many $-C_5H_{11}$ side chains. These are absent in LLDPE, allowing the molecules to pack closer together to give a very tough resin. It is virtually free of long chain branches but does contain numerous short side chains. Generally the advantages of LLDPE over LDPE are improved chemical resistance, improved performance at both low and high temperatures, higher surface gloss, higher strength at a given density and a greater resistance to environmental stress cracking. LLDPE shows improved puncture resistance and tear strength. The superior properties of LLDPE have led to its use in new applications for polyethylene as well as the replacement of LDPE and HDPE in some areas.

Polypropylene (PP)

Polypropylene is produced by the polymerisation of propylene. All PP films have permeability about ¼ to ½ that of polyethylene. It is stronger, rigid and lighter than polyethylene.

- *Cast polypropylene (CPP)*

It is an extruded, non oriented film and is characterized by good stiffness, grease and heat resistance and also has good moisture barrier. However, it is not a good gas barrier.

- *Oriented, Heat set Polypropylene (OPP)*

Orientation can be in one direction (unbalanced) or in two directions equally (balanced). The resulting film is characterized by good low temperature durability, high stiffness and excellent moisture vapour transmission rate. One drawback of OPP is its low tensile strength.

Polystyrene

The material is manufactured from ethylene and benzene, which are cheap. The polymer is normally atactic and it is thus completely amorphous because of the bulky nature of the benzene rings prevents a close approach of the chains. The material offers reasonably good barrier to gases but is a poor barrier to water vapour. New applications of polystyrene involve coextrusion with barrier resins such as EVOH and poly vinylidene chloride copolymer to produce thermoformed, wide mouthed containers for shelf stable food products and multi layer blow moulded bottles. To overcome the brittleness of polystyrene, synthetic rubbers can be incorporated at levels generally not exceeding 14% w/w. High impact polystyrene is an excellent material for thermoforming. Co-polymerisation with other polymers like acrylonitrile butadene improves the flexibility. Since it is crystal clear and sparkling, it is used in blister packs and as a breathing film for packaging fresh produce. These materials have low heat sealability and often tend to stick to the jaws of heat sealer.

Polyester

Polyester can be produced by reacting ethylene glycol with terephthalic acid. Polyester film's outstanding properties as a food packaging material are its great tensile strength, low gas permeability, excellent chemical resistance, lightweight, elasticity and stability over a wide range of temperature (-60° to 220°C). The later property has led to the use of PET for boil in the bag products which are frozen before use and as over bags where they are able to withstand cooking temperatures without decomposing.

Although many films can be metallized, polyester is the most commonly used one. Metallization results in considerable improvement in barrier properties. A fast growing application for polyester is ovenable trays for frozen food and prepared meals. They are

preferable to foil trays for these applications because of their ability to be micro wave processed without the necessity for an outer board carton.

Polyamides (Nylon)

Polyamides are condensation products of diacids and diamine. The first polyamide produced was Nylon-6,6 made from adipic acid and hexamethylene diamine. Various grades of nylons are available. Nylon-6 is easy to handle and is abrasion-resistant. Nylon-11 and nylon-12 have superior barrier properties against oxygen and water and have lower heat seal temperatures. However, nylon-6,6 has a high melting point and hence, it is difficult to heat seal. Nylons are strong, tough, highly crystalline materials with high melting and softening points. High abrasion resistance and low gas permeability are other characteristic properties.

Polyvinyl Chloride (PVC)

The monomer is made by the addition of reaction between acetylene and hydrochloric acid. It must be plasticised to obtain the required flexibility and durability. Films with excellent gloss and transparency can be obtained provided that the correct stabilizer and plasticizer are used. Thin plasticized PVC film is widely used in supermarkets for the stretch wrapping of trays containing fresh red meat and produce. The relatively high water vapour transmission rate of PVC prevents condensation on the inside of the film. Oriented films are used for shrink-wrapping of produce and fresh meat. Unplasticized PVC as a rigid sheet material is thermoformed to produce a wide range of inserts from chocolate boxes to biscuit trays. Unplasticized PVC bottles have better clarity, oil resistance and barrier properties than those made from polyethylene. They have made extensive penetration into the market for a wide range of foods including fruit juices and edible oils.

Copolymers

When polythene resins are being manufactured it is possible to mix other monomers with ethylene so that these are incorporated in the polymer molecules. These inclusions alter the characteristics of the polythene. Vinyl acetate is commonly used and the resulting ethylene vinyl acetate (EVA) copolymers display better sealing than modified polythene. Butyl acetate is incorporated with similar effects.

Aluminium foil

Aluminum foil is defined as a solid sheet section rolled to a thickness less than 0.006 inches. Aluminum has excellent properties like thermal conductivity, light weight, corrosion resistance, grease and oil resistance, tastelessness, odourlessness, heat and flame resistance, opacity and non-toxicity. Aluminium foil free from defects is a perfect moisture and oxygen barrier. In all flexible packaging applications using aluminium foil where good moisture and

oxygen barrier properties are important, the foil is almost always combined with heat sealing media such as polythene or polypropylene. It is the cheapest material to use for the properties obtained. Foils of thickness 8 to 40 microns are generally used in food packaging. Foil as such is soft and susceptible for creasing. Hence, foil is generally used as an inner layer.

Packaging fish and fishery products

Fresh fish

Fresh fish is the most perishable of all foods. Post-harvest losses account to 20-30 % of the fresh fish. Chilling by mixing fish with ice is the cheapest and most efficient method of minimising such wastage. Fish is usually sold local markets without any packaging, but for retailing and further storage packaging is of utmost importance. Packaging materials for fresh fish should provide a barrier against oxygen to reduce fat oxidation, prevent dehydration, retard chemical and bacterial spoilage and permeation of external odours. For bulk transportation The container should be sturdy enough to withstand the rigours of transit and travel by different modes, should be of light weight, hygienic and easily cleanable and possess good insulation properties. High density polypropylene containers are commonly used for transportation of fish in the landing centres and fish markets. However, for longer distance transportation insulated containers are commonly used.

Frozen fish

Seafood's are a major source of export from the country. They are packed in two major forms namely, as block frozen in 2 or 4 kg each. Shrimps, squids, cuttlefish etc. are packed in low density polythene (LDPE) covers or duplex board carton lined with LDPE. About 10 cartons are then packed in a master carton made of 5 ply or 7 ply corrugated fibre board boxes. The packed cartons are then store in the cold store at -18°C

Shrimp is processed in individually quick frozen (IQF) form which is a value addition against the traditional block frozen and fetches a higher unit value. The packaging requirements of IQF shrimp vary considerably from those of block frozen shrimp. Greater demand for IQF shrimp is in consumer packs and not in bulk or institutional packs. In the case of block frozen shrimp, the risk of moisture loss or oxidative reaction leading to flavour changes etc. are minimal. For IQF packaging coextruded or laminate films are used. Polyester laminated with low density polyethylene is used. Duplex carton when used, are laminated with plastic film to improve the functional properties as well as aesthetic value of the pack. The most functionally effective film has been identified as 10 micron biaxially oriented polypropylene (BOPP).

Major requirement of shipping container / transit package for IQF shrimp is high compression strength to bear weight without damage to the product. It is very important that IQF shrimp

should not be subjected to undue pressure during transit and storage. The stack weight should not increase pressure on the product in the cartons in the lower layers. This can be achieved only if master cartons do not yield to pressure and pass it on to the product inside. A compression strength of 500 kg is the minimum recommended specification which might give reasonable safety to the product. Cartons made of 5 or 7 ply corrugated fibre board satisfying the above requirements can be safely used.

Battered and Breaded Products

They are value added products in a convenience form where the battering and breading increase the bulk of the product thus reducing the cost element. A number of value added marine products both for export and internal markets can be prepared from shrimp, squids, cuttle fish, certain species of fish and minced meat from low priced fishes. Various value added battered and breaded fish products available in the market are battered and breaded peeled shrimp, battered and breaded shrimp, fantail (butterfly), battered and breaded shrimp round tail-on, battered and breaded squid rings, battered and breaded stuffed squid rings, battered and breaded stuffed squid, battered and breaded fish fillets, fish fingers, fish fingers, fish cutlets and fish patties.

The changes taking place during frozen storage of the value added products are desiccation, discoloration, development of rancidity etc. Application of proper packaging prevents/retards these changes and enhance shelf life. Conventional packaging materials like flexible plastic films alone are not suitable for these products as they provide little mechanical protection to the products and as a result the products get damaged or broken during handling and transportation. Hence, thermoformed containers are commonly used for this purpose. The thermoformed trays produced from food grade materials are suitable for the packaging of value added fishery products both for internal and export markets. Trays made of materials like PVC, HIP and HDPE are unaffected by low temperature of frozen storage and provide protection to the contents against desiccation, oxidation etc. during prolonged storage.

Dried fishery products

Dryfish is a traditional product and commands a good market. Baskets improvised with braided coconut or Palmyrah leaves or gunny bags are containers mainly used for packaging and transportation of dried fish for domestic distribution. These packages are prone to easy entry of insects, rodents and other pests. Since dried fish is highly sensitive to changes in relative humidity, the packaging has to be sufficiently water vapour proof. The bulk packaging materials commonly used in tropics are waxed corrugated cartons, deal wood or plywood boxes, bamboo baskets or gunny bags, dried Palmyrah or coconut palm leaves and multiwall

paper sacks. Among different packaging materials studied high density polythene woven gusseted bags laminated with 100 gauge low density polythene are found quite suitable for dried fish packaging. From the hygienic points of view HDPE is impervious to microbial and insect attack. The commonly used packaging materials for consumer packs of dry fish are low-density polythene or polypropylene. These materials are cheap, readily available and have good tearing and bursting strength. Disadvantages are high water vapour and gas transmission rate, proneness to puncture or damage from sharp spines. Laminate films made of polyester polythene is advisable for consumer packaging

Accelerated Freeze dried (AFD) products

Application of the technique of freeze drying in fish preservation is a relatively recent development due to the high cost of machinery and operation skill involved. AFD products are practically devoid of moisture, its percentage generally being below 2. The products are very fragile and can easily undergo chemical reactions with air leading to oxidation, deterioration of colour, absorption of water etc. They are generally packed under an inert gas to exclude air and oxygen. Hence the main requirements in the packaging employed are low oxygen and water vapour transmission to protect the product from rancidity and absorption of moisture and sufficient mechanical strength to protect from shock. Paper/ aluminium foil /polythene laminates or metallised polyester polythene laminated pouches are recommended for accelerated freeze dried products. In some cases metal containers like tin cans have to be used to protect the material from shock, as these products are very brittle.

Fish pickles

Fish pickle is a value added item whose bulk is contributed by low value items like ginger, chilly, acetic acid etc. Generally low cost fish, clam meat is used in fish pickles. Conventionally glass bottles are used as containers, which offer properties like inertness, non-toxicity, durability, non-permeability to gases, moisture etc. But they are heavy, prone to break, voluminous and expensive. New flexible packaging materials developed for fish pickle is based on plain polyester laminated with LDPE-HDPE Co-extruded film or Nylon/Surlyn or LD/BA/Nylon/BA/Primacore. These are inert to the product, can be attractively fabricated as stand up packs and can be printed on the reverse side of the polyester film.

Fish soup powder

Fish soup powder is a speciality product containing partially hydrolysed fish, protein, carbohydrates, fat and several other seasonings including salt. The product is hygroscopic and hence the selection of the package assumes great significance. Appropriate package developed for such products are 12 micron plain polyester laminated with LDPE-HDPE co-extruded film

or 90-100 micron LD/BA/Nylon/BA/Primacore multilayer films which ensure a safe storage of the product up to six months.

Extruded products

Ready to eat breakfast cereals, pasta, ready-to-eat, snacks, pet foods, and textured vegetable protein (TVP) are prepared by the extrusion process. An extruder consists of one or two screws rotating a stationary barrel and the mixed raw material is fed from one end and comes out through a die at the other end where it gets puffed up due to the release of steam. It is either in the ready to eat form and hence have to be hygienically packed for consumption. The extruded products are highly hygroscopic in nature and hence they should not come into contact with moisture. Since the extruded product contains fat, the product should not be exposed to air. It is also highly brittle and may powder when crushed. Hence packaging films of high barrier strength and low permeability to oxygen and water vapour are required. Generally extruded products are packed in LDPE/metallised polyster laminated pouches flushed with Nitrogen.

Surimi and surimi based products

Surimi is an intermediate product / raw material for processing several value added products like fabricated foods, shrimp and crab analogues and a variety of other products. Surimi requires to be preserved frozen until used for processing different products. For this purpose surimi is generally frozen as rectangular blocks. In order to prevent oxidative rancidity and desiccation care has to be taken to ensure that the frozen block does not contain any voids and that the packaging materials used have low water vapour permeability and low permeability to gases and odours. The packaging materials employed should be sufficiently strong and durable to withstand stress during handling, storage and distribution. LDPE and HDPE packaging films employed for block frozen shrimp are considered safe for surimi.

Fish Sausage

Fish sausage is a minced based product. Surimi is the base material, which is homogenised after mixing with several other ingredients. The homogenised mass is stuffed in synthetic casings like Ryphan (Rubber hydrochloride) or Kurehalon (Vinylidene chloride). The casing is closed using metal rings after which it is heated in water at 85-90°C and then slowly cooled. After drying the surface the sausage is wrapped in cellophane laminated with polythene. Fish sausage is kept at refrigerator temperatures for retail; however when prolonged storage is needed it is better kept frozen. Fish sausage is also processed in polyamide and cellulose and fibrous casing. For thermal processing polypropylene casings are used so as to withstand high temperatures.

Glucosamine hydrochloride

D-Glucosamine hydrochloride is used to cure rheumatic arthritis, and is also used as an additive in the food & cosmetic industry. D-Glucosamine hydrochloride Powder is stored in a cool and dry well-closed container, the temperature should be lower than 25°C, and the relative humidity should not exceed 50%. Glucosamine is packed in polybottle, namely PP or HDPE of 1kg, 500g and 20 g, 1kg metallised bag, 25kg in drums for commercial use and smaller quantities are packed in auto sample vials.

Chitin and Chitosan

Chitin and chitosan are derived from prawn shell waste and is exported in large quantities. The product should be protected against moisture gain as well as microbial and insect attacks. Bulk packaging of chitosan is done in HDPE woven gusseted bag laminated with 100 gauge LDPE liner. Chitosan is also marketed in capsule forms for consumption. Capsules made of gelatin are used for filling chitosan. Since chitosan is in the powdered form or flakes they are filled into the capsules. A particular numbers of capsules are then placed in HDPE containers.

Fish Hydrolysate

Fish Hydrolysate is prepared from fish mince which has contain oil and is undiluted, and so is a richer food source for beneficial microbes and especially beneficial fungi in the soil. It is generally cold-processed and hence retains the amino acids and protein chains as such. Fish hydrolysate is concentrated, and when diluted can be used ideally as soil fertliser, and is suitable for all soils, crops, ornamentals, trees and vegetables It contains a wide spectrum of major nutrients and trace elements in organic, plant available form. It can be used as a foliar spray, but since the oil is present it may show patches on the leaves. The liquid is generally packed in jars or cans which are made of polypropylene or HDPE.

Fish Meal

Fish meal is a source of high quality protein (60%) and is also a rich in omega-3 essential fatty acids EPA and DHA due to the high fat content. Incorporation of DHA and EPA in fish meal will in turn ensure its concentration in the diets of fish and poultry, ultimately reaching the human diet. Hence the packaging should be impermeable to moisture, oxygen and other insets and pests. Fish meal is generally packed in HDPE sacks for bulk transportation. The fishmeal whether in ground or pelletised form should contain moisture 6-12 %. The fat content should not exceed 18% and the final meal should contain at least 100 ppm antioxidant (ethoxyquin). If the temperature exceeds°130 F or 55° C then the ventilation should be kept on hold. The fish meal is generally packed in jute bags, multiwall paper bag which are lined with polythene and in HDPE woven bags with liner.

Fish oils

Fish oils are highly unsaturated and easily susceptible to oxidation when exposed to air. Hence they have to be packed in containers which have high barrier properties which are moisture proof, oil resistant and impermeable to oxygen. Larger quantities of fish oil are mainly packed in LLDE/Nylon films or in glass bottles. Bulk transportation food grade flexitanks made of 4 layered polyethylene and tubular PP. Advantages of using flexitanks are that they can carry 50% more than bottles and therefore will save on storage space, packaging and transportation cost.

Fish oil is also marketed for regular oral dosage in the form softgel capsules. The shell is made of gelatin, water, glycerol or sorbitol. The process of encapsulation is by using the rotary die encapsulation process. The encapsulation process is a FFS operation. Two flat gelatin ribbons manufactured on the machine are brought together on a twin set of rotating dies that contain recesses in the desired size and shape, these cuts out the ribbon into a two-dimensional shape, and form a seal around the outside. At the same time a pump delivers a precise dose of oil through a nozzle incorporated into a filling wedge whose tip sits between the two ribbons in between two die pockets at the point of cut out. The wedge is heated to facilitate the sealing process. The wedge injection causes the two flat ribbons to expand into the die pockets, giving rise to the three-dimensional finished product. After encapsulation, the soft gels are further dried depending on the product. They are then further packed in glass or plastic bottles. The soft gels are also packed as blister packs.

Fish silage

Fish silage is a product made from whole fish or parts of the fish which are mainly processing discards and to which an acid is added. The liquefaction of the fish is brought about by enzymes inherent in the fish. The product is a stable liquid and contains all the water present in the original material. Hence it is in the liquid form. Fish silage is generally stored in huge drums or polycontainers so that they can be transported.

Shark fin rays

Dried shark fin is a traditionally exported item from India. Significant value addition is possible if the rays from the shark fins are extracted and exported in place of shark fins. With the indigenous development of inexpensive and simple technology for extraction of fin rays, export of fin rays have picked up. Moisture resistant packaging having good puncture resistance and sufficient mechanical strength to withstand the hazards of transportation are the major requirements in the packaging employed for shark fin rays. Polyester / polythene laminates or Nylon based co-extruded films having good puncture resistance are appropriate for shark fin rays. Traditionally dried shark fins are packed as bulk pack in jute sacks. The improved bulk pack consists of high-density polythene woven sack or polypropylene woven sack.

Chapter 18

Seafood Export and Trade Issues

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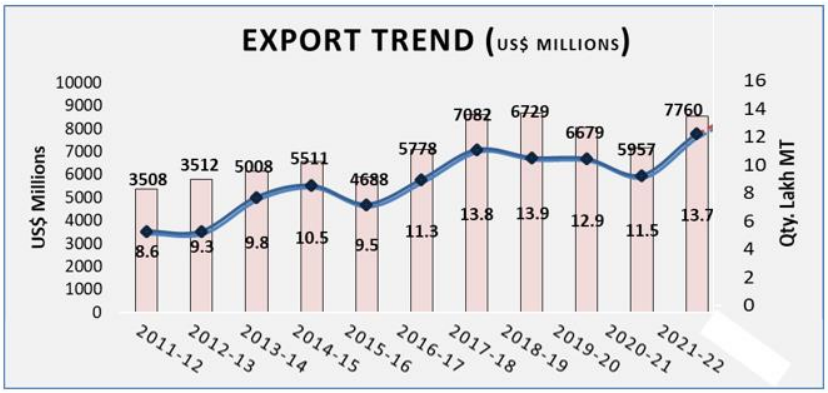
In 2021-22 India exported to 123 countries and the top 5 countries are USA, China, Japan, Vietnam and Thailand. The following figures illustrates the export performance of the marine products from India

Export Performance of Marine Products



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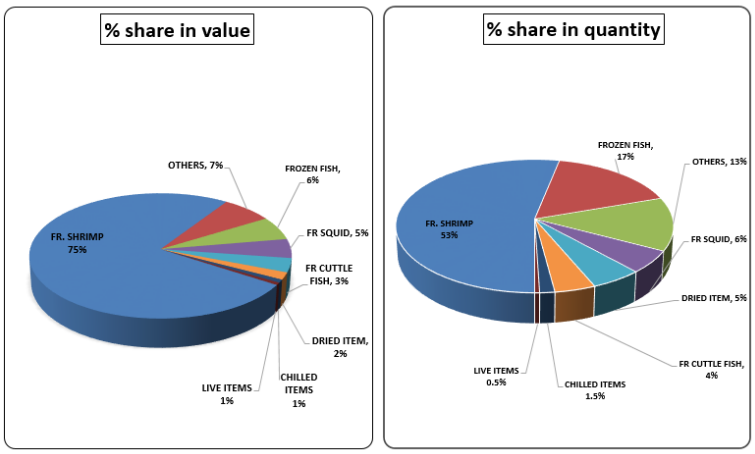

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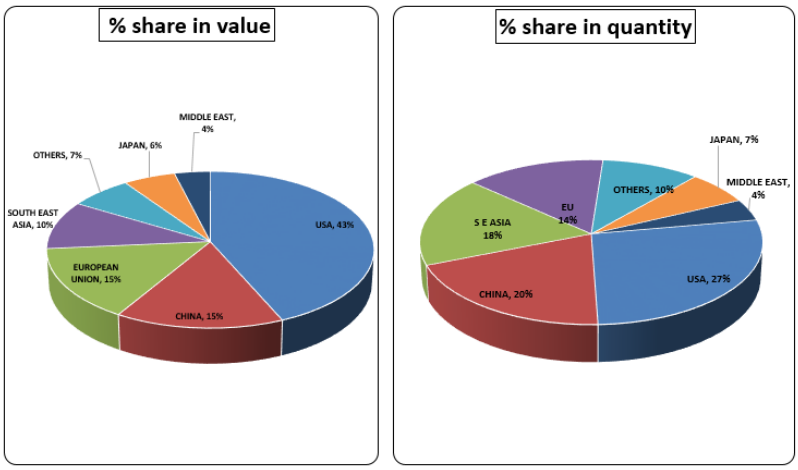

Sanitary & Phytosanitary measures

The word Sanitary means that measures relating to human or animal life or health while Phyto sanitary means those relating to plant life or health. There are three international standards setting bodies specifically mentioned in the SPS agreement. These are often referred as **"Three sisters"** and they are as follows:

1. **The International plant protection convention (IPPC) - dealing with plant health**
2. **The World Organization for Animal Health (OIE) – dealing with animal health**
3. **The Codex alimentarius Commission (CODEX) – dealing with food Safety**

World Trade Organization recommends that members must not use SPS measure that are Unnecessary, not science based, arbitrary or which constitutes a disguised restriction on International Trade. The key points of SPS are as follows:

- The SPS agreement recognizes the need for WTO members to protect themselves from the risk posed by the entry of pests and diseases but also seeks to minimize any negative effects of SPS measures on trade.
- The health aspect of SPS agreement basically means that WTO members can protect human, animal or plant life or health by applying measures to manage the risk associated with imports.
- The measures usually take the form of quarantine or food safety requirements.

Terms used in SPS agreement

Harmonization

WTO members are encouraged to base their SPS measures on international standards, guidelines & recommendations where they exist . The SPS Committee promotes and monitors international harmonization.

Equivalence

SPS agreement requires importing WTO members to accept the SPS measures of exporting WTO members as equivalent, if the exporting country objectively demonstrate to the importing country that its measures achieve the importing country's ALOP (Appropriate Level Of Protection).

Appropriate Level Of Protection (ALOP)

ALOP is the level of protection deemed appropriate by the WTO members to protect human, animal or plant life or health within its territory

Risk Assessment

The evaluation of livelihood of entry, establishment or spread of a pest or disease within the territory of an importing WTO member according to SPS measures which might be applied and of the associated potential biological and economic consequence.

OR

The evaluation of the potential for adverse effects on human or animal health arising from the presence of additives, contaminants, toxins or disease –causing organisms in food, beverages or feedstuffs.

Risk assessment is essentially the process of gathering scientific evidence and relevant economic factors on the risks involved in allowing a particular import to enter a country.

An importing member is likely to seek information on matters such as the pests or diseases that might be associated with the commodity for which permission to import has been sought, and if they are present in the exporting country, the type of question that might be asked as below:

- Does the pest or disease occur in your country?
- Have the pests or diseases been controlled?
- Are they restricted to particular parts of the country?
- How effective are procedures applied to ensure that the products for export are free from pests, diseases and other contaminants?

Regional conditions

WTO members are required to recognize the concepts of pest / disease-free areas and areas of low pests / disease prevalence.

Exporting WTO members claiming pest / disease-free areas or areas of low pests/disease prevalence must demonstrate to the importing WTO member that such areas are, and are likely to remain, pests / disease free areas of low pest / disease prevalence.

Transparency

SPS agreement requires WTO members to provide information on their SPS measures and to notify changes in the SPS measures. WTO members are required to publish their SPS regulations.

The notification requirements are met through a national notification authority. Each WTO member must nominate a national enquiry point to deal with SPS related queries from other WTO members.

The World Organization for Animal Health (OIE)

The objective of the world organization for Animal Health (OIE) include ensuring transparency in the global animal disease and zoonosis situation, publishing health standards for trade in animals and animal products, promoting veterinary skills, improving the safety of food of animal origin and promoting animal welfare through a **science based approach.**

OIE standards, guidelines and recommendations are contained in the **aquatic animal health code** and the **manual of diagnostic tests for aquatic animals.**

CODEX

- Codex has a dual mandate to protect the health of consumers and to ensure fair practices in the food trade.
- Codex develops and encourages implementation of standards, codes of practice, guidelines and recommendations covering all aspects of food safety, including handling and distribution.

SPS – Notification – Australia

Notification No. G/SPS/N/AUS/298 dt. 09/07/2012 (Bio security bill and the inspector general of Bio security bill)

As per Bio Security advice 2009/25, the imported prawns –

1. be sourced from a country or zone that is recognised by Australia to be free of WSSV, YHV, TSV and NHPB – Necrotising Hepetopancriatis Bacterium (the last disease agent, for unfrozen product only); or

2. have the head and shell removed (except for the last shell segment and tail fans) and, if not from a disease free source, have each batch tested on arrival with negative results for WSSV, and YHV; or
3. be 'highly processed', that is head and shell-off (except for the last shell segment and tail fans), and coated for human consumption by being breaded or battered, marinated in a wet or dry marinade, marinated and placed on skewers or processed into dumpling, spring roll, samosa, roll, ball or dim sum-type product; or
4. be cooked to a standard where all protein is coagulated and no uncooked meat remains.

Aquatic animal health certificate for import of seafood

The Canadian Food Inspection Agency (CFIA) brought out guidelines for import of aquatic animals on 10th December 2011 with amendments to Health of animals act. These new guidelines are operational from 10th Dec 2012.

As per this guidelines every consignment of aquatic animal into Canada must be accompanied with an aquatic animal health certificate by the competent authority for aquatic animal health services of the country of origin.

The aquatic animal health certificate must clearly certify **zoo sanitary requirements,** packaging and shipping requirements by competent authority.

Imposition of stringent standards for fish and fishery products by developed countries

The higher standards imposed by the developed countries becoming a major threat to exports of developing countries.

MRL of Ethoxyquin in shrimps under the Food Sanitation Law of Japan

Japanese authority responsible for ensuring the quality of imported food products into Japan has unexpectedly started examining the shrimp consignments from India for Ethoxyquin since August 2012. Japan have adopted the default standard as 0.01 ppm, as designated for parameters or residues that do not figure in the positive list introduced by Japan in 2006. Japan has fixed MRL for fish at **1ppm** whileNo MRL was fixed for shrimp.

Registration of Overseas enterprises and exporting companies in importing countries

The recent requirements of registration under US food safety modernization act and the AQSIQ of China is leading to procedural issues and adding documentation costs for seafood exporting countries in Asia including India. European Union, Russian Federation, Brazil, China etc. are following such kind of registration procedures in their countries to import the seafood from India. Some of these countries also reserve the right to inspect the establishments in India although stringent regulations are in place in India.

ITC HS

ITC-HS Codes or Indian Trade Classification based on Harmonized System of Coding was adopted in India for import-export operations. Indian custom uses an eight digit ITC-HS Codes to suit the national trade requirements. ITC-HS codes are divided into two schedules:

- Schedule I - Describe the rules and guidelines related to import policies
- Schedule II - Describe the rules and regulation related to export policies

The total number of chapters in the schedule I is 98. The chapters are further divided into sub-heading under which different HS codes are mentioned. Export Policy Schedule II of the ITC-HS code contains 97 chapters giving all the details about the guidelines related to the export policies. The marine products mainly come under chapter 03 and 16.

List of marine products coming under various HS code (4 digit level) is as below

Chapter	HS Code (4 digit level)	Description
03	0301	Live fish
	0302	Fish, fresh or chilled, excluding fish fillets and other fish meat of heading 0304
	0303	Fish, frozen, excluding fish fillets and other fish meat of heading 0304
	0304	Fish fillets and other fish meat (whether or not minced), fresh, chilled or frozen
	0305	Fish, dried, salted or in brine; smoked fish, whether or not cooked before or during the smoking process; flours, meals and pellets, of fish fit for human
	0306	Crustaceans, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; smoked crustaceans, whether in shell or not, whether or not cooked before or during the smoking process; crustaceans, in shell, cooked by steaming or by boiling in water, whether or not chilled, frozen, dried, salted or in brine; flours, meals and pellets of crustaceans, fit for human consumption
	0307	Molluscs, whether in shell or not, live, fresh, chilled, frozen, dried, salted or in brine; smoked molluscs, whether in shell or not, whether or not cooked before or during the smoking process; flours, meals and pellets of molluscs, fit for human consumption
	0308	Aquatic invertebrates other than crustaceans and molluscs, live, fresh, chilled, frozen, dried, salted or in brine; smoked aquatic invertebrates other than crustaceans and molluscs, whether or not cooked before or during the smoking process; flours, meals and pellets of aquatic invertebrates other than crustaceans and molluscs, fit for human consumption

Chapter	HS Code (4 digit level)	Description
05	0508	Coral and similar materials, unworked or simply prepared but not otherwise Worked; shells of molluscs, crustaceans or echinoderms and cuttle-bone, unworked or simply prepared but not cut to shape, powder and waste thereof
	0511	Animal products not elsewhere specified or included; dead animals of chapter 1 or 3, Unfit for human consumption: **Products of fish or crustaceans, molluscs or other aquatic invertebrates; dead animals of Chapter 3** ***(Fish nails, fish tails and other fish waste)***
12	1212	Locust beans, seaweeds and other algae, Sugar beet and sugarcane, fresh, chilled, frozen or dried, whether or not ground; fruit stones and kernels and other vegetable Products (including unroasted chicory roots of the variety cichorium intybus Sativum) of a kind used primarily for human consumption, not elsewhere specified or included : ***Seaweed***
13	1302	Vegetable saps and extracts; pectic substances, pectinates and pectates; agar-agar and other mucilages and thickeners, whether or not modified, derived from vegetable products : ***Agar-agar, Kappa Carrageenan***
15	1504	Fats and oils and their fractions, of fish or marine mammals, whether or not refined, but not chemically modified
16	1604	Prepared or preserved fish; caviar and caviar substitutes prepared from fish eggs
	1605	Crustaceans, molluscs and other aquatic invertebrates, prepared or preserved
23	2301	Flours, meals and pellets, of meat or meat offal, of fish or of crustaceans, molluscs or other aquatic invertebrates, unfit for human consumption; greaves : **Fish meal unfit for human consumption**
	2309	Preparations of a kind used in animal feeding: **Feeds for fish (prawn, etc.)**

Trade agreements: Review & Execution

Trade agreements	Way Forward
1. India - Japan CEPA 2. India - Korea CEPA 3. India - EU BTIA 4. India - Peru FTA 5. India - Mauritius CECPA 6. India - EFTA 7. RCEP Negotiation 8. India Australia Free Trade Agreement 9. India New Zealand Free Trade Agreement 10. India Canada Free Trade Agreement 11. India MERCOSUR Free Trade Agreement 12. India Eurasian Economic Union Trade Agreement	• **India EU FTA** : Early execution of India-EU FTA and reduction of tariff is urgently required for better market access of Indian seafood in EU. • **India-Korea CEPA**: It is understood that during previous review the duty for frozen shrimp was agreed to be reduced to 0% by South Korea with a quota restriction. This may be urgently brought into effect for getting duty benefit for exporting to South Korea. **This review and execution of FTA's on a fast track mode will facilitate India's export to these markets.**

Strategies to overcome SPS issues:

Various strategies to overcome the SPS agreement related issues are as follows:

- Prevent the usage of banned antibiotics like chloramphenicol, nitrofuran *etc.*, in food producing animals

- Competent authority needs to take steps to create aquatic disease-free areas/zones/region
- Use SPS as tool to counter the countries who are using SPS as a tool to restrict the trade
- Raise the SPS issues in bilateral trade meetings for market access
- Raise the SPS issue sin WTO SPS Committee meetings; and
- Active Indian participation in CODEX, OIE & IPPC proceedings

Chapter 19

Traceability and Seafood Authenticity

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Introduction:

The horse meat scandal is known across world. This involved food products across Europe which was labeled as beef where it contained horse meat. Profit driven malpractice was identified by Irish food inspectors who revealed in mid-January 2013 that they had found horse meat in frozen beef burgers. This leads to the path for authentication of food products and open the horizon of thought of people to suspect about crime against food safety and human health. Such food frauds are being carried out in all kind of food stuffs including seafood. Seafood comprises of various species and after their processing it is very hard to know which species it is made of until it is not tested with modern analytical tools.

Food fraud

Food fraud is a significant and growing problem, driven by globalization, economic opportunity, and the low probability and severity of punishment. Analytical verification of food fraud and food authentication is needed to support proper food safety management systems. Food fraud is designed to increase the perceived value of both food and ingredients and is a growing concern in our global food supply.

"Consumers expect safe and nutritious foods. They also expect all participants in the supply chain to have effective practices in place that allow for the rapid identification, location, and withdrawal of food lots when problems are suspected or confirmed. The increased focus on food safety and consumer awareness raises the need for the identification and adoption of business practices that will aid the ability of the trading partners in the food industry to track and trace a product throughout the supply chain" (FAO, 2017).

A few of the recent, and possibly well-known, occurrences of food fraud include:

- Melamine or cyanuric acid found in infant formula and pet foods
- Fake food and alcohol seizures in EU borders
- Kiwi wine company accused of complex food fraud
- Exporting falsified hazelnut products to Germany from Georgia
- 75% seafood samples mislabeled with cheaper fish in place of more expensive across Canadian cities.

- Mislabeled giant squid as octopus in North America

DRIVERS for Food fraud

- Deliberate criminal fraud for financial gain (adulteration/substitution – premium products)
- Rising commodity prices
- Shortage of supply
- Raw material quality due to poor yields and variable composition
- Avoidance of tariffs
- Sustainability fishing

***Seafood Authentication*:**

Process that verifies that a food is in compliance with its label description is called as Authentication. This is necessary to preventing Food Fraud and quick Recalls of products distributed in markets.

Traceability is vitally important for food safety as well as operational efficiency. This will help to pinpoint the source of the issue and the scope of any potential incident.

Any deliberate action of businesses or individuals to deceive others in regards to the integrity of food to gain undue advantage. Types of food fraud include but not limited to: adulteration, substitution, dilution, tampering, simulation, counterfeiting, and misrepresentation. There are more than 8,100 (up to 2017) papers dealing with food authenticity have been recorded in the Science Direct database

***Seafood Traceability*:**

Seafood Traceability cab be defined as track and trace a product throughout the supply chain. This requires reliable analytical methods that can give a decisive answer about the authenticity of foodstuffs. This can also be called as measuring features that can discriminate foods of different origins.

There are certain authenticity indicators which includes Rare earth elements and precious metals, Microbial fingerprinting, Metabolomics fingerprinting and Sensory analysis

Timeline (Traceability and Authentication Definition)

1994: ISO 8402 Definition of Traceability - "The ability to trace history, application and location of an entity by means of recorded identification"

1998: Denis (1998) Definition of Food Authentication - "Food authentication is the process by which a food is verified as complying with its label description"

2002: The Food Safety Agency (FSA) basic characteristics for traceability system-
(1) Identification of units/batches of all ingredients and products
(2) Information on when and where they are moved and transformed
(3) A system to link these data

2004: CAC Definition of food Traceability-"The ability to follow the movement of a food through specified stages of production, processing and distribution"

2005: ISO 9000 Definition of Traceability-"The ability to trace the history, application or location of that which is under consideration"

2016: Danezis et al. defined Food Authentication as "Food authentication is the process that verifies that a food is in compliance with its label description. This may include, among others, the origin, production method, or processing technologies

Food Traceability (terms & definitions)

- **Tracking" or "Tracing forward":** refers to pursuing in the downstream direction
- **"Tracing" or "Tracking back":** refers to pursuing in the upstream direction
- **Traceability system:** A series of mechanisms for traceability, by which "identification", "link", "records of information", "collection and storage of information", and "verification" are performed.
- **Traceable unit:** The unit used for identification. This unit is used when tracing and tracking. In some cases, a lot works as a unit and in others, an individual and/or individual product works as a unit

Traceability systems in practice

The key factors to successfully implementing a traceability system within the seafood processing establishments include

• Full details of suppliers of raw materials and ingredients.

• Identification of individual batches by product coding till dispatch from factory

• Maintain batch separation throughout the processing and storage.

• Linking batch codes to production records for each process in the establishment.

Of the various different methods by which traceability can be achieved the following are provided as examples;

• Paper-based traceability

• Bar code/scanner systems

Technology-Enabled Traceability

Data Elements Specific data captured through the traceability system (e.g. origin, water usage, etc.)

Unique Identifiers An assigned unique identifier to the individual food product for tracking along the supply chain; examples include RFID tag or barcodes

Sensor Technology Real-time tracking of identified data elements through supply chain; enables automated data capture

Distributed Ledger Technology Enables easier aggregation, integration, analysis and sharing of data; today, ledgers are often completed using suboptimal paper based systems but can be significantly improved through technology adoptions

There are 7 principal ways a food or food ingredient can be adulterated to increase its perceived value:

1. Substitution
2. Unapproved Enhancements
3. Concealment
4. Mislabeling
5. Dilution
6. Counterfeiting
7. Gray Market

CURRENT FOOD AUTHENTICITY CHALLENGES

CATEGORY	**EXAMPLE**
Origin of food from sustainable sources	Palm oil, fish, exotic meats
Method of food production	Organic food
Substitution - Quantification of ingredients	Meat species in processed foods
Designation of geographical origin	Meat, fish , composite foods

(Food Information legislation)	
Specialty foods	Vanilla, saffron, honey, balsamic vinegar, Basmati rice
Adulteration - Alcoholic and nonalcoholic beverages	Fruit juices, wine, spirits
Miscellaneous	High protein foods

ANALYTICAL WAYS:

1. DNA-methods: DNA-based methods for food authentication depend on the highly specific amplification of DNA fragments by the Polymerase Chain Reaction (PCR). The advantage of genomics is that it can amplify minute traces of nucleotide material. The sensitivity of these methods are high since the amount of analyte required is in nanogram (ng).
2. Stable isotope analysis: The isotopic ratios are applicable to food authentication because stable isotope ratios change with the climatic conditions, geographical origin, soil pedology, and geology of the locations of food ingredients origin. The analysis of isotopic ratios uses various methods such as Isotope Ratio Mass Spectrometry (IRMS), Multi Collector – Inductively Coupled Plasma – Mass Spectrometry (MC-ICP-MS), and Thermal Ionization Mass Spectrometry (TIMS).
3. Proteomics: proteins can act as markers for many properties of the food products all along the food chain from farm to fork, and therefore proteomics can be applied for a systematic search of new marker proteins or peptides. Proteomics identifies specific products encoded by DNA. The sensitivity is very high since the amount of required material can be as small as a few cells.
4. Metabolomics: Metabolomics deals with the study of multiple metabolites in a cell, a tissue or an organism. Ultra-high performance liquid chromatography (UHPLC), high-resolution mass spectrometry (HR-MS) and software programs to process the large analytical data sets can be used.
5. Spectroscopy: Spectroscopy, in particular vibrational spectroscopy, is a fast and inexpensive method for both the assessment of food quality and food authenticity. Novel instrumental techniques combined with chemometric methods have enabled the

development of rapid methods that apply multivariate (MVA) analysis, to near infrared (NIR) and mid infrared (MIR) data to analyze food matrices.

6. Metagenomics/Next Generation Sequencing: Metagenomic and metatranscriptomic have great potential in becoming valuable options for detecting food authenticity for a specific food product. Traditional DNA barcoding methodologies based on PCR and Sanger sequencing has limitation being low-throughput. Such limitations has been overcome by high-throughput NGS technologies including metagenomic approaches, which provide more information food product.
7. Sensory analysis: Traditionally reliable results in sensory analysis require a well-trained panel of human assessors. Organoleptic test panels comprise a set of techniques for accurate measurements of human responses to foods. Appearance, aroma, flavor and texture properties are important characteristics determining the quality authenticity of food products. These panels require extensive training of judges, adequate replication and detailed statistical analysis of the observations.

Current work – supporting testing and enforcement

Meat speciation

- DNA quantitation breed authentication
- Detection of offal and serum in meat products
- Gelatine speciation (water-retention, chicken plumping agents)

Fish speciation

- Geographic traceability
- EU harmonisation of fish DNA methods
- Nitrogen factors for fish quantitation

Technical Challenges in detecting Food Malpractices

- **3 key difficulties:**

1. Issue is linked to a legal requirement, standard or guidance; conclusion must be beyond reasonable doubt, but data interpretation is made against a background of analytical uncertainty, natural variation etc

2. Finding a marker that characterises the food, one of its ingredients, the adulterant(s), or the processing, production or geographic origin

3. Availability of authentic samples (databases)

Emerging authenticity indicators

Sl. No.	Indicators	Remarks
a.	**Rare earth elements and precious metals:**	○ Elemental fingerprinting targets groups of elements including macroelements (such as calcium, sodium, potassium), trace-elements (such as selenium, zinc, copper), rare earth elements (REEs, such as cerium, samarium and lanthanum), or other ultra-trace elements (such as precious metals platinum or gold) ○ ICP-MS (Inductively coupled plasma mass spectrometry) and ICP-OES (Inductively coupled plasma - optical emission spectrometry) can be used exclusively
b.	**Metabolomic fingerprinting:**	○ Quantitative analysis of the complete metabolome or selected subsets is called Metabolomics. ○ Metabolomics uses mostly nuclear magnetic resonance (NMR) and mass spectrometry (MS) analytical technologies ○ Gas chromatography mass spectrometry (GC–MS), Liquid chromatography mass spectrometry (LC–MS), ultra performance liquid chromatography (UPLC), QTOF-MS and Orbitrap-based technologies, High-Resolution MS (HRMS), Vibrational spectroscopic techniques (near-infrared or NIR, mid-infrared or MIR, and Raman spectroscopy
c.	**Microbial fingerprinting:**	○ Assessment of geographical origin can be achieved through microbial fingerprinting in non-processed foods (fruits, fish, wine, yoghurt). ○ Microflora was found to be specific of the production system and microbial fingerprints were shown to differ between organically and conventionally grown fruits ○ Polymerase chain reaction-denaturing gradient gel electrophoresis technique, PCR-DGGE is usually used for microbial fingerprinting
d.	**Sensory analysis:**	○ Specialized panelists required with total sensory experience of food combines aroma, taste, texture, temperature, spiciness, appearance.

		○ The instrumental sensory techniques electronic tongue (e-tongue), electronic nose (e-nose), electronic eye (eeye) and gas chromatography olfactometry (GCO) are used for objective sensory evaluations.

Opportunities and challenges for Food Authentication

Food authentication is an interdisciplinary area where has input from instrumentation, biology, informatics, mathematics and statistics, agriculture, and food technology are needed.

- Vast volumes of data are generated, but our ability to manage and analyze these date are falling behind the ability to generate these data.
- Mass spectrometry is a frontline technology rapidly replacing other methods in many fields of food science.
- Multi-analyte capabilities are essential for food authentication studies since they provide more descriptors and thus facilitate better classification.
- Programs are being developed and implemented to reduce food fraud, but these programs must continue to evolve in order to keep pace with the ingenuity of food fraud perpetrators.

Opportunities and challenges for Food Traceability

Global supply chain is complex system. Hence ensuring it as effective practices in place is an on-going challenge. One of the biggest traceability challenges goes back to recordkeeping. Without effective procedures to capture multiple dimensions of product information, it becomes difficult to track products and comply with recall requirements. One problem that hinders traceability and increases the scope and cost of recalls is the lag time between when a problem occurs and when a company detects it. Government regulation requires businesses to implement a food traceability system (record system, recall system, etc.)

Development of information technology supports implementation of food traceability system (IoT, Bigdata, machine learning, etc.) is the need of hour for effective implementation of traceability system for any food products.

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