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QUALITY MATTERS

*Online Training programme on*

# QUALITY ASSURANCE OF FISH AND FISHERY PRODUCTS

**19 September - 01 October 2022**

*Jointly organising*

## Indian Technical and Economic Cooperation (ITEC)

(Ministry of External Affairs, Govt of India)

&

## ICAR - Central Institute of Fisheries Technology (CIFT)

(Indian Council of Agricultural Research, New Delhi)

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# Training Manual

*on*

# QUALITY ASSURANCE OF FISH AND FISHERY PRODUCTS

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

**Date: 19 September – 01 October, 2022**

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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, 20 participants–from 7 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. Leela Edwin, Director (Acting), 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**

# Contents

# INTRODUCTION TO FISH PRESERVATION TECHNIQUES

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Fish is one of the healthiest foods available to man and there is an ever-increasing demand for fish and fishery products. Being a highly perishable commodity, fish require immediate processing and various options are available for the value addition of fish. Fish processing, particularly seafood processing and marketing have become highly complex and competitive and exporters are trying to process more value-added products to increase their profitability. Value can be added to fish and fishery products according to the requirements of different markets. These products range from live fish and shellfish to ready to serve convenience products. In general, value-added food products are raw or pre-processed commodities whose value has been increased through the addition of ingredients or processes that make them more attractive to the buyer and/or more readily usable by the consumer. It is a production/marketing strategy driven by customer needs and perceptions.

Technology developments in fish processing offer scope for innovation, increase in productivity, increase in shelf life, improve food safety and reduce waste during processing operations. A large number of value added and diversified products both for export and internal market based on fish, shrimp, lobster, squid, cuttlefish, bivalves etc. have been identified. This paper gives an overview of the processing techniques, emerging technologies and the value added products from fish and shell fish.

## Chilling

Chilling is an effective way of reducing spoilage by cooling the fish as quickly as possible without freezing. Immediate chilling of fish ensures high quality products (Connell, 1995: Huss, 1995). Chilling by use of ice is the most important method employed commercially. The storage life of fish kept in ice depends on a number factors which include species, size, method of capture, fat content, breeding conditions, feeding regime and the method of killing. In general, the keeping quality of non-fatty fish is better than fatty fish in ice storage. The quality and

quantity of ice used are important factors in determining the shelf life of iced fish. In tropical countries, a 1:1 fish to ice ratio is ideal for ice storage. It is recommended to add about 12-20% extra ice to the fish in order to compensate for water loss from melting and bad handling (Zugarramurdi *et al*., 1995). It is generally accepted that some tropical fish species can keep for longer periods in comparison to fish from temperate or colder waters.

**Transportation of live fish and shellfish**

Transportation of fish, crustaceans and molluscs in live condition is the best method to ensure that the consumer is supplied with fresh product. In India, traditional mode of live transport in open earthen containers and metal containers was practiced (Jhingran, 1975). In terms of the range of species and the distance shipped, tropical fishes stand first in live fish transport. Waterless transportation of live fish is also practised for many species where the animals are kept in moist conditions under optimal cold temperatures.

**Freezing**

Freezing is one of the better methods to preserve fresh fish. It may be either slow freezing or quick freezing. Slow freezing is accomplished by placing the product at a low temperature and allowing it to freeze slowly usually in still air. Quick freezing is accomplished in any one or combination of the following four methods:

1. Immersion freezing
2. Indirect contact freezing
3. Air blast freezing and
4. Cryogenic freezing

**Air freezing**

***Air blast freezing***

Circulating cold air at high speed enables freezing to proceed at a moderately rapid rate and this method is referred to as air-blast freezing. Air-blast freezing is usually accomplished by placing the products on a mesh belt and passing it slowly through an insulated tunnel containing air at-18 to -34°C or lower, moving counter current to the product at a speed of I to 20 meter/sec. Air at -

29°C and at a speed of 10-12 meter/sec, is often satisfactory, although lower temperatures are preferred. Air blast freezing is economical and is capable of accommodating products of different sizes and shapes. It can result in (1) excessive dehydration of unpackaged products if conditions are not carefully controlled, and this in turn necessitates frequent defrosting of equipment and (2) undesirable bulging of packaged products which are not confined between flat rigid plates during freezing. Spiral Belt Freezer and Fluidized Bed Freezing are some of the commonly used methods in the industry.

***Contact Plate Freezing***

Fish products can be frozen by placing them in contact with a metal surface cooled by expanding refrigerants. Double contact plate freezers are commonly used for freezing fish/prawn blocks. This equipment consists of a stack of horizontal cold plates with intervening spaces to accommodate single layers of packaged product. The filled unit appears like a multi layered sandwich containing cold plates and products in alternating layers. When closed, the plates make firm contact with the two major surfaces of the packages, thereby facilitating heat transfer and assuring that the major surfaces of the packages do not bulge during freezing. Vertical plate freezers are also in use especially onboard fishing vessels. Contact plate freezing is an economical method that minimises problems of product dehydration, defrosting of equipment and package bulging. In this method the packages must be of uniform thickness. A packaged product of 3 to 4 cm thickness can be frozen in I to 1.5 hour when cooled by plates at -35°C. Freezing times are extended considerably when the package contains a significant volume of void spaces.

***Liquid Immersion Freezing***

Liquid immersion freezing or direct immersion freezing is accomplished when a product is frozen by immersing or by spraying with a freezant that remains liquid throughout the process. This technique is occasionally used for fish and prawns. Liquid immersion freezing can result in moderately rapid freezing. Freezants used for liquid immersion freezing should be non-toxic, inexpensive, stable, reasonably inert, and should have a low viscosity, low vapour pressure and freezing point and reasonably high values for thermal conductivity. Freezants should have a low tendency to penetrate the product, little or no undesirable effects on organoleptic properties and

require little effort to maintain desired standards for sanitation and composition. Aqueous solutions of propylene glycol, glycerol, sodium chloride, calcium chloride and mixtures of sugars and salt have been used as freezant.

***Cryogenic Freezing***

Cryogenic freezing refers to very rapid freezing by exposing food products to an extremely cold freezant undergoing change of state. The fact that heat removal is accomplished during a change of state by the freezant is used to distinguish cryogenic freezing from liquid immersion freezing. The most common food grade cryogenic freezants are boiling nitrogen and boiling or subliming carbon dioxide.

***Individually Quick Frozen Products (IQF)***

Lobster, squid, cuttlefish, different varieties of finfish etc. are processed in the individually quick frozen style. IQF products fetch better price than conventional block frozen products. However, for the production of IQF products raw-materials of very high quality need to be used, as also the processing has to be carried out under strict hygienic conditions. The products have to be packed in attractive moisture-proof containers and stored at –30°C or below without fluctuation in storage temperature. Some of the IQF products in demand are prawn in different forms such as whole, peeled and de-veined, cooked, headless shell-on, butterfly fan tail and round tail-on, whole cooked lobster, lobster tails, lobster meat, cuttlefish fillets, squid tubes, squid rings, boiled clam meat and skinless and boneless fillets of white lean fish. IQF products can be easily marketed as consumer packs, which is not possible with block frozen products. This is a distinct advantage in marketing.

**Canning**

Canning is a method of food preservation in which preservation is achieved by the destruction of micro-organisms by the application of heat of food packed in a sealed container. Since the canned foods are sufficiently cooked products and free from micro-organisms they offer consumer safety besides being ready to consume. Canning has the unique distinction of being an invention in the field of food processing/ preservation whereas all other methods can be considered as adaptation of natural processes or their modifications. Because of their very long

shelf life and ready to consume feature canned products have become very popular and a variety of food stuffs, both plant and animal origin and their combinations are produced and distributed.

*Unit Operations in a canning process are:*

1. Selection and preparation of raw material.
2. Pre-cooking / blanching
3. Filling in to containers.
4. Addition of liquid medium
5. Exhausting
6. Seaming
7. Heat Processing / Retorting
8. Cooling
9. Drying, warehousing, labelling and casing

Retort Pouch Processing is an improvement over the conventional canning process. Reportable flexible containers are laminate structures that are thermally processed like a can, are shelf stable and have the convenience of keeping at room temperature for a period of more than one year without refrigeration. The most common form of pouch consists of a 3 ply laminated material. Generally it is polyester / aluminium foil / cast polypropylene. See-through pouches made of polyester/aluminium oxide or silicon dioxide/nylon/cast polypropylene is also available. The manufacture of retort pouch packs involves a series of lengthy operations viz., filling, air removal, sealing, traying and heat processing in an over pressure autoclave

**Curing**

The traditional methods of processing fish by salting, drying, smoking and pickling are collectively known as curing. Cured fish consumption is more in areas where the availability of fresh fish is comparatively limited, namely interior markets and hilly areas. This is also the cheapest method of preservation, since no expensive technology is used. In India roughly 20 % of the fish caught is preserved by curing. Considerable quantities of cured fish are also exported, mainly to Singapore, Sri Lanka and to the Middle East. Simple sun drying was the

widely practised traditional method of fish preservation. By this, preservation was achieved by lowering of water content in the fish, thereby retarding the activity of bacteria and fungi. The heat was able to destroy the bacteria to a certain extent. Later on, a combination of salting and drying or salting, smoking and then drying were developed.

**Salting**

This is one of the oldest methods of preservation of fish. Salting is usually done as such or in combination with drying or as a pretreatment to smoking. During salting osmotic transfer of water out of the fish and salt into the fish takes place, which effect fish preservation. It is based on different factors like diffusion and biochemical changes in various constituents of the fish. Salting amounts to a process of salt penetration into the fish flesh. Penetration ends when the salt concentration of the fish equals that of the surrounding medium. Loss of water during salting limits bacterial growth and enzyme activity, thus preserving the fish. The high salt content prevents the growth of normal spoilage microflora in the fish; but halophiles, which can survive 12-15% of salt, will survive.

**Smoking**

Smoke curing is another traditional method of preservation of fish. It is generally a combination of salting, smoking and drying. Smoking is usually done in a specially designed kiln or a room. The source of smoke is wood, sawdust or coconut husk, depending on the particular flavour required. The fish that is salted and partially dried is used for smoking. Smoking can be done at temperatures below 35 $^{0}$ C (cold smoking) or at higher temperature (hot smoking). Liquid smoking by immersion in smoke liquor and electrostatic smoking is also practised in different countries. Masmin, Dried Squid, Dried Jelly Fish, Dried Bombay duck etc are Some commercially important dried aquatic products

**Irradiation**

Irradiation treatment involves controlled exposure of the food to radiation sources such as isotopes of cobalt ($^{60}$Co) or cesium ($^{137}$Cs), which emit gamma rays, and also X-rays and electron beams (Lagunas-Solar, 1995). Radiation processes that can be applied to fishery products include radurization (pasteurization of chilled fish), radicidation (sanitization of fresh and frozen

products including fish mince by elimination of non-spore forming pathogenic bacteria) and disinfestation.

Radurization of fresh fish at 1 to 3 kGy reduces initial microbial loads by 1 to 3 log cycle, essentially reducing spoilage causing bacteria and extends their chilled storage life 2-3 fold. The treatment is effective for the extension of shelf life of most of the marine and freshwater fish species.

**Battered and Breaded Products**

The most prominent among the group of value added products is the battered and breaded products processed out of a variety of fish and shellfish. Battered and breaded products offer a 'convenience' food widely valued by the consumer. These are products, which receive a coat or two each of a batter followed by coating with breadcrumbs, thus increasing the bulk and reducing the cost element. The pick-up of coating can be increased by adjusting the consistency of the batter or by repeating the coating process. By convention, such products should have a minimum fish component of 50%. Coated products viz., fish fingers, squid rings, cuttlefish balls, fish balls and prawn burgers form one of the major fish and shellfish based items of trade by the ASEAN countries.

The first commercially successful coated product is 'fish finger; or 'fish stick'. Later several other products particularly the coated fish fillet, fish portions, fish cakes, fish medallions, fish nuggets, breaded oysters and scallops, crab balls, fish balls, coated shrimp products, coated squid rings etc. became prominent in most of the developed countries with the advent of the fast food trade. The present day production of coated seafood items involve fully automated batter and breading lines which start from portioning and end with appropriate packaging of the product

**Fish Mince and Mince Based Products**

Mechanically deboned fish meat is termed as fish mince. Fish mince is more susceptible to quality deterioration than the intact muscle tissue since mincing operation cause disruption of tissue and exposure of flesh to air, which accelerates lipid oxidation and autolysis. The quality of the mince is dependent on the species, season, handling and processing methods. Also, low bone content in the mince (01-0.4%) is desirable for better functional and sensory properties. Fish mince is a major source of raw material for the preparation traditional products such as patties,

balls, wafers, loaves, burgers, fish fingers, dehydrated fish minces , cutlets and pickled products The mince from different species could be combined to prepare composite fillets

**Surimi**

Surimi is stabilized myofibrillar protein obtained from mechanically deboned flesh that is washed with water and blended with cryoprotectants (Park, 2005). Washing not only removes fat and undesirable matters such as blood, pigments and odoriferous substances but also increases the concentration of myofibrillar protein, the content of which improves the gel strength and elasticity of the product. This property can be made use of in developing a variety of fabricated products like shellfish analogues. India produces about 40.000 MT of surimi per annum, 70% of which comes from thread fin bream.

**Kneaded products**

Several kneaded products like kamaboko, chikuwa, hampen, fish ham and sausage are processed using surimi incorporating other ingredients. The ingredients used in most of these preparations are identical; however, the classification is principally based on the manufacturing process involved. The ingredients employed other than surimi include salt, monosodium glutamate, sugar, starch, egg white, polyphosphate and water. The method of processing all these products involves grinding together of the various ingredients to a fine paste and some sort of heat treatment at some stage.

**Fibreized products**

Fibreized products are in great demand among the surimi based imitation shellfish products. The ingredients used in the formulation of fibreized products includes, besides surimi, salt, starch, egg white, shellfish flavour, flavour enhancers and water. All the ingredients are thoroughly mixed and ground to a paste. The paste is extruded in sheet on the conveyor belt and is heat treated using gas and steam for partial setting. A strip cutter subdivides the cooled sheet into strings and is passed through a rope corner. The rope is coloured and shaped. The final product is formed by steam cooking the coloured and shaped material.

**Fish sausage**

Fish sausages are surimi or fish mince mixed with additives, stuffed in suitable casings and heat processed. The surimi or fish mince is mixed with salt (3-4%), sugar (2-3%), sodium glutamate (0.3%) starch and soy protein in a silent cutter and stuffed in casings by an automatic screw stuffer. The stuffed sausage is heated in hot water at 85-90°C for 40-60 min. After heating, it is cooled slowly to avoid shrinking of the tube and then stored at refrigerated temperature. The production of fish sausage in India is rather insignificant, although market potential for this product is good (Hassan & Mathew, 1999). Sausages prepared from rohu mince treated with potassium sorbate had a shelf life of 16 days at refrigerated temperatures (Sini *et. al*., 2008).

Accelerated Freeze Drying, High Pressure processing, Enzyme treatment, Extrusion technology, Pulsed light technology, Hurdle technology etc. are some of the emerging technologies for value addition of fish which are being practices by the industry to certain extent.

Fish processing and value addition has evolved over the years as an important sector in Indian Agriculture. Fish and fishery products earn maximum foreign exchange in the category of agricultural produce exported from India. This sector has immense scope for development through diversification and generation of employment for the skilled and unskilled workforce of the country.

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# POST MORTEM CHANGES IN FISH MUSCLE

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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.

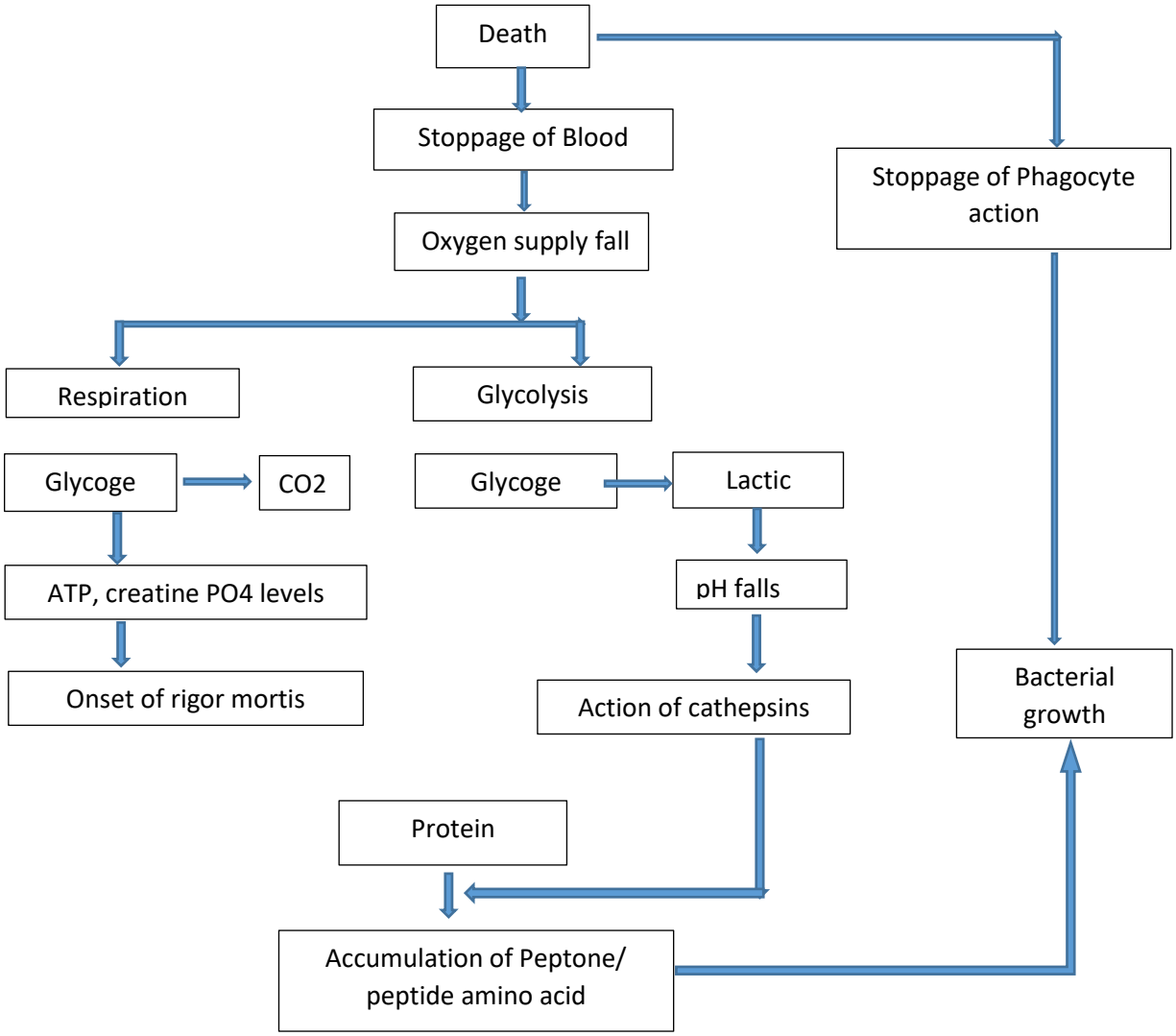
Fish is a food item of good acceptability and nutritional value. But it is a highly perishable item and quality deterioration very fast if not preserved properly. The changes leading to spoilage of fish are highly complex. Both biochemical and microbiological processes contribute the quality deterioration. 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

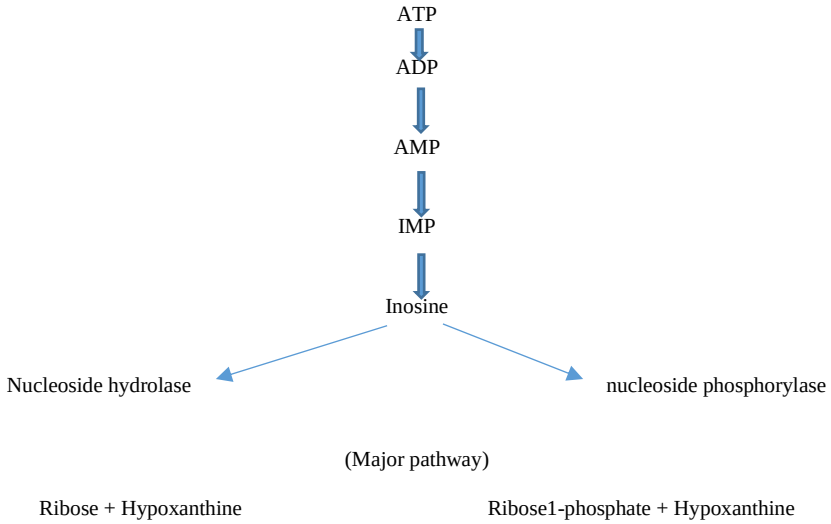
conversion of glycogen to lactic acid takes place, leading to the production of just 3 molecule of ATP.

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**

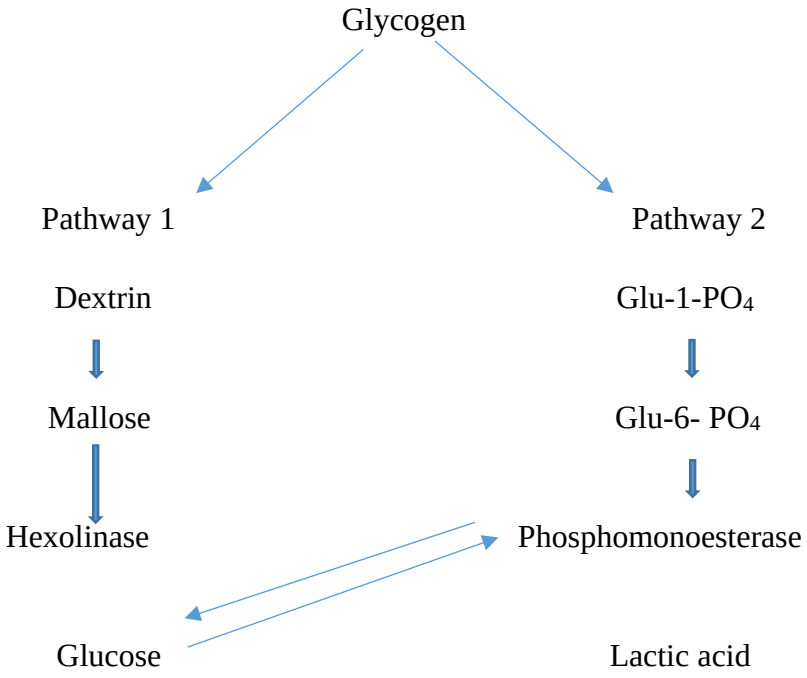
ATP is converted in to adenosine-5' –diphosphate (ADP) by sarcoplasmic ATPase, which is by myokinase. Conversion of adenosisne-5' monophosphate (AMP) to IMP is effected out by a deaminase action. IMP, which plays a role in the flavour of good quality fish, is dephosphorylated to inosine. Decomposition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

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# SPOILAGE INDICES OF FISH AND SHELLFISH

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## 1. INTRODUCTION:

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

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

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

Spoilage refers to any change in the condition of food in which the food becomes less palatable, or even toxic; these changes may be accompanied by alterations in taste, smell, appearance or texture. The rate of spoilage is temperature dependent and lowing the temperature will reduce the spoilage rate. Also, the rate of spoilage of fish may be reduced by following good handling practices.

## 2. QUALITY AND FRESHNESS:

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

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

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

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

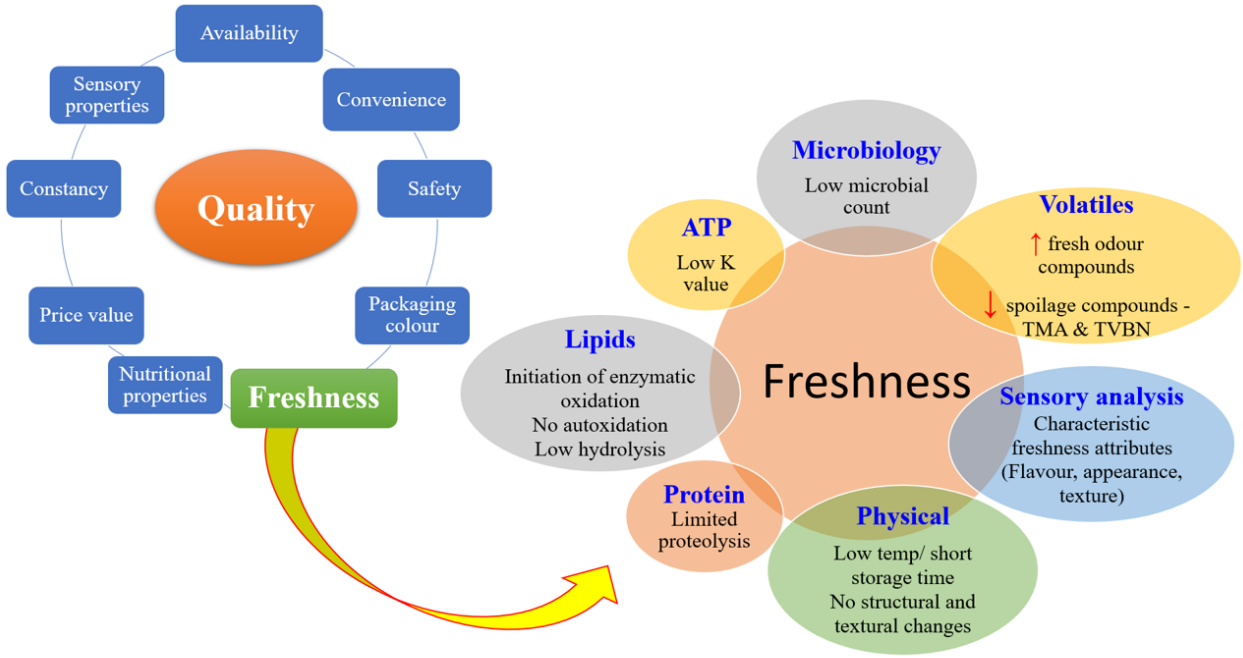



Figure 1. Relationship between quality and freshness (Olafsdbttir *et al*., 1997)

## 3. FISH SPOILAGE:

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

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

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

### 3.1. Enzymatic Spoilage:

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

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

Enzymatic spoilage causes bally bursting in fish, especially during a periods of high food intake. These fishes will have a large contain of digestive enzymes in the digestive tract. Such fish will degrade quickly and spoil easily soon after they are caught. Belly bursting is caused by leakage of proteolytic enzymes from pyloric caeca and intestine to the ventral muscle. In the dissolved gut components, bacteria proliferate and produce gases such as $CO_2$ and $H_2$. This gas production leads to belly bursting after short storage period. The rate of degradation by proteolytic enzymes was reduced when the fish was kept at 0°C.

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

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

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

### 3.2. Oxidative Spoilage:

Lipid oxidation is a major cause of deterioration and spoilage for the pelagic fish species such as mackerel and herring with high oil/fat content stored fat in their flesh. Fish lipids which consist of polyunsaturated fatty acids are highly susceptible to oxidation. Lipid oxidation involves a three-stage free radical mechanism: initiation, propagation and termination (Figure 2). Oxidation typically involves the reaction of oxygen with the double bonds of fatty acids. Chain reaction (initiation) involves the production of lipid free radicals through catalysts (such as heat, metal ions and irradiation) by removal of a hydrogen atom from the alpha methyl group. Production of hydroperoxide takes place in the propagation sequence of reaction. In this reaction, free radicals react with oxygen to form peroxyl radicals, which in turn react with other lipid molecules to form hydroperoxides and a new free radical. Which can initiate chain of event again. Termination occurs when peroxide decompose or when a buildup of these free radicals interacts with one another or one other oxidation products resulting in formation of non-radical products such as carboxylic

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

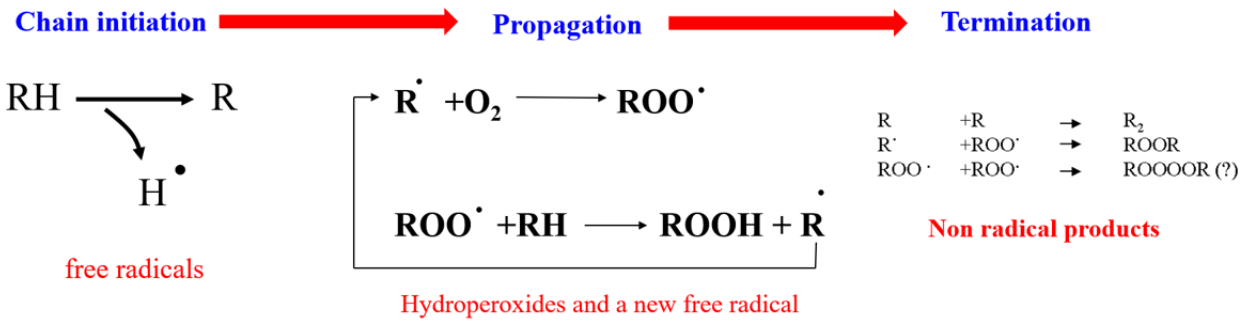



**Figure 2.** Three-stage free radical mechanism of lipid oxidation

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

### 3.3. Microbial Spoilage:

Composition of the microflora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish microflora includes bacterial species such as *Pseudomonas*, *Alcaligenes*, *Vibrio*, *Serratia* and *Micrococcus*. Microbial growth and metabolism are a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors. The compounds formed during spoilage through microbial metabolism are listed in Table 2.

**Table 2.** Bacterial spoilage compounds (Church, 1998)

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

## 4. METHODS OF ASSESSING FISH FRESHNESS AND QUALITY:

Freshness makes a major contribution to the overall quality of fish and fishery products and is greatly influence by both pre-harvest conditions and post-harvest handling practices. The methods for evaluation of fresh fish quality may be conveniently divided into two categories. They are: Sensory method of quality assessment and non-sensory or instrumental method of quality assessment. Non-sensory assessment of freshness and quality of fish includes Chemical, physical and microbiological methods. Non-sensory assessment is based mainly on measuring major physical or chemical alterations from the original condition of the fish.

### 4.1. METHODS FOR SENSORY EVALUATION OF FISH:

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

1. General appearance

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

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

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

### 4.1.1. Grading Schemes:

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

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

**The Torry Scoring System:**

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

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

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

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

**European Union Schemes:**

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

**Table 4.** Criteria of EU schemes

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

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

**Quality Index Method:**

The QIM was developed at the Tasmanian Food Research Unit (TFRU) of the Commonwealth Scientific and Industrial Research Organization (CSIRO). QIM schemes are developed for individual species. Each attribute is scored from 0 to 3 by novice or experienced assessors with low scores indicating the best quality (Table 5). The sum of all attributes is called demerit points, or QIM index points. This value increases linearly with storage time in ice of a given fish, therefore, the linear relationship between the quality index (QI) and storage time on ice, makes it easy to calculate the remaining shelf-life of fish.

**Table 5.** Quality Index Method (QIM) schemes

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

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

## 4.2. CHEMICAL METHODS OF QUALITY ASSESSMENT:

Chemical methods of quality assessment primarily focused on Measuring the concentration of indicator compounds within the sample, which are closely related to the level of a specific sensory attribute of the fish (primarily odor or flavor). These compounds are produced in fish muscle by autolytic enzymes, putrefactive microorganisms or by chemical reactions like lipid oxidation. During the spoilage these compounds accumulate gradually in the meat and hence their

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

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

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

**Total volatile basic nitrogen (TVB-N):**

Total volatile basic nitrogen (TVB-N) is a useful index of spoilage in different fresh and lightly preserved seafood. The TVBN value along with the TMA is the most common index of quality used method for deciding the state of freshness of fish. Some TVB is present in very fresh fish (<20 mg%). A range of 35 – 40 mg TVB-N / 100 g of fish muscle is usually considered as Limit of acceptability, beyond which the fish can be regarded as too spoiled for most uses. TVB-N values identify the latter stages of spoilage due to Lack of significant changes during the early stages of spoilage. Conway microdiffusion method and steam distillation method are commonly used method for estimation TVBN.

**Trimethylamine (TMA):**

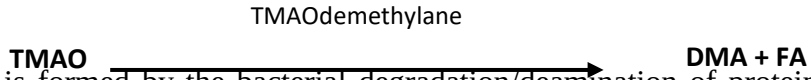
TMA is a microbial metabolite and it can only be used as an index of spoilage and not as an index of freshness. Trimethylamine oxide (TMAO) is found largely in most marine fish; in contrast, its presence is negligible or nil in freshwater fish. TMAO is reduced to TMA partly by intrinsic and mostly by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

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

**Dimethylamine (DMA):**

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

**Ammonia:**




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

**Nucleotide degradation**

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

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

***Hypoxanthine (Hx):***

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

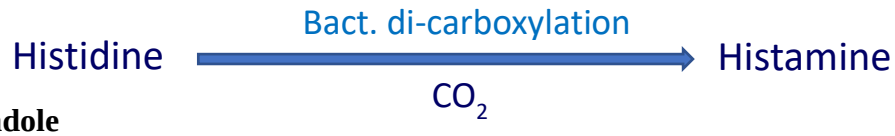
***K-value***

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

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

**Histamine:**

Many seafood spoilage bacteria produce one or more of the biogenic amines agmatine, cadaverine, histamine, putrescine, spermidine, spermine, and tyramine. Production of biogenic amines in seafood depends on concentrations of the free amino acid substrates and is, therefore, strongly species dependent. Most pelagic and scombroid fish contain a good amount of histidine in free state as well as with proteins. Histamine production occurs in fresh fish hew hours after death in tropical conditions when fish is not chilled properly. It causes food poisoning known as scombroid poisoning as it is linked with eating tuna, mackerel, and other species of the Scombroidea family. In fishes like mackerel, tuna, bonito, herring sardine etc. histamine formation is an indication of spoilage. Dark fleshed fish will have high histidine content and converted to histamine. Certain bacteria under favorable conditions are able to produce histamine from histidine by a decarboxylation process. USFDA enforces a Maximum level of 50 mg/100g of histamine in fish tissue. Histamine is determined eighter by HPLC method or by spectrophotometer method.




**Indole**

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

**Lipid oxidation**

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

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

Oxidative rancidity is one of the great concerned in fatty fish storage. At first, hydroperoxides are formed, which further degrade to form aldehydes and ketones with typical rancid flavour. Compounds derived from the oxidation of the highly unsaturated fatty acid moieties in fish lipids have been used to quantify the extent of oxidative rancidity. The major chemical indices of oxidative rancidity, peroxide value (PV) and thiobarbituric acid-reactive substances (TBA-RS)

***Peroxide value***

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

***TBARS***

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

***Free fatty acid value***

Lipid hydrolysis is the dominant reason for the generation of FFA when the fish lipid entered the second stage of lipid oxidation. It is a measure of hydrolytic rancidity. It is a Non esterified fatty acids in "free" form and more readily oxidized than esterified fats. FFA can act as pro-oxidants in oils by speed up the rate of hydroperoxide decomposition. Thus, high FFA content in the oil may cause further oxidation and lead to development of offensive taste and flavor in the Fish. Prior to the appearance of oxidative rancidity in lean fish, there is rise in lipid hydrolysis that leads to build up of FFA.

**Total volatile acids (TVA):**

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

**pH:**

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

**Microbiological methods:**

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

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

# HYGIENIC HANDLING REQUIREMENTS FOR FISH QUALITY ASSURANCE

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Seafood is recognized as superior protein source to terrestrial meat because of possessing easily digestible protein with all EAAs, highest amount of omega 3 PUFAs such as EPA and DHA, rich macro and micro mineral content and a low caloric density and red meat. However, seafood is highly perishable on account of its higher moisture content and soft texture and the maintenance of fish quality is difficult than in the case of meat products. Hygienic handling is one among the important extrinsic factors that influence the quality of fish. Hygienic handling practices are essentially required to maintain the quality and safety of fish during storage, transportation and marketing. Good handling always results in economical benefit to the farmers, processors and retailers because the consumers are willing to pay more for a premium quality product.

Microbial growth is the major reason for fish quality loss and GPHPs are necessary to control the growth of bacteria and contamination of pathogens. Implementing hygienic handling practices helps to reduce microbial growth and delay spoilage. Hygienic handling practices ensure the supply of safe and quality fish to consumers while meeting the standards of national and international food safety regulatory bodies. Post harvest handling refers to the activities carried out after the fish is captured from cage or pond or other production site unit till the product reaches the consumers. Whereas hygiene measures involve not only the activities that deal with operations but also those focusing on the facilities/equipments, operational measures which are aimed to ensure safety of the final product.

## Good harvesting practices

Stress encountered by the fish during harvesting operations has a direct impact on the post mortem quality. Fishes which undergone more stress during harvesting spoils faster compared to other fishes. On set of rigor mortis in such fishes are faster as the muscle has lower amounts of glycogen. Additionally harvesting stress induces oxidation of fatty acids in the fish muscle through the production of reactive oxygen metabolites. The aim of implementing good harvest practices is to

catch the fish out of water in good condition. Following are the key points to be remembered while harvesting.

- Harvesting should be done as quickly as possible with minimum stress to the animals.
- Avoid harvesting from waters prone to industrial pollution and oceanic waters having oil spills.
- Harvesting should be done at a time when temperature is the lowest.
- For harvesting farmed fishes, cast nets, bag nets and traps should be carefully selected to ensure minimum damage during harvesting.
- Gears and accessories such as nets, bags, pumps, baskets, tubs, bins, and boxes should be designed to ensure minimum physical damage and contamination to the fish during harvesting.
- Chutes and conveyors should be designed to prevent physical damage caused by long drops or crushing
- Fishing gears should be disinfected both before and after use to avoid contamination.
- In case of live fish marketing, fish should be starved before harvesting to reduce the risk of mortality during transportation.
- Prevent the entry of pet animals to the aquaculture farm site at the time of harvesting to avoid fecal contamination.
- The personnel involved in harvesting should be healthy and free from infectious diseases.
- Adequate lighting should be provided to all working areas during night harvesting.
- Obtaining Pre-harvest test (PHT) certificate of ready to harvest shrimp for the absence of antibiotics (chloramphenicol and nitrofuran metabolites-AOZ, AMOZ, SEM and AHD) ensures easy sale of shrimp to the processing units.

**Onboard handling practices**

Fish and fish products intended for human consumption should be handled properly to prevent contamination and spoilage till it reaches the end user. Poor onboard handling practices damages

the fish and speed up the spoilage process resulting in post-harvest losses. The important points are

- The fishing vessel and equipment should be thoroughly cleaned using clean water before and after every fishing trip.
- Fishing vessel must be kept free of pests using pest control devices.
- Sea water from fishing harbor/landing centre should never be used for cleaning. Only tap water from the public water supply, clean well / borehole water that has been treated with chlorine or clean seawater should be used to clean boats and equipment.
- All fish contact surfaces should be made of non-toxic, smooth materials to minimize the build-up of slime, blood, scales etc. from the harvested fish to reduce the risk of microbial contamination.
- Handling areas should not have sharp corners and projections to avoid physical damage to the fish.
- Fish should be washed well with potable water/ clean sea water to remove dirt and other foreign matter if any, immediately after harvesting.
- In aquaculture site, the harvested fish should not be dropped on to muddy floor or hardy surfaces to prevent contamination and physical damages, respectively.
- Fish should be kept away from objectionable substances such as grease, fuel oil, drainage, bilge water, smoke, and other solid or semi-solid to prevent contamination.
- Bruised, damaged and decomposed fish shall be separated from the catch during sorting.
- Bleeding of fish if any, should be carried out as early as possible.
- Mishandling of the fish such as throwing, standing on fish, exposing to sunlight etc. needs to be strictly avoided.
- Fish should not be exposed to sunlight for a longer duration as it causes dehydration and accelerate spoilage.
- The condition of the equipment and utensils should be such that it minimizes the build-up of residues and prevents them becoming a source of contamination.

**Chilling and Storage**

The ambient temperature of tropical countries favours the growth of mesophilic bacteria. A 5°C rise in temperature doubles the rate of spoilage. Hence, the temperature of fish should be immediately brought down to near 0°C after harvesting to delay the microbial growth and spoilage. Time and temperature control during post-harvest stage are the most effective tools to ensure food safety.

- Store the harvested fish in ice or chilled/refrigerated seawater as early as possible to bring down the core temperature as close to 0°C avoid bacterial spoilage.
- Fish and ice should be tightly packed in the container in shallow layers (1:1 fish to ice ratio) to avoid free space which otherwise cause faster melting of ice.
- In refrigerated or chilled sea water systems, care should be taken to control overloading of fish to prevent physical damage.
- Boxing/shelving storage areas of fishing vessel should apply minimum pressure on the fish.
- Clean and chemical-free ice made from potable water should be used and it should be protected from contamination.
- Fish harvested at different times should be stored separately as they are in different stages of spoilage.
- Use of crushed ice with sharp edges must be avoided as it causes physical damage to the fish.
- The containers used for storage should be designed to provide adequate drainage and should ensure proper cleaning and disinfection to avoid contamination.
- The boxes used for storage should not be over filled or stacked too deep as it exerts pressure on the fish.
- Fish room/hold must be strong, corrosion resistant, insulated, and easy to clean with smooth surfaces and allows adequate drainage for melt water.

**Handling at landing centre**

A great deal of fish handling occurs at landing centers or harbors. It should be ensured that the fish leaving the fish landing centers is of an assured quality and safe for human consumption. Proper handling practices assure the quality of fish and reduce post harvest loss at landing centers.

- The ice should never be dragged on the floor and must be stored in clean containers.
- Never use seawater from landing centre for cleaning the fish.
- Do not throw the fish on hard surfaces to prevent physical damage and contamination.
- Sorting the catch on beaches should be avoided.
- All the containers/contact surfaces used for unloading and weighing shall be cleaned & disinfected immediately.
- Entry of flies, cats, dogs, rodents etc. in the fish handling premises may be prevented.
- Adequate supply of clean, potable water should be ensured at the landing centers.
- Fish wastes and offal shall be separate boxes with tight lids and shall be discarded properly.

**Handling at retail markets**

Various handling activities including fish landing, washing, dressing, packing, distribution, distribution and selling takes places in retail fish markets which invites many risk factors creating additional sources of bacteria and contamination. Therefore, implementation of hygienic measures is recommended to prevent contamination of fish at retail markets.

- Location of retail fish market should be away from vegetable market, meat or other food markets.
- Facilities for potable water, electricity and proper hygienic sewage disposal should be provided.
- Facilities like toilet and arrangement for washing of hands should be provided near the market premises.

- Selling / Auction Platform/ tables should be elevated with smooth vitrified tiles with side protection and drain pipe.
- Cutting and filleting of fish to be separated from selling area to prevent cross contamination.
- Separate area for crate and utensil washing must be provided.
- Waste materials should be properly segregated, iced and stored in tight containers.
- Drain pipe from display tables and cutting platforms should be directly connected to main drain to avoid splashing of water
- All fish contact surfaces should be smooth, water resistant and non-corrosive.
- Effective cleaning procedure and regular cleaning schedule should be maintained.
- All the utensils should be washed within 2 hrs of use apart from daily washing at the start and end of sale.
- Utensils must be stored upside down so that they can adequately drain.
- Protection from adulteration with lubricants, fuel, pesticides, cleaning compounds, sanitizing agents, condensate, and other chemical, physical, and biological contaminants.

**Hygienic fish handling in processing units**

Processing units aims towards value addition of the fish thus improving the market value of the products. Hygiene and sanitation are one among the pre-requisite programs for implementing HACCP in seafood processing units. The following important measures need to be takes care of in processing units for the supply of safe products.

- Design and layout comprising sufficient working space under adequate hygienic conditions, area for machinery, equipment & storage, separation of operations preventing cross-contamination, adequate natural or artificial lighting, ventilation and protection against pests.
- All food contact surfaces shall be smooth, durable, non-absorbent type, easy to maintain and clean and non-toxic.

- Availability of uninterrupted supply of potable water throughout for all processing operations.
- Availability of suitable facilities for temperature, humidity and other controls.
- All pre-processing and processing activities should be scheduled under HACCP system with proper documentation.
- Regular monitoring of processing unit for plant sanitation with an in-house laboratory and an in-process product quality check.
- Effective maintenance and sanitation systems including cleaning and sanitation procedures, pest control systems, waste management and monitoring effectiveness.
- All fish handlers should follow the recommended hygienic handling practices such as periodic medical examinations, regular cleaning and disinfection procedures prior and post to processing activities.

**Personnel hygiene**

There is a possibility that the people who handle the fish can introduce hazards to to the products. Hygiene and cleanliness of workers handling fish and fish product at each stage from harvesting is very crucial in determining the safety of fish. Hence, the fishers should adopt few simple hygienic actions to prevent contamination.

- No person who is suffering from, or who is a carrier of, any communicable disease or has an infected wound or open lesion should be engaged in fish handling or transportation.
- A high degree of personal cleanliness should be maintained by the personnel involved in handling and should take all necessary precautions to prevent contamination.
- Farm personnel should invariably take bath before getting into the pond for harvesting the fish
- Adequate and appropriate protective clothing, face mask, head coverings and footwear should be worn during fish handling.
- Fish handling personnel should strictly avoid the objectionable practices such as smoking, spitting, chewing, sneezing or coughing to prevent contamination.

- Fish handlers should sanitize their hands regularly in Hand Dips to prevent contamination.
- The workers must wash their hands 1) Before they start handling fish or go back to handling fish after other work 2) Immediately after using the toilet 3) Immediately after smoking, coughing, sneezing, using a handkerchief or disposable tissue, eating, drinking or using tobacco or similar substances and 4) After touching their hair, scalp or a body opening.

**Conclusion**

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. Once the fish is harvested, handling practices have remarkable influence on the quality and safety the product. Proper fish handling practices should be strictly followed at all stages after harvesting to meet the consumer's expectations. Fish is food, so treat it as food.

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# THERMAL PROCESSING OF FISH AND 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.

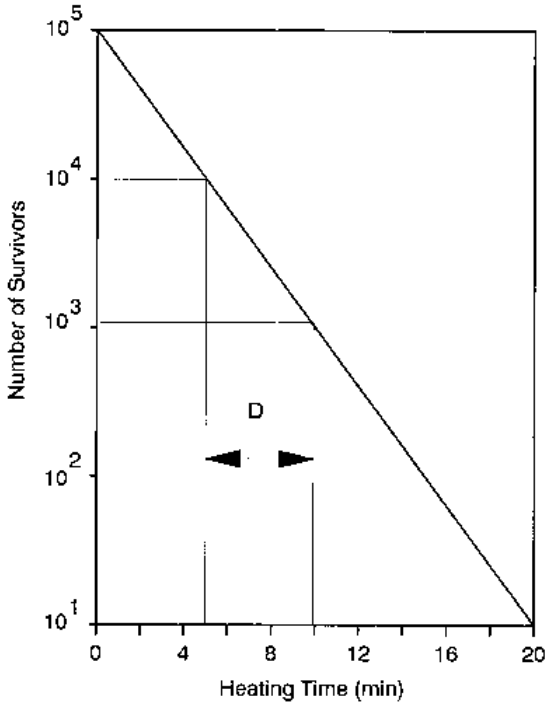


Fig 1. Survivor curve

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.*

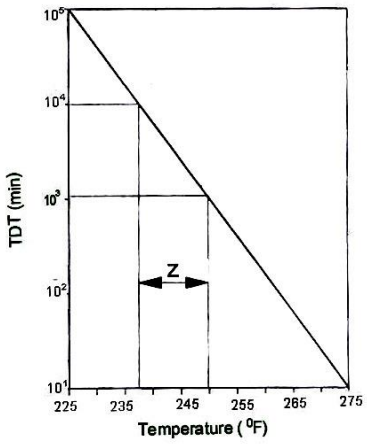


Fig. 2 TDT Curve

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.,

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

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

***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 $log10^{-12}$ 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

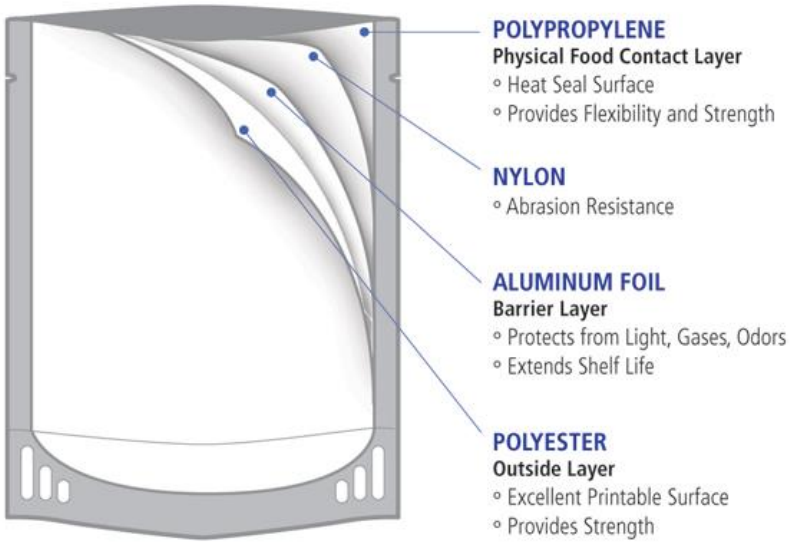


Fig.: Composition of Retortable pouch

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

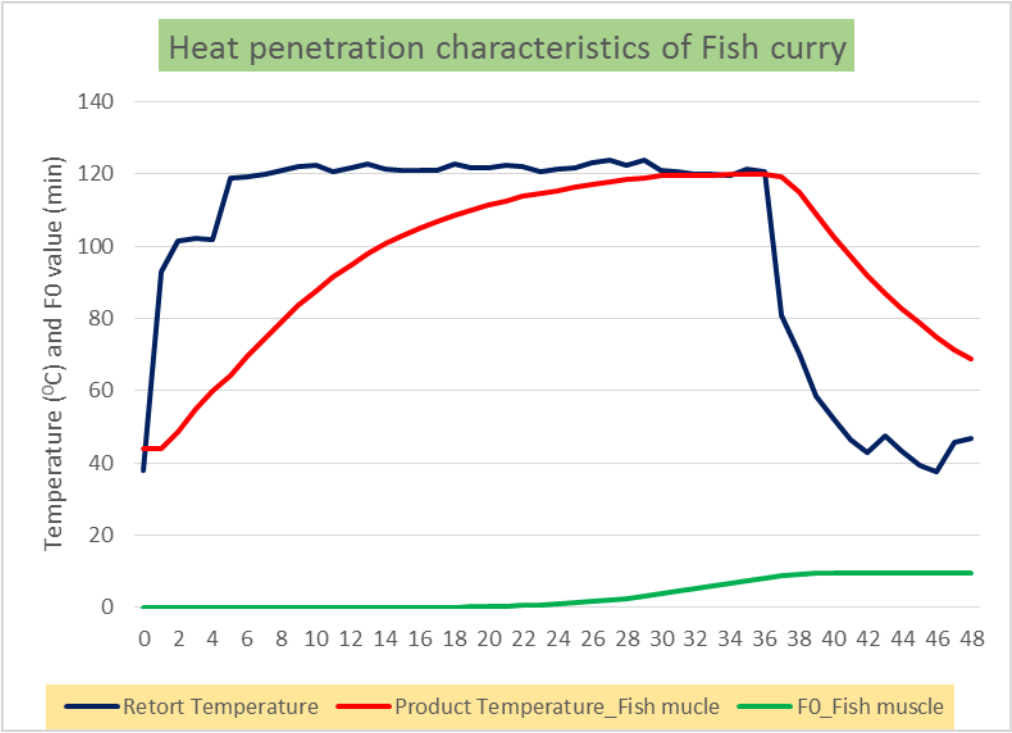


Fig.: Typical heat penetration curve of fish curry in retortable pouches

# NON THERMAL PROCESSING OF FISH

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Changes in consumer's desires in the recent past, have led to the requirement for more convenient foods having supreme qualities and freshness, minimally processed and packaged, easy to consume and nutritionally healthier. Hence, the focus of food scientists and engineers have been directed towards alternative technologies or minimal processing and preservation technologies that are environment friendly, low in cost and able to preserve fresh quality attributes of the food. Many novel non thermal technologies like high pressure processing, pulsed light, pulsed electric field, ultrasound, irradiation etc. find application in preservation of food and is in the line of commercialization.

Thermal pasteurization and thermal sterilization for the inactivation of microorganism and reduction of enzyme activity, has resulted in making safe product with extended shelf life than its raw counterparts. But despite its substantial benefits, thermal treatments end with over processed food having significant changes that can alter its sensorial attributes like flavor, colour, texture and nutrient content (Barbosa-Canovas and Bermudez-Aguirre, 2011). The introduction of non-thermal technologies in food processing opens a new era of minimally processed food with high nutritive value, retains the fresh attributes of the product without compromising the safety and quality. Among all non-thermal technologies, HPP offers promising possibilities for the processing and preservation especially in meat, poultry and seafood.

## 1. High pressure Processing (HPP)

Application of very high pressures (100-900 MPa) for the preservation of food substance with or without the addition of heat, to achieve microbial inactivation or to alter the food attributes in order to achieve consumer-desired qualities. This technology is also known as high hydrostatic pressure processing or ultra-high pressure processing. HPP retains food quality, maintains natural freshness, and extends microbiological shelf life of the product. This technology is now recognized by the USFDA for RTE foods. The processing can be conducted at ambient or refrigerated temperature eliminating thermal effects and cooked off flavors and thus highly beneficial for heat sensitive products.

The first line of HPP was demonstrated in 1899 by Bert H Hite, as a possible food preservation process at West Virginia Agricultural Experimental Station (Hoover et al., 1989; Knorr, 1999). In 1992, commercialized high pressure processed products (high acid products including apple, strawberry, and pineapple jams) were marketed in Japan and since after 1992 High pressure processed foods are available in the markets of Japan (Suzuki, 2002) and in Europe and in the United States since 1996 (Knorr, 1999). Other, commercially available high pressure processed products in Australia, Europe and the U.S. include juices, tomato salsa, smoothies, fruit & vegetable purees, and ready to eat meals.

Later there was a growing interest in the area of seafood safety that led seafood processors to explore high pressure technology in product development and extension of shelf life. This technology was utilized in the area of extending shelf life of product mainly be destroying the spoilage and pathogenic microorganisms (Toepfl et. al., 2006) and also used as an alternative thermal treatment to packaged food materials. This non thermal preservation technique could also showed many benefits like complete separation of meat from shells of clams, crabs, lobsters, and oysters providing high yield of product without any mechanical damage. HPP could open up the new eras of product development and product improvements in all segments of meat and fish industry. Another advent is pressure assisted freezing and thawing, which finds its unique application in food industry especially in product development and product quality improvement (Urrutia et.al. 2007). Since HPP has minimal detrimental impact on thermally labile bioactive compounds the technology is becoming a topic of major interest for cosmetic, nutraceutical and pharmaceutical industry.

During the time HPP has turned to be an explored technologies and today it is a commercial reality. HPP products find its place in the world food market with high quality and high value addition. Today the use of high pressure (300-700 MPa) for commercial application comes in vessels ranging 35-420L capacity which had given an annual production of >150,000 tons (Wan et. al., 2009). Regulatory agencies like FDA has approved HPP as substitute to pasteurization but in February, 2009, a combination of pressure with heat called as PATS (Pressure assisted thermal sterilization) found to be effective instead of conventional sterilization (NCFST, 2009).

The basic principles that govern the high pressure effect on the behaviour of foods are (i) Pascal's Isostatic principle and (ii) Le Chatelier's principle.

According to Pascal's isostatic principle high pressure acts uniformly and instantly throughout the sample, independently of the size and shape of the food product (Smelt,1998). A uniform pressure will be applied to the product from all direction, thereby the product will not get damage and return to its original shape on the release of pressure. The fundamental principle of physico-chemical changes occurring during HPP follow the Le Chatelier's principle, which states that 'when a system at equilibrium is disturbed, the system then respond in a way that tends to minimizes the disturbance'. So at high pressure any reactions like change in conformation, or transition of phase that is accompanied by a volume decrease will be favored, while inhibit those reactions involving an increase in volume (Lopez-Malo et. al., 2000).

**Mechanism of Pressure Treatment**

Each processing cycle in HPP consists of an initial pressurization period where the pressure builds up and the processing operation can be done either with or without the application of heat. The packaged product should be in flexible or semi flexible pouch, which can sustain very high pressures. The product is then submerged into a pressure transmitting fluid, where water is commonly used. Other liquids like ethanol or glycol, castor oil, silicone oil etc. can also use in various combinations with water or use separately. This fluid is able to protect the inner vessel from being corroded and fluid is selected based on the manufacture's specification. During the pressure processing adiabatic heating occurs and the product gets heated up. The temperature increase due to adiabatic heating depends on the type of fluid, pressurization rate, temperature and pressure.

Once the process starts, the hydraulic fluid is pressurized with a pump and the generated pressure is transmitted into the packaged food uniformly from all sides. Since this processing is independent of size and geometry of foods, also acts instantaneously there by the total processing time can be reduced. The process is suitably applied for liquid foods and to liquid foods, having a certain amount of moisture content. The transmitted pressure is uniform and simultaneously applied from all directions so that food retained its structure even at high pressures. Once the pressure is build up to the desired level the product is held at this pressure for a few minutes and then decompression or pressure release takes place. Once there is a fall in pressure the product temperature falls below that of the initial product temperature.

**Major Advantages of the Technology**

1. HPP does not involve in breaking covalent bonds which prevents the development of unpleasant flavours to the product and maintains the natural freshness and quality.

2. High pressure is able to modify the palatability and functional properties by inducing denaturation and muscle protein gelation.
3. Process can be carried out at ambient temperatures that helps in reducing the thermal energy used during conventional processing.
4. High pressure processing is isostatic in nature, equally applied to all particles of food, with no particle escapes.
5. Since high pressure is not time-mass dependent, pressure acts instantaneously thereby reducing the processing time.
6. This non thermal technology is independent of size and geometry of the food.
7. The process is eco-friendly, with no waste and requires only electric energy.

**Application in marine Products**

- Used to extend shelf life of products
- Develop new gel based products with desired sensory attributes and mouth-feel
- Used in shell fish processing for 100% removal of meat from shells
- Reduces the microbial risks during raw sea food consumption
- Inactivates vegetative micro-organism and reduces the bacterial contamination and the pathogens
- Modify functional properties of the food material
- HPP in combination with salting and smoking helps to extend the shelf life
- Pressure assisted thermal processing used for development of shelf stable ready to eat products
- Pressure assisted freezing and thawing helps in retaining the microstructure and reduces drip loss in fish products

**High Pressure Processing Facility at ICAR-CIFT**

*Fig.: A Research model of 02 litre capacity High Pressure machine at Central Institute of Fisheries Technology, Cochin (from M/s Stansted Fluid Power Ltd, United Kingdom)*

Seafood is a highly perishable commodity and technologies like high pressure processing are essential to increase the market value of some high value fishes. High pressure processing has now experiencing a growing demand in the global market. A lot of researches have been carried out on HPP from the past decade. Further studies on the effects of this technology on the textural and functional modification, biochemical characteristics and microbial kinetics of fish and shellfishes are necessary. The effectiveness of high pressure on microbial and enzyme inactivation, while maintaining optimal product quality is a crucial factor for the commercialization of this technology. HP processing offers many advantages over conventional processing methods known to seafood. This is exemplified by the success of HP-processed oysters in USA by Motivatit Seafood, Goose Point Oysters and Joey Oysters. However, as HP processing becomes more widely available, initial capital costs may be reduced, making technology accessible to more producers. In addition, the commercialization of the technology for other foods may provide encouragement for seafood processors, by allaying apprehension regarding the use of this novel technology and demonstrating consumer acceptance of HP-processed products.

## 2. Pulse electric field

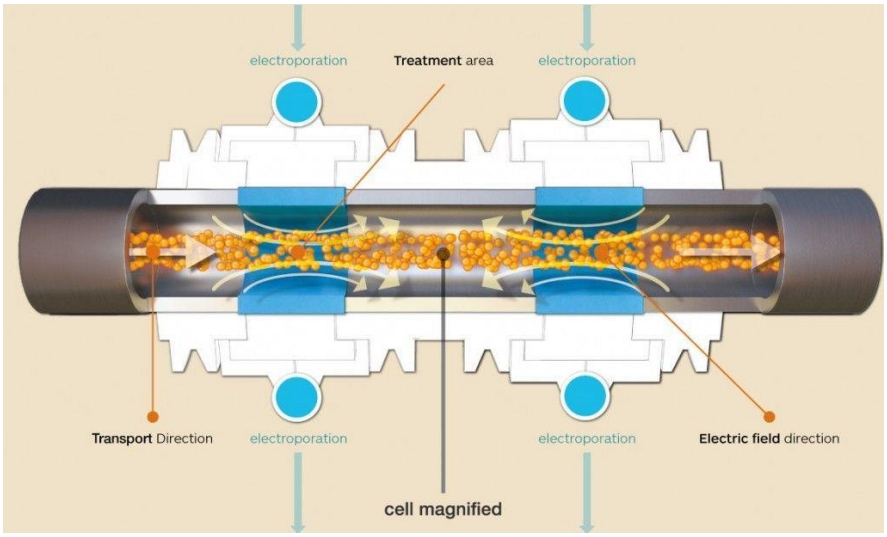
Pulsed electric field processing is a non-thermal food preservation technique used mainly for inactivation of microbes. PEF technology is the application of short pulses of high electric

fields with duration of micro- to milliseconds and intensity in the order of 10-80 kV/cm in order to preserve the food. The processing time is calculated by multiplying the number of pulses times with effective pulse duration. The process is based on pulsed electrical currents delivered to a product placed between a set of electrodes and the distance between electrodes is termed as the treatment gap of the PEF chamber. The applied high voltage results in an electric field that causes microbial inactivation.

The pulsed electric field induces poration of cell membranes and thereby the cell membranes of microorganisms, plant or animal tissue are permeable. This process of electroporation is suitable for use in a broad range of food processes and bioprocesses using low levels of energy. PEF technology has many advantageous in comparison to heat treatments, because it kills microorganisms and at the same time maintains the original color, flavor, texture, and nutritional value of the unprocessed food. It is suitable for preserving liquid and semi-liquid foods removing micro-organisms and producing functional constituents. Most PEF studies have focused on PEF treatments effects on the microbial inactivation in milk, milk products, egg products, juice and other liquid foods.

**Working**

PEF technology is based on a pulsing power delivered to the product placed between a set of electrodes confining the treatment gap of the PEF chamber. The equipment consists of a high voltage pulse generator and a treatment chamber with a suitable fluid handling system and necessary monitoring and controlling devices. Food product is placed in the treatment chamber, either in a static or continuous design, where two electrodes are connected together with a nonconductive material to avoid electrical flow from one to the other. Generated high voltage electrical pulses are applied to the electrodes, which then conduct the high intensity electrical pulse to the product placed between the two electrodes. The food product experiences a force per unit charge, the so-called electric field, which is responsible for the irreversible cell membrane breakdown in microorganisms. This leads to dielectric breakdown of the microbial cell membranes and to interaction with the charged molecules of food. Hence, PEF technology has been suggested for the pasteurization of foods such as juices, soups, and other liquid based products.




*(Source: i³ foods)*

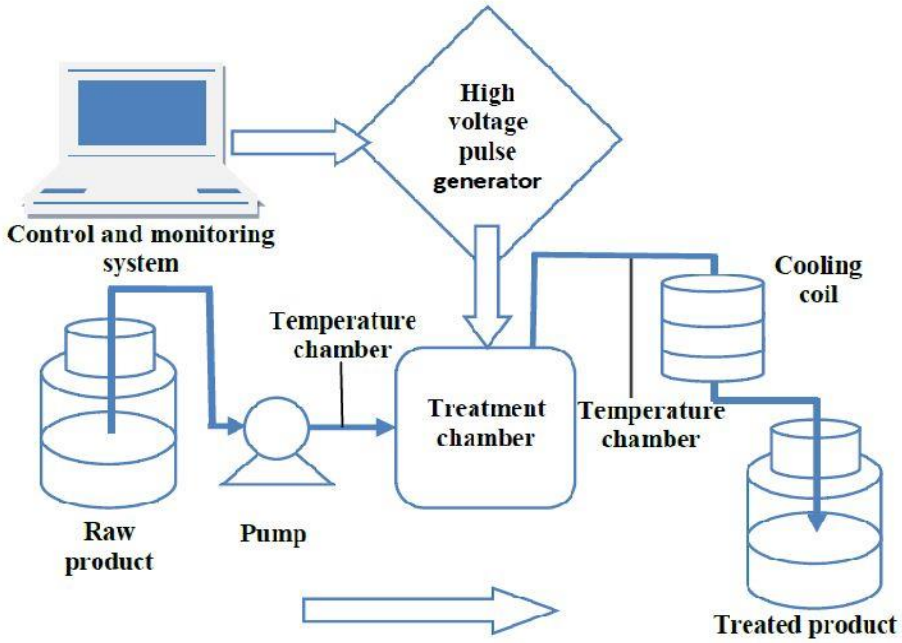



**Fig.: Pulsed Electric Field Preservation**

Pulsed electric field can be applied in fishes fresh and frozen fish dried, brined or marinated fish. Mass transport processes, such as moisture transport and removal, are improved by the electroporation of fish tissue, resulting in enhanced drying, brining and marinating of fish. The required field strength for cell disintegration of fish is 1,0 – 3,0 kV/cm and the energy delivery is 3 – 10 kJ/kg. The applied pulsed electric field leads to cell disintegration in tissue, enhancing product quality and production processes. It also helps in inactivation of parasites

such as nematodes. PEF processing enhances mass transport, processes during extraction, pressing, drying, brining and marinating processes. PEF technology speeds up drying of food products, minimizing processing times and energy consumption. The process can be applied to fruits, vegetables, potatoes and meat. Enhancement of extraction processes is also an advantage of electroporation. Extraction and pressing yields are increased, for example for fruit juice, vegetable oil and algae oil and protein. PEF technology speeds up freezing of food products, allowing a reduction of processing times and energy consumption. The cell disintegration increases the freezing rates. Cellular water flows easily out of the cell and ice nucleation outside the cell starts. As smaller ice molecules are formed, product quality of frozen food is improved. (www. pulsemaster).

### 3. Pulse Light technology

Pulse light technology is one such explored Non thermal technology in the food industry, especially for decontamination of food surfaces and food packages. This technique works by applying high-voltage, high-current short electrical pulse to the inert gas in the lamp, which results in strong collision between electrons and gas molecules cause excitation of the latter, which then emit an intense, very short light pulse to decontaminate and sterilize foods (Palmieri & Cacace, 2005). Usually short pulses of light one to twenty flashes per second is used in food industry. The term light is generally used to mean radiations having wavelength ranging from 180 to 1100 nm, which includes ultraviolet rays (UV 180–400 nm, roughly subdivided into UV-A, 315–400 nm; UV-B, 280–315 nm; UV-C, 180–280 nm); visible light (400–700 nm) and infrared rays (IR 700–1100 nm) (Palmieri and Cacace, 2005). This technology can be used for the rapid inactivation of microorganisms on food surfaces, equipments and food packaging materials (Dunn et al., 1995). The effect on microorganisms is mostly due to the photochemical action of the ultra violet part of the light spectrum that causes thymine dimerization in the DNA chain preventing replication and ultimately leading to cell death (Gomez-Lopez et al., 2007).

The principle involved in generating high intensity light is that a gradual increase of low to moderate power energy can be released in highly concentrated bursts of more powerful energy. The key component of a Pulse Light unit is a flash lamp filled with an inert gas. A high-voltage, high-current electrical pulse is applied to the inert gas in the lamp, and the strong collision between electrons and gas molecules cause excitation of the latter, which then emit an intense, very short light pulse. It is generally accepted that UV plays a critical role in microbial inactivation. So pulsed light is a modified and claimed improved version of delivering UV-C to bodies. The classical UV-C treatment works in a continuous mode, called continuous-wave

(CW) UV light. Inactivation of microorganisms with CW-UV systems is achieved by using low-pressure mercury lamps designed to produce energy at 254 nm (monochromatic light), called germicidal light (Bintsis et al., 2000). More recently, medium-pressure UV lamps have been used because of their much higher germicidal UV power per unit length. Medium-pressure UV lamps emit a polychromatic output, including germicidal wavelengths from 200 to 300 nm (Bolton & Linden, 2003). Pulse Light treatment of foods has been approved by the FDA (1996) under the code 21CFR179.41. The treatment is most effective on smooth, nonreflecting surfaces or in liquids that are free of suspended particulates. In surface treatments, rough surfaces hinder inactivation due to cell hiding.

**Generation of Pulsed Light**

Light can be emitted from different sources by different mechanisms, due to the spontaneous transition of some atoms from an excited state to a condition of lower energy. Light can be delivered either continuously or in the form of pulses. (Palmieri and Cacace, 2005). Pulsed light works with Xenon lamps that can produce several flashes per second. During the pulse treatment the spectrum produced is 20000 times brighter than sunlight at the surface of the earth (Dunn et al., 1995). Electromagnetic energy is accumulated in a capacitor during fractions of a second and then released in the form of light within a short time (nanoseconds to milliseconds), resulting in an amplification of power with a minimum of additional energy consumption. As the current passes through the gas chamber of the lamp unit, a short, intense burst of light is emitted. The light produced by the lamp includes broad-spectrum wavelengths from UV to near infrared. The wavelength distribution ranges from 100 to 1,100 nm.

**Merits and Demerits**

**Merits**

The inactivation of microbes by Pulse Light is very fast process and cause rapid disinfection in a very short period. It is a green technology as the consumption of energy is very less during its application. Pulse Light has been proven as a safe technology for living being and their environment without producing harmful residuals, chemicals and toxic by-products in the treated foods. It does not affect the nutritional and sensory quality of the products. The concerns of ionized radicals and radioactive by-products in foods by consumers are removed in Pulse Light due to its nonionizing spectrum (Dunn et al.1995).

**Demerits**

Pulse Light application in meat industry has some constraints as the low penetration power and chances of lipid oxidation (Fine & Gervais, 2004). To get the desired outcome, the packaging materials showing high penetration of light should be used while treating the packed food by this method. The limited control of food heating still remains the main concern in Pulse Light technology. Sample heating is perhaps the most important limiting factor of this technology for practical applications (Gomez-Lopez et al., 2007).

**Pulsed Light Equipment at CIFT**


## 4. Ultrasound processing

The application of ultrasound in food processing has been stared as another area in non-thermal approaches, which exploits the preservative effect of the high intensity sound waves. The preservative effect is by the inactivation of microbes and spoilage enzyme by mechanical actions. Mechanism is that when propagates through biological structures, Ultrasonic cavitation produces shear forces, which causes mechanical cell breakage and allows material transfer from cell into solvents. Cavitation causes particle size reduction thereby increases the surface area in contact when extracting a compounds.

The technology finds its application in the field of extraction of proteins, lipids and their functional modifications, emulsification, viscosity improvement, homogenization and improvement of dispersion stability in liquid foods (Mohd. Adzahan and Benchamaporn, 2007). So this technology is utilized in the field of processing, preservation and extraction, which makes use of physical and chemical phenomena that are fundamentally different from conventional extraction, processing or preservation techniques.

In food industry, the application of ultrasound can be divided based on range of frequency:

- *Low power ultrasound:* Uses a small power level that the waves cause no physical and chemical alteration in the properties of the material through which it passes. This property is being utilized for non-invasive analysis and monitoring of various food materials during processing and storage, to ensure quality and safety.
- *High power ultrasound*: Uses high energy [high power, high intensity] ultrasound of 20 and 500 kHz. It causes disruptive and enforce effect on the physical, mechanical, or biochemical properties of foods. These effects are promising in food processing, preservation and safety.

## 5. IRRADIATION

Irradiation is the process of applying low levels of radiation to any food material to sterilize or extend its shelf life. It is a physical method that involves exposing the prepackaged or bulk foodstuffs to gamma rays, x-rays, or electrons. Foods is generally irradiated with gamma radiation from a radioisotope source, or with electrons or x-rays generated using an electron accelerator (Barbosa-Canovas et al., 1998). These rays have high penetration power and thus can treat foods for the purpose of preservation and quality improvement. During exposure of food the amount of ionizing radiation absorbed is termed 'radiation absorbed dose' (rad) and is measured in units of rads or Grays. A strictly regulated process of dosimetry is used to measure the exact dose of radiation absorbed by the food. One Gray is equal to one joule of energy absorption per kilogram of a material. Irradiation has been approved for the microbial disinfestations of various food products in the US (USFDA, 1998). A number of countries have marketed irradiated products worldwide. Irradiation has the potential to enhance food safety for fresh foods that will be consumed raw and for raw foods that require further processing. Food irradiation mainly is done by the radioactive element cobalt-60 as the source of high energy gamma rays. Gamma rays are electromagnetic waves or photons emitted from the nucleus of an atom. These gamma rays have energy to dislodge electrons from food molecules, and to convert them into ions which are electrically charged. However, the rays do not have enough energy to dislodge the neutrons in the nuclei of these molecules and hence they are not capable of inducing radioactivity in the treated food. The radiation dose varies depending on the thickness moisture, and characteristics of the foods. External factors, such as

temperature, the presence or absence of oxygen, and subsequent storage conditions, also influence the effectiveness of radiation (Doyle, 1990).


**Fig.:** ***Applications of Irradiation***

In general, irradiation of food does not significantly affect the protein, lipid, and carbohydrate quality. Minerals are stable to food irradiation. The overall chemical changes in food due to irradiation are relatively minor and hence there is little change in the nutritional quality. Irradiation of moist food under frozen condition and in the absence of oxygen significantly decreases the overall chemical yields by about 80%; So the cumulative effects of irradiating to a dose of 50 kGy at −30°C is essentially equivalent to a dose of 10 kGy at room or chilled temperature. A dose of 1-10 kGy can control food-borne parasites responsible for diseases such as trichinosis. A minimum dose of 0.15 kGy can prevent development of insect infestation in dried fish. Irradiation is considered as a phytosanitary measure often obligatory if certain agricultural commodities are to be exported. The unique feature of radiation decontamination is that it can be performed in packaged foods even when the food is in a frozen state. Table I gives details of irradiation processes for seafood.

***Table 1: Radiation processes of seafoods (Source: Venugopal, Protech 2013-Pg28)***

| Treatment and storage temperature | Radiation process | Benefits |
|---|---|---|
| -10° to -20°C | Radicidation<br>(Radiation hygienization) | Improvement of hygienic quality of frozen, materials |

| | | |
|---|---|---|
| Packaged, frozen, ready-to-export fish can be treated before shipment. Frozen storage | Dose required: 4-6 kGy<br>Elimination of non-spore forming pathogens such as *Salmonella, Vibrio, Listeria* etc. | for export such as frozen shrimp, cuttlefish, squid, finfish, fillets, and IQF items. |
| 15° to 30°C<br>Ambient storage | Radiation disinfestation<br>Dose required < 1 kGy<br>Elimination of eggs and larvae of insects. | Dry products free from spoilage due to insects, from dried fishery products including fish meal and feed for aquaculture.<br>Inactivation of *Salmonella* spp. and other pathogens |
| -1°to +3°C<br>(Post-irradiation storage: under ice). | Radurization (Radiation pasteurization for shelf life extension)<br>Dose: 1-3 kGy<br>Reduction of initial microbial content by 1 to 2 log cycles. Specific reduction of spoilage causing organisms. | Extends chilled shelf life of fresh marine and freshwater fishery products two to three times.<br>Additional benefit includes reduction of non-spore forming pathogens |

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# VALUE ADDED FISHERY PRODUCTS- AN OVERVIEW

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**What is Value addition?**

Any additional activity that is one way or other changes the nature of product thus adding to its value at the time of sale. Products processed as "Ready to eat', 'Ready to cook', 'Ready to fry', Heat & Serve' and 'retail raw branded products and other fishery pharmaceutical and cosmetic products of high unit value in export market are considered as value added products.

**Significance of value addition**

- ✓ To provide variety of products
- ✓ For improved processing utilization
- ✓ Most practical way to increase profitability in fish processing
- ✓ Allowing income creation during off-seasons
- ✓ To keep in-phase with consumer needs
- ✓ Make use of excess produce.
- ✓ It has become a market requirement

**Major forms of adding Value to Seafoods.**

There are three major forms of adding value to Seafoods.

I. **<u>Improving market forms</u>**

- ✓ Fillets
- ✓ Steaks
- ✓ Customization

II. **<u>Processing convenience foods</u>**

- ✓ Peeled, in brine either chilled or heat - treated
- ✓ Battered and breaded products
- ✓ Extruded cooked products
- ✓ Breakfast/lunch/dinner packs

III. **Functional foods**

- ✓ Fortified with calcium, Beta carotene,vitamins, etc.

**Major value added products prepared from fish, shell fish and cephalopods**

**I. Value added Fish products**

Fish pickles, Fish curry Frozen Fish Fillets, Fish Loins/ Fish Steaks, Breaded fish fingers, Breaded fish fillets, Tray pack fish, Pre-cooked Loins, Fish powder, Fish soup, Fish cutlet, Fish ball, Fish soup powder, Fish wafer, Ready to serve fish curry in flexible pouches

**II. Shrimp products**

IQF Marinated Shrimp, Skewered Shrimp, Stretched Shrimp (Nobashi), AFD Shrimp, AFD Powder, Blanched/ Cooked Shrimp, IQF Head-On/ Headless /Butterfly cooked/ blanched shirimp, IQF Peeled Tail-on cooked shrimp, Cooked salad shrimp, Cooked and peeled shrimp, Sushi, Shrimp Pickle, IQF Tray pack shrimp and Shrimp Curry.

**III. Cephalopods Products**

Double Skinned Cuttlefish IQF Sashmi Grade, IQF Cooked/ Blanched squid Cuttlefish fillets Sashimi grade, Cuttlefish strips blanched, Squid strips blanched, Cuttlefish Pine Cut/ Diamond Cut, Stuffed Squid IQF Tray Pack, Squid Tube Tray Pack, Squid Ring Blanched IQF, IQF Tray Pack Squid, Cuttlefish Skewers, Vaccum Skin Packed Squid & Cuttle Fish Products in trays, Marinated Squid, Battered and breaded Cephalopod products, AFD Cuttlefish/Squid.

**I. Battered and breaded fish products**

Consumers are looking for better alternative for conventional fresh food that offers time-saving preparation. Hence there exists an increased global demand for readyto-heat frozen foods, especially breaded and battered products with high standards of quality. Battering and breading enhances the consumer satisfaction by improving the nutritional value, organoleptic characteristics and appearance of the products. The most important advantage of coating is value addition as it increases the bulk of the product. Also this paves way for better utilisation of low cost or underutilised fishes. Coating is referred as the batter and/or breading adhering to a food product. Each ingredient in coating offers unique role in development of functionality and characteristics of the product. Polysaccharides, proteins, fat, seasonings and water are the commonly used ingredients. The method of product development differs with the type of product. Mostly this includes seven major steps.

**Portioning / forming**

A perfectly portioned product is the right starting point. Mechanically deboned fish meat is formed to different shapes and sizes after mixing with ingredients, if needed. The product should keep its consistency with proper weight and shape. The key factor in this production step is speed and accuracy of processing the frozen fish block at minimum costs without any compromise to the product quality.

**Predusting**

Predusting is usually done with very fine raw flour type material or dry batter itself, sprinkled on the surface of food substrate before coating. This helps to reduce the moisture on the surface of the product so that the batter can adhere uniformly. Flavourings such as salt and spices can be added in minimum amounts.

**Battering**

Batter is defined as the liquid mixture composed of water, flour, starch, and seasonings into which the fish products are dipped prior to breading. Two types of batter are there- adhesive batter and tempura batter. The adhesive batter is a fluid, consisting of flour and water. Tempura batter is the puff-type batter containing raising/leavening agents. This forms a crisp, continuous, uniform layer over the food. The predusted portions are applied with wet batter and excess batter can be blown

off by a current of air. The batter mix helps in governing the amount of bread to be picked up and it contributes to flavour of the final product. Specific ingredients are used to aid viscosity, texture and adhesion.

**Ingredients of batter mix**

a) Flour- Wheat flour provides structure to the product through gelatinisation of starch as well as through formation of gluten protein matrix. Higher protein levels in flour increases viscosity of batter and produce darker crispy coatings. Corn flour can be added to produce yellow colour and to enhance browning during frying.

b) Water- The ratio of water to dry batter mix is 1.8:1. Formation of gelatinised starch phase, hydration of flow proteins, batter viscosity etc. depends on the purity of water used.

c) Starch- Corn starch is added mainly to control batter viscosity and thus increasing the batter pickup and breading retention.

d) Flavour and flavour enhancers- salt, sugar, spices etc. can be added to improve the organoleptic characteristics of the products.

e) Sodium tripolyphosphate- This lowers the water activity of the product and has bactericidal property. It increases the hydration of proteins and reduces protein denaturation.

**Breading**

Breading was defined as the application of a dry mixture of flour starch, seasonings having a coarse composition to battered food products prior to cooking. Normally the battered fish portions are dropped in to dried bread crumbs and are turned over to ensure complete coating with bread crumbs. A fine layer or coarse layer of bread crumps will contribute to structure and tastiness of the product. For soft products the crump depth should be fine so as to avoid the product damage on further processing.

**Pre-frying/ flash frying**

Pre-frying is the process of giving a shallow fry so as to coagulate batter over the product and lock the flavour and juices to the product. The time of frying and temperature of oil are crucial factors. This could be done at 180-200°C for 40-60 sec, thus restricting the actual heat transfer to the surface of the product. The term pre-frying is used as frying will be completed only when the consumers fry the product for 4-6 minutes depending on the product size.

**Freezing**

The fish portions are air cooled before freezing. This helps the coating temperature to drop while the batter can stabilise itself and recover from the frying shock. Freezing is done at a temperature of -10°C to -20°C in order to preserve freshness and quality of the product over longer storage periods.

**Advantages of coated products**

- Enhanced nutritional quality
- Moisture barrier during frozen storage and reheating
- Crispy texture and appealing colour and flavour
- Structural reinforcement of the substrate
- Prevents loss of natural juices
- Increased bulk of the substrate and reduced product cost
- Improved overall acceptability of the product

Battering and breading have contributed significantly to the value addition of fishes, shell fishes and molluscs. The first commercially successful coated fish item was fish fingers. Later several other products like fish cutlets, fish balls, fish nuggets, etc. came into the market. Coated butterfly shrimp, squid rings, stuffed squid rings etc. are among the fancy items that cater to the luxury markets. Sophisticated equipments like meat bone separator, meat strainer, portioning and forming equipment, preduster, battering and breading machine, fryer, freezer and packaging machineries are in the market for preparation of a wide variety of coated products.

**Fish finger or Fish portion**

Fish fingers, or portions or sticks are regular sized portions cut from rectangular frozen blocks of fish flesh. They are normally coated with batter, and then crumbed before being flash fried and frozen. They may be packed in retail or catering - size packs. The typical British fish finger normally weighs about 1 oz. (28 g) of which up to about 50% of the total weight may be batter and crumbs. Food Advisory Committee of the UK government has recommended a minimum fish content of 55% for battered and 60% for the fingers coated with breadcrumbs.

**Fish fillets**

The brined fillets are battered and breaded. Fillets from freshwater fish are also used for the production of coated products. The only problem noticed in this case is the presence of fin bones; its complete removal is still a major hurdle.

**Fish cutlet**

Cooked fish mince is mixed with cooked potato, fried onion, spices and other optional ingredients. This mass is then formed into the desired shape, each weighing approximately 30g. The formed cutlets are battered and breaded.

**Fish balls**

Fish balls are generally prepared from mince of low cost fish. Balls can be prepared by different ways. The simplest method is by mixing the fish mince with starch, salt and spices. This mix is then made into balls, cooked in boiling 1 % brine. The cooked balls are then battered and breaded.

**Crab claw balls**

Swimming legs of crab may be used for this purpose. Crab claws are severed from the body, washed in chilled portable water and the shell removed using a cracker. The leg meat is then removed and mixed with 2 % starch based binder. This is then stuffed on the exposed end of the claw. Alternatively the body meat mixed with the binder also can be used for stuffing. The stuffed claw is then frozen, battered and breaded and flash fried. The coated products are packed in thermoformed containers with built in cavities.

**Clam and other related products**

Meat shucked out from depurated live clams after boiling is blanched in boiling brine, cooled, battered, breaded, flash-fried and packed. Other bivalves such as oyster, mussels etc. can also be converted into coated products by the same method.

**Mince based products**

Fish mince separated from skin, bone and fins are comminuted and used for preparation of different products. Battered and breaded products like fish fingers, fish balls, cutlet etc. are produced. Fish cultlets fetch good demand in domestic markets while fish fingers are demanded in export market. Fish cutlets with partial replacement of fish meat with soy protein will increase the acceptability and storage stability of fish cutlets. A ready to eat novel battered and breaded snack product, 'Oyster pablano pepper fritter' have a good scope of attraction in value added markets. Fish finger from Bombay duck adds on to the value addition potential of fish in our markets. Fish rolls with good shelf life can be developed from frame meat of fishes, eg: rohu. Fish sausage, cakes and patties are some other mince based products.

**Surimi and Surimi Products**

Surimi and surimi based seafoods are traditional products of japan and occupy an important position in the dietary culture of the country. Today, the largest producers of surimi are the United States, Japan and Thailand. Surimi is also manufactured in China, Vietnam and Malaysia. The process of making surimi originated in Southeast Asia and was further developed in Japan in the 16th century. The Japanese word "surimi" means "ground meat." In Chinese, it is called "yú jiang," which means "fish puree."

Technically, surimi is the stabilized myofibrillar protein which is obtained by mechanically deboned fish flesh, which is washed, mixed with cryoprotectants, and stored frozen. Washing not only removes fat and undesirable matters such as blood, pigments and odoriferous substances but also increases the concentration of myofibrillar protein. A fish-based product serving the raw material for preparation of analog of seafoods like crab, lobster, scallop & other shellfish. Globally, Alaska Pollock is the main species used for the surimi production.

**Raw material for surimi production**

According to gel-strength Alaska Pollock has been the predominant fish species used for surimi production. In India, for the preparation of surimi, Gopakumar and co-workers utilized the mince of barracuda (*Sphyraena spp*.), threadfin bream(*Nemipterus japanicus*), croaker, Lizard fish, prawn (Metapenaeus dobsonii) and tilapia (*Oreochromis mossabicus*).

**Kamaboko**

The most typical surimi-based product in Japan is Kamaboko. Surimi paste is formed in to Quonset-hut shape on a wood board panel before any thermal treatment. Sometimes its surface is coated with colored paste for appearance. After its unique shape is formed, the surimi paste is subjected to a low temperature setting process (20-40°C for 30-60min), depending on species. or During this process, the gel –forming ability of solubilized myofibrillar proteins is highly enhanced, which yields a strong gel. Cooking by either steaming or baking is carried out to complete the gelation of fish proteins. The finished steamed product is called "mushi" (steaming) kamaboko.

**Chikuwa**

It is an original model of surimi-based products. Its shape is typically like a pipe or tube. Surimi paste is placed on to a grooved hole in a rectangle shape on the surface of a drum. The paste is rolled onto a metal stick on the conveyor. The rolled paste on the stick is baked rotationally in the oven on the screw conveyor for gelation. The finished products are packed, pasteurized and chilled before entering their marketing channel.

**Satsuma-Age**

It is fried kamaboko with various shapes and characteristics. Additional ingredients such as vegetables, shrimp, squid, and minced fish are sometimes mixed into the surimi paste for satsuma-age. The paste is then molded into various shapes (stick, patty, ball, nugget) before frying. In recent years, most satsuma-age is manufactured using a two step-frying process because it yields high gel strength and productivity. The first frying is done at 130°C and the
second frying is subsequently done at 170°C

**Hampen**

The surimi paste is aerated compulsorily by the continuous mixer. Recently, gums, polysaccharides are used as a whipping and stabilizing agent. Vegetable oil is commonly mixed for the development of texture as well. The whipped paste is then boiled in hot water (80-85°C) to fix the soft gel texture.

**Types of kamaboko**

**Based on heating method**

- Steamed kamaboko (Itasuki)
- Steamed and broiled kamaboko
- Broiled kamaboko (chikuwa)
- Boiled kamaboko (Hampen)
- Fried kamaboko (Tempura, Satsuma age)

**Based on shape**

- Tubular shaped (Chikuwa)
- Ball, bar, square shaped (age kamaboko)
- Lead shaped (sasa kamaboko)
- Noodle shaped (soba kamaboko)
- Rolled (date maki)
- Chipped (kezuri kamaboko)

**Fish sausage**

Fish sausage is a product in which surimi is mixed with additives, stuffed into suitable casings and heat processed. Thus for the preparation of fish sausage, the thawed surimi is mixed with salt (3%), sugar (1.5%), STPP (0.3%), starch (8%), spice mixes (3%) (coriander, chilli powder, ginger garlic paste, pepper), vegetable oil (10%) and water 10%) in a bowl chopper to get a homogeneous paste. The mixing process should be ideally completed within 12-15 min. The paste is then stuffed into synthetic casings preferably PVDC and heat processed for 60 min at 90 °C followed by cooling for 15 min in chilled water. The sausage is consumed primarily as a snack and as an appetizer or used as an ingredient for salad and stir-fried food.

**Crab analog**

The frozen surimi is converted to imitation crab meat through various steps. First, it is tempered at -4°C, then shredded into coarse flakes and subjected to comminution during which, the surimi flakes are mixed with other ingredients include starch, salt, natural crab meat, egg white, and flavors in a bowl chopper. Comminution results in the formation of thick surimi paste, which is then transferred to a hoper (holding tank). The paste is conveyed from the hoper to the sheet-forming machine. Continuous sheets of surimi, about 10 inches (25 cm) wide and 0.05 inch (1.2 mm) thick are extruded. Due to the functional nature of surimi protein, the extruded sheets are very smooth in texture. After the sheets are formed, they are passed to machines and subjected to initial cooking. This cooking meditates the setting of the sheets and prepares them to be suitable for the further slitting process. Slitting gives the appearance and texture of crab meat. The slitting is done by a machine which is composed of two steel rollers that cut the thin sheets into strands having 1.5 mm wide. These thin strands are pulled, bundled and rolled into a rope. This rope is colored, wrapped, and cut to the appropriate size. It is then steam cooked, forming a product that imitates in texture and tastes very much like the crab meat.

**Shrimp and lobster analog**

For the preparation of shrimp and lobster style products, the surimi paste is commonly mixed with pre-prepared surimi meat fibers and transferred to a molding machine or cold extruded in a three-dimensional shape. For imparting the color, a color solution is sprayed inside the mold before stuffing. Another way to impart the color is directly using the colored paste (brushed) on the surface of cooked molded products. In the later method an additional, additional heating is needed to set the color.

**Scallop analogs**

The plant set up for the production of scallop analog is similar to crab analog. For the preparation of scallop analog, a wider and thicker surimi sheet is extruded compared to the surimi sheet extruded in crab analog preparation. After sheet formation, surimi sheet subjected to partial cooking for facilitating the gelation and subsequently subjected to slitting. After slitting, an uncooked layer of surimi paste is added on top of the gelled surimi sheet immediately. This additional layer of surimi paste is to enhance the binding of fibers. The gelled fibers are wrapped

and cut into 2-foot lengths and heat processed. The cooked fiber bundles are cut into the desired dimension of scallops shapes using flaking machine.

## II. Shrimp products

### Stretched shrimp (Nobashi)

Increasing the length of peeled and deveined shrimp and minimising its curling by making parallel cuttings at the bottom and applying pressure using simple mechanical devices is a new technique adopted by the seafood processing industry in recent years. Increasing the length by about 1-2 cms depending on the size of the shrimp is possible by this method. The stretched shrimp will have better appearance compared to conventional PD shrimp and it also fetches higher unit price. The stretched shrimp because of its increased surface area will have more pickup of coating during battering and breading and also good appearance. Shrimp is washed in chilled water containing 5-ppm chlorine, beheaded, deveined, using bamboo stick and pealed keeping the last segment and tail intact. The tail is then trimmed and the shrimp is stretched using a metallic stretcher after making 2-3 parallel cuttings at the bottom side. Stretched shrimps are then packed in thermoformed trays under vacuum and frozen at -40°C.

### Barbecue

Shrimp is washed in chilled water containing 5-ppm chlorine, beheaded, deveined, peeled and again washed in chilled water. Bamboo stick is then pierced into the meat from head portion to tail. It is then packed in thermoformed trays under vacuum and frozen at -40°C.

### Sushi (Cooked butterfly shrimp)

Shrimp is washed in chilled water containing 5ppm chlorine, beheaded, deveined and again washed in chilled water. Bamboo stick is then pierced between the shell and the meat from head portion to tail and then cooked in 1% brine for two minutes at 100°C. The cooked shrimp is then cooled in chilled water, bamboo stick removed and then peeled completely, including the tail fans. The ventral side is then gently cut down lengthwise completely using a sharp scalpel. The cut surface is then gently opened up to form the butterfly shape, packed in thermoformed trays under vacuum and frozen at - 40°C.

**Skewered shrimp**

The process is similar to that of barbecue, but piercing is carried out in such a way that 4-5 shrimps are arranged in a skewer in an inverted ⎓ U‖ shape. It is then packed in thermoformed trays under vacuum and frozen at -40°C.

**Shrimp head-on (centre peeled)**

Shrimp is washed in chilled water containing 5 ppm chlorine, pealed at the centre keeping the head and the last two segments intact, deveined, and the tail is trimmed. It is again washed in chilled water packed in thermoformed trays under vacuum and frozen at -40°C. Shrimp head-on cooked (centre peeled) Shrimp is washed in chilled water containing 5 ppm chlorine, deveined and then cooked in 1% brine for two minutes at 100°C. It is immediately cooled in chilled water and pealed keeping the head and the last two segments intact. The tail is trimmed and again washed in chilled water. It is then packed in thermoformed trays under vacuum and frozen at -40°C.

## III. Squid products

Squid rings and stuffed squid are the popular coated products processed out of squid. Cleaned squid tubes are cut in the form of rings of uniform size, cooked in boiling brine (3%) for 1-2 minutes followed by cooling, breading and battering. The coated rings are flash-fried, cooled, frozen and packed. Stuffed squid is generally processed out of small size animals. The cleaned tubes are filled with a stuffing mixture prepared using cooked squid tentacles, potato, fried onion, spices etc. It is then battered, breaded and flash-fried.

**Extruded products**

Fish based extruded products have got very good marketing potential. Formulation of appropriate types of products using fish mince, starches etc., attractive packaging for the products and market studies are needed for the popularization of such products. However, technological studies involving use of indigenously available starches like cassava starch, potato starch, cornstarch and the associated problems need thorough investigation. Such products can command very high market potential particularly among the urban elites. The technology can be employed for profitable utilization of bycatch and low value fish besides providing ample generation of employment opportunities. CIFT has worked on the production of extruded products by

incorporating fish mince with cereal flours. The product obtained is finally coated with spice mix to provide a delicious snack that has been christened as "Fish Kure.

**Ready to serve fish products in retortable pouch**

Ready to serve fish products viz. curry products, in retortable pouches are a recent innovation in ready to serve fish products for local market. The most common retortable pouch consists of a 3 ply laminated material. Generally it is polyester/aluminium/cast polypropylene. These products have a shelf life of more than one year at room temperature. As there is increasing demand in National and International market for ready to serve products the retort pouch technology will have a good future. The technology for retort pouch processing of several varieties of ready to serve fish and fish products has been standardised at CIFT and this technology has been transferred successfully to entrepreneurs.

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# 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.

# QUALITY ISSUES IN DRIED FISHERY PRODUCTS

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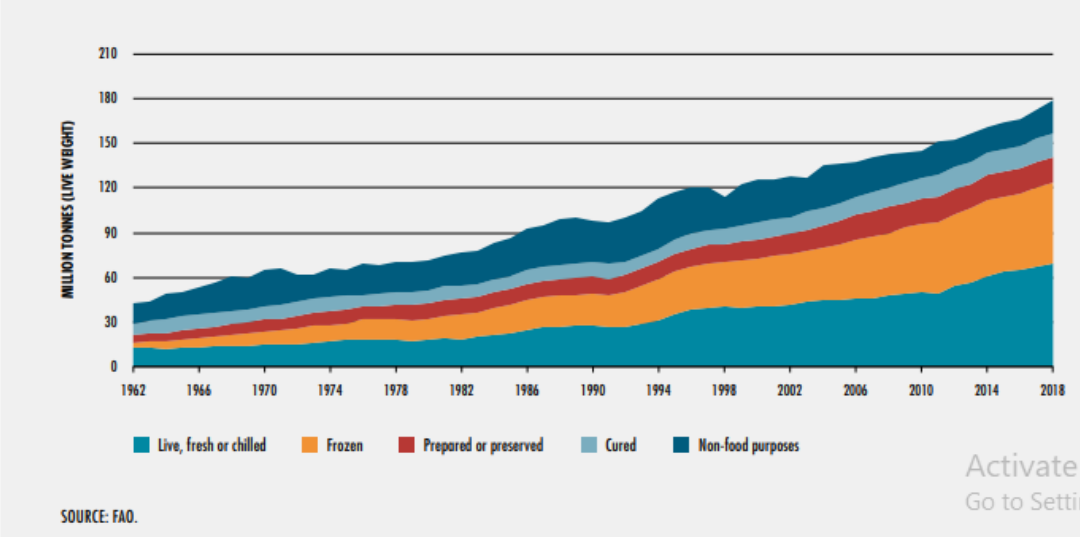
## Introduction

Drying is the oldest known, widely used and least expensive food preservation method. During drying, water from a subject gets removed which reduces microbial activity, and thus prevents spoilage. The reduced water content not only influence the microbial activity but also retards the chemical as well as the enzymatic processes happening in the food system. Dried products are stable products that can be stored at ambient temperatures. Hence the distribution costs are also minimum while transportation and storage.

As per the FAO records (Fig.1), 10% of the global fish production is utilized for cured products, which comprises the dried, salted, fermented and smoked categories while 44% of the total production is utilized for direct human consumption in the form of fresh, chilled and frozen.



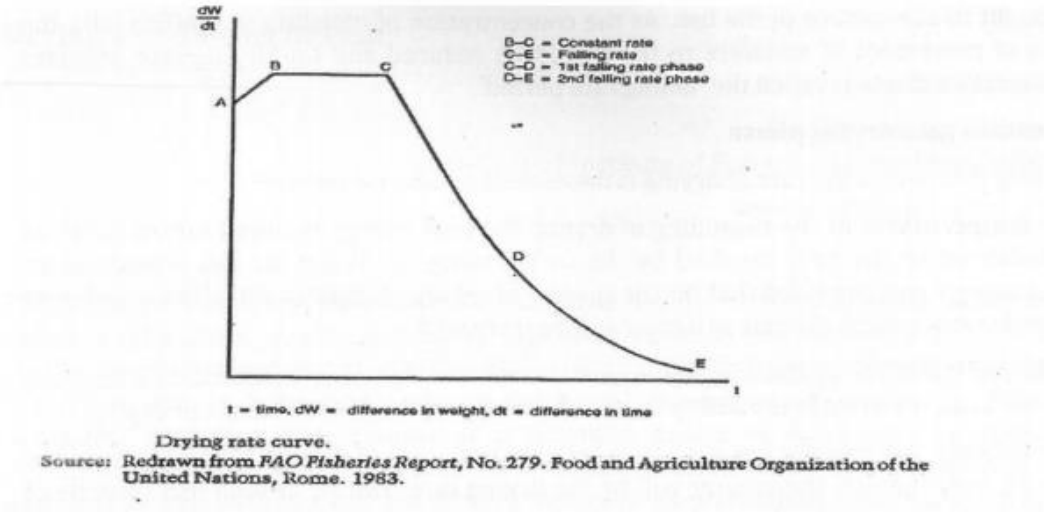

Cured means dried, salted, in brine, fermented, smoked,

Fig:1. Utilization of world fisheries and aquaculture production, 1962-2018. *Source: FAO*

## Principle of drying process:

During drying process, the water content of the food item gets removed or reduced which proportionately retard or totally stop all microbial and autolytic activities, thus prevents spoilage of food resulting in preservation.

The process of drying involves 2 steps – Diffusion and Evaporation. There are various factors which influences the rate of drying such as nature of the fish (water content/having larger surface area/fat content), Air temperature (25-35°C/40-50°C), Relative humidity, and Air velocity (75-130m/min). As per the method of drying, drying can be various types, viz. Natural/ sun drying, artificial/mechanical dryers. The dried fishery products can be salted/ unsalted/ smoke-dried. Salting can be of different types such as dry salting, wet salting, kench salting, mona curing *etc*. Fig.2. illustrate 'drying curve' with different stages. Smoking is detailed in another chapter - 'quality issues of smoked fishery products. The characteristics of dried/salted dried/smoke dried fish, making them shelf stable is water activity ($a_w$). Water activity is the measure of the amount of water in a food that is available for the growth of microorganisms, including pathogenic bacteria. A water activity of 0.85 or below will prevent the growth and toxin production of all pathogenic bacteria, including *Staphylococcus aureus* and *Clostridium botulinum,* and is critical for the safety of a shelf-stable dried product.




Drying rate curve.
Source: Redrawn from *FAO Fisheries Report*, No. 279. Food and Agriculture Organization of the United Nations, Rome. 1983.

Fig:2. Drying curve with different stages. *Source: FAO*

## DRYING PROCESS

The drying process involves various steps *viz.*, receiving of raw material/fresh fish, washing, weighing, preparation of fish (optional)– gutting/ beheading/ splitting/ filleting/ washing and weighing, salting (optional), drying, packaging and labelling, and storage (Fig.3).

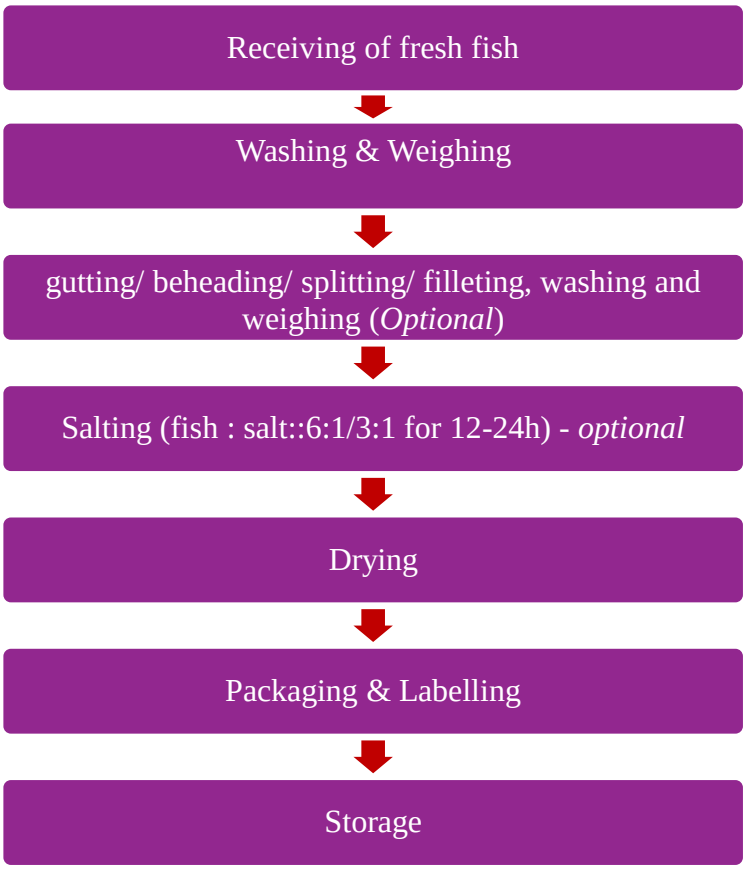



Fig. 3. Process flow chart of drying of fish

At any stages of processing (*i.e.*, drying), a hazard can be present as well as introduced. For example, in the case of receiving step, the potential hazards are presence of viable parasites, pathogens, biotoxin, scomberotoxin, physical and chemical contaminants. In fishes, that are prone to scomberotoxin (histamine formation), time-temperature control is the effective method to ensure food safety. Temperature of the raw material should be <4°C as temperature abuse may result in histamine formation. However, receiving of fresh fish is not a critical control point for drying process, as the subsequent steps are there to prevent the potential hazards. Raw fish should be washed in chilled potable water and properly iced or moved to the chilled storage facility without undue delay if not immediately taken for drying. Depending on the design/type of final product the fish has to be gutted, beheaded, split, or filleted. If the final product is a salted dried fish, after the pre-processing step, it needs to be salted for 12-24h. Fish to salt ratio is again on the basis of thickness/nature of the fish- whether it is lean or fatty. The salt used should be of good quality, as it can cause cross-contamination - introduction of viable parasites, pathogens, biotoxin, scomberotoxin, physical and chemical contaminants into the raw material.

Drying is the most critical step in the case of dried/salted-dried fish products and it is first critical control point ($CCP_1$) as the inadequate drying can results in the proliferation of pathogenic bacteria like *Staphylococcus aureus* and *Clostridium botulinum*, and toxin production. A water activity of 0.85 or below will prevent the growth and toxin production of all pathogenic bacteria, including *S. aureus* and *C. botulinum*, and is critical for the safety of a shelf-stable dried product. Some dried products are not dried to get a water activity ($a_w$) of 0.85, but to a little bit higher level, $a_w$-0.95. So as to control growth and toxin formation by *C. botulinum* type E and non- proteolytic types E and F, these partially dried products should be kept under refrigeration duration storage. Toxins of *Clostridium botulinum* are not allowed in smoked fish, smoke-flavored fish and smoke-dried fish products. The formation of *C. botulinum* toxin can be controlled through scientific approaches involving packaging type, storage temperature, and the use of salt. The preventive measure for inadequate drying procedure is proper design and control of drying process, to achieve desirable level of $a_w$ according to the nature of the final product *i.e.,* fully dried/ partially dried. The design and operation of the drying equipment should be in such a way to ensure that, every unit of a product receives at least the established minimum process;

The packaging of the final product should be effective to prevent rehydration of the product during transit and storage. For the partially dried products, appropriate packaging – vacuum packaging/modified atmosphere packaging (MAP), reduced oxygen packaging *etc*.- should be used to control the growth of pathogenic organisms. If the products are partially dried, vacuum packed/ MAP, it should be kept under refrigeration during storage and distribution and the final product should be clearly labelled as "keep refrigerated". If the partially dried product is packed under reduced oxygen condition, the product should be kept frozen during storage and distribution, and labelling with "keep frozen" instructions are important to ensure food safety. Therefore, finished product storage and labelling is another critical control point (CCP2) in the drying process of dried/salted- dried fish.

The minimum and maximum values for the critical factors - drying time, input/output air temperature, humidity, and velocity, as well as flesh thickness- should be derived scientifically for both fully dried and partially dried/ salted- dried fish products to achieve the desired $a_w$. The spoilage organisms like mold in shelf- stable products should be eliminated/inhibited by further processes such as heat treatment, use of additives, further drying or other treatments *etc*., The $a_w$ of the finished products should be monitored by using a water activity meter along with the process parameters such as drying time and input/output air

temperature. A recording thermometer can be used for continuous monitoring of the drying temperature. Any person who has an understanding of the nature of the controls or with sufficient training to perform the analysis should carry out the monitoring activity.

If any final product involved in a critical limit deviation, chill and hold the product for an evaluation of the adequacy of the drying process. Re-dry the product if the redrying process does not provide an opportunity for the growth of pathogenic bacteria. Otherwise divert the product to a use in which the critical limit is not applicable because pathogenic bacteria growth in the finished product will be controlled by means other than drying or for non- food use. If these things are not at all possible destroy the product. At the same time corrective actions needs to be taken to control drying time/temperature/ air velocity/ humidity/ belt speed in equipment for feeding etc., regain control over the process for adequate drying.

**Effect of drying on quality of fish**

Shrinkage:

During drying there are many structural changes are happening and shrinkage is such a major physical change. During drying, when water get removed from the fish, a proportionate shrinkage in volume of fish also takes place.

Case hardening:

When the drying temperature is high, relative humidity is low the dissolved salts, proteins and organic matters get deposited on the surface of the fish. This impervious layer prevents the diffusion process, which results in cooking of the final product. Thus, the final product became brittle. This condition is known as case hardening.

Denaturation of protein:

During drying, the concentration of dissolved material in water increases. Reduced evaporation due to case hardening will result in increase of temperature of the fish muscle which leads to denaturation of protein and toughening of texture.

Rehydration:

Due to denaturation of protein and poor water holding capacity, penetration of water will be retarded in dried fish resulting in poor dehydration.

Maillard reaction:

The Maillard reaction/ non-enzymatic browning is a chemical reaction between amino acids and reducing sugars, resulting in brown coloration and distinctive flavor in dried fish.

Rancidity:

Rancidity is caused by the oxidation of fat, which is more pronounced fatty fishes such as mackerel, sardine, tuna, anchovy etc. The unsaturated fat in the fish reacts with the oxygen in the atmosphere forming peroxides, which are further broken down into simple and odoriferous compounds like aldehydes, ketones and hydroxy acids. This imparts the characteristic odors, and yellowish to brown color in the dried fish, referred to as rust.

**Spoilage during drying and storage**

**Dun** – Dun is a kind of spoilage occurs in dried/salted- dried fishes during storage due to the growth of halophilic mold – *Valencemia semi* imparting black/brown/grey-colored spots on the fleshy part of the fish. Being halophilic in nature, *V. semi* can growth at optimal condition of 10-15% salt concentration, 75% relative humidity and 30-35°C. The preventive measures are use of good quality salt, storage at low temperature and humidity, under well ventilated and dry storage conditions.

**Pink or Red** – Common type of spoilage associated with salted dried fish and fishery products, with a salt concentration above 10%. The halophilic bacterial growth (*Halobacterium salinaria, H cutirubum, Sarcina morrhuae, S. littoralis & Micrococcus rosens*) imparts a red slime on the surface of the fish within unpleasant odor. The spoilage is known as pink/red due to the colour of colonies of bacteria appearing on the surface of the fish. These bacteria are aerobic and thermophilic in nature within optimum growth temperature of 42°C. The preventive measures are use of good quality salt, keeping the fish out of contact with air, storage at low temperature (<10°C).

**Insect infestation** – Insect infestation is major problem for unsalted fish during drying. The fishes are often infested with blowflies- *Chrysomya spp. Lucilia spp. Sarcophaga spp.*, and their larvae feed on it resulting in huge loss in terms of quantity, quality, and economic aspects. Insect attack may also take place during storage also. *Eg*: Beetle infestation (dermestid beetles) – their larvae feed on the fish at a moisture content of 15% and more, leaving only fish bones. Preventive measures are Good Hygiene Practices (GHP), and salting.

Spoilage during drying and storage

**Rancidity/Rust**- Fishes with rich oil content are prone to oxidation and development of rancid flavour. Rancid flavour in dried fish is acceptable to some extent, but excessive will be objectionable. The preventive measure is air tight packaging, and use of permitted antioxidants.

**Fragmentation** – Fragmentation is often observed in cured/dried fish due to the brittleness and breakage happening during storage & transportation. The brittleness and breakage are caused by various reasons such as denaturation of protein, insect infestation, poor quality raw material for drying. The preventive measures are use of fresh fish as raw material, and appropriate packaging.

**Regulations/Standards:**

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

- Food Safety and Standards Regulations (FSSR), 2011
- *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
- Indian Standard (IS 14950:2001)

**Food Safety and Standards Regulations (FSSR), 2011**

As per Food Safety and Standards (Food products Standards and Food Additives) Regulations, 2011

(1) Dried/ salted and dried fishery products mean the product prepared from fresh or wholesome fish after drying with or without addition of salt.

(2) The fish shall be bled, gutted, beheaded, split or filleted and washed prior to salting and drying.

(3) Salt used to produce salted fish shall be clean, free from foreign matter, show no visible signs of contamination with dirt, oil, bilge or other extraneous materials.

(4) The product shall be free from foreign matter, objectionable odour and flavour.

(5) The product may contain food additives permitted.

(6) The product shall conform to the microbiological and chemical requirement as laid down in the regulation.

(7) The products shall conform to the following requirements

**FSSR requirements**

| Sl no: | Characteristics | Requirements |
|---|---|---|
| 1. | Water activity (aw), at 25°C | Less than 0.78 |
| 2. | Salt Content (percent Sodium Chloride)* | Not less than 12 % |
| 3. | Histamine** content, max. | 200 mg/Kg |
| 4. | Acid Insoluble Ash on dry basis | Not more than 1% |

*Requirement of salt content is only applicable to dry salted fishery products

## Limit of histamine level

| Product category | Applicability | Level of histamine |
|---|---|---|
| Dried/ Salted and Dried fishery products | Species with high amount of free histidine (Listed fish species with potential to cause histamine fish poisoning) | n=9, c=2; m=200 mg/kg, M=400 mg/kg |

n : Number of units comprising the sample
c : Maximum allowable number of defective sample units
m : Acceptable level in a sample
M : Specified level when exceeded in one or more samples would cause the lot to be rejected

Satisfactory, if the following requirements are fulfilled:
1. the mean value observed is ≤ m
2. a maximum of c/n values observed are between m and M
3. no values observed exceed the limit of M,

Unsatisfactory, if the mean value observed exceeds m or more than c/n values are between m and M or one or more of the values observed are >M.

# Additives permitted

| Food Category System | Food Category Name | Food Additive | INS No | Recommended Maximum Level | Note |
|---|---|---|---|---|---|
| 9.2.5 | Smoked, dried, fermented, and/or salted fish and fish products, including molluscs, crustaceans, and echinoderms (Dried shark fins, Salted fish/ dried salted fish) | Allura red AC | 129 | 100 mg/kg | 22 |
| | | **BENZOATES** | | 200 mg/kg | |
| | | Butylated hydroxyanisole (BHA) | 320 | 200 mg/kg | 15, 196 |
| | | Butylated hydroxytoluene (BHT) | 321 | 200 mg/kg | 15, 196 |
| | | **CHLOROPHYLLS AND CHLOROPHYLLINCOPPER COMPLEXES** | | 200 mg/kg | |
| | | Calcium carbonate | 170(i) | GMP | 266, 267 |
| | | Canthaxanthin | 161g | 15 mg/kg | |
| | | beta- Carotenes, vegetable | 160a(ii) | 1,000 mg/kg | |
| | | Fast green FCF | 143 | 100 mg/kg | |
| | | Fumaric acid | 297 | GMP | |
| | | Grape skin extract | 163(ii) | 1,000 mg/kg | 266, 267 |
| | | **IRON OXIDES** | | 250 mg/kg | 22 |
| | | Magnesium carbonate | 504(i) | GMP | 22 |
| | | Indigotine (Indigo carmine) | 132 | 100 mg/kg | 22 |
| | | Magnesium hydroxide | 528 | GMP | 266, 267 |
| | | Magnesium hydroxide carbonate | 504(ii) | GMP | 266, 267 |
| | | Malic acid, DL- | 296 | GMP | 266, 267 |
| | | Ponceau 4R | 124 | 100 mg/kg | 266, 267 |
| | | Potassium dihydrogen citrate | 332(i) | GMP | 22 |
| | | Propyl gallate | 310 | 100 mg/kg | 266, 267 |
| | | **RIBOFLAVINS** | | 300 mg/kg | 15, 196 |
| | | **SORBATES** | | [32][1000 mg/Kg] | 42 |
| | | **SULFITES** | | 30 mg/kg | |
| | | Sodium dihydrogen citrate | 331(i) | GMP | 44 |
| | | Sodium fumarate | 365 | GMP | 266, 267 |
| | | Sunset yellow FCF | 110 | 100 mg/kg | 266, 267 |
| | | Acetylated distarch phosphate | 1414 | GMP | 22 |
| | | Agar | 406 | GMP | 300 |
| | | Carrageenan | 407 | GMP | 300 |
| | | Citric and fatty acid esters of glycerol | 472c | GMP | 300 |
| | | Guar gum | 412 | GMP | 300 |
| | | Gum arabic (acacia gum) | 414 | GMP | 300 |
| | | Hydroxypropyl cellulose | 463 | GMP | 300 |
| | | Hydroxypropyl methyl cellulose | 464 | GMP | 300 |
| | | Hydroxypropyl starch | 1440 | GMP | 300 |
| | | Lactic and fatty acid esters of glycerol | 472b | GMP | 300 |
| | | Magnesium chloride | 511 | GMP | 300 |
| | | Mannitol | 421 | GMP | 300 |
| | | Methyl cellulose | 461 | GMP | 300 |
| | | Methyl ethyl cellulose | 465 | GMP | 300 |
| | | Oxidized starch | 1404 | GMP | 300 |
| | | Pectins | 440 | GMP | 300 |
| | | Powdered cellulose | 460(ii) | GMP | 300 |
| | | Processed eucheuma seaweed | 407a | GMP | 300 |
| | | Salts of myristic, palmitic and stearic acids with ammonia, calcium, potassium and sodium | 470(i) | GMP | 300 |
| | | Salts of oleic acid with calcium, potassium and sodium | 470(ii) | GMP | 300 |
| | | Sodium alginate | 401 | GMP | 300 |
| | | Carboxymethyl cellulose | 466 | GMP | 300 |
| | | Tara gum | 417 | GMP | 300 |
| | | Tragacanth gum | 413 | GMP | 300 |
| | | Xanthan gum | 415 | GMP | 300 |
| | | Lecithins | 322(i), (ii) | GMP | 300 |
| | | Acetic and fatty acid esters of glycerol | 472a | GMP | 300 |

15- On the fat or oil basis

22- For use in smoked fish products only

196- Singly or in combination: butylated hydroxyanisole (BHA, INS 320), butylated hydroxytoluene (BHT, INS 321) and ropyl gallate (INS 310)

266- Excluding salted atlantic herring and sprat

267- Excluding products conforming to the standard for salted fish and dried salted fish of the gadidae family of fishes , the standard for dried shark fins, the standard for crackers from marine and freshwater fish, crustaceans and molluscan shellfish , and the standard for boiled dried salted anchovies

42- As sorbic acid

44- As residual SO2

300 - For use in salted squid only

# Microbiological requirements – hygiene indicator organisms

| Sl. No. | Product Category* | Aerobic Plate Count | | | | Coagulase positive Staphylococci | | | | Yeast &mold count | | | | Stage where criterion applies | Action in case of unsatisfactory results |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sampling Plan | | Limits (cfu/g) | | Sampling Plan | | Limits (cfu/g) | | Sampling Plan | | Limits (cfu/g) | | | |
| | | n | c | m | M | n | c | m | M | n | c | m | M | | |
| 7. | Dried/Salted and Dried Fishery Products | 5 | 0 | $1x10^5$ | | - | - | - | - | 5 | 2 | 100 | 500 | End of Manufacturing process | Improvement in hygiene; Selection of raw material; Adequate drying (water activity ≤ 0.78) |

Sampling Plan:
The terms n, c, m and M used in this standard have the following meaning:
n = Number of units comprising a sample.
c = Maximum allowable number of units having microbiological counts above m.
m = Microbiological limit that may be exceeded number of units c.
M = Microbiological limit that no sample unit may exceed.

# Microbiological requirements – safety indicator

| Sl. No. | Product Category* | *Escherichia coli* | | | | *Salmonella* | | | | *Vibriocholerae* (O1 and O139) | | | | *Listeria monocytogenes* | | | | *Clostridium botulinum* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sampling | | Limits | | Sampling | | Limits | | Sampling | | Limits | | Sampling | | Limits | | Sampling | | Limits (MPN) | |
| | | n | c | m | M | n | c | m | M | n | c | m | M | n | c | m | M | n | c | m | M |
| 7. | Dried/ Salted and dried fishery products | 5 | 0 | 20 | | 5 | 0 | Absent/25g | | - | - | - | - | - | - | - | - | - | - | - | - |

Sampling Plan:
The terms n, c, m and M used in this standard have the following meaning:
n = Number of units comprising a sample.
c = Maximum allowable number of units having microbiological counts above m.
m = Microbiological limit that may be exceeded number of units c.
M = Microbiological limit that no sample unit may exceed.

# CODEX standard -Codex Committee on Fish and Fisheries Products (CCFFP)



**STANDARD FOR SALTED FISH AND DRIED SALTED FISH OF THE GADIDAE FAMILY OF FISHES**

***CODEX STAN 167 - 1989***

**1. SCOPE**

This standard applies to salted fish and dried salted fish of the *Gadidae* family which has been fully saturated with salt (heavy salted) or to salted fish which has been preserved by partial saturation to a salt content not less than 12% by weight of the salted fish which may be offered for consumption without further industrial processing.

**2. DESCRIPTION**

**2.1 PRODUCT DEFINITION**

Salted fish is the product obtained from fish:

(a) of the species belonging to the family *Gadidae*; and

(b) which has been bled, gutted, beheaded, split or filleted, washed, salted.

(c) dried salted fish is salted fish which have been dried.

**2.2 PROCESS DEFINITION**

The product shall be prepared by one of the salting processes defined in 2.2.1 and one or both of the drying processes defined in 2.2.2 and according to the different types of presentation as defined in 2.3.

**2.2.1 Salting**

(a) Dry Salting (kench curing) is the process of mixing fish with suitable food grade salt and stacking the fish in such a manner that the excess of the resulting brine drains away.

(b) Wet Salting (pickling) is the process whereby fish is mixed with suitable food grade salt and stored in watertight containers under the resultant brine (pickle) which forms by solution of salt in the water extracted from the fish tissue. Brine may be added to the container. The fish is subsequently removed from the container and stacked so that the brine drains away.

(c) Brine Injection is the process for directly injecting brine into the fish flesh and is permitted as a part of the heavy salting process.

**2.2.2 Drying**

(a) Natural Drying - the fish is dried by exposure to the open air; and

(b) Artificial Drying - the fish is dried in mechanically circulated air, the temperature and humidity of which may be controlled.

**2.3 PRESENTATION**

2.3.1 **Split fish** - split and with the major length of the anterior of the backbone removed (about two-thirds).

2.3.2 **Split fish with entire backbone** - split with the whole of the backbone not removed.

2.3.3 **Fillet** - is cut from the fresh fish, strips of flesh is cut parallel to the central bone of the fish and from which fins, main bones and sometimes belly flap is removed.

2.3.4 Other presentation: any other presentation of the product shall be permitted provided that it

(i) is sufficiently distinctive from the other forms of presentation laid down in this Standard;

(ii) meets all other requirements of this Standard; and

(iii) is adequately described on the label to avoid confusing or misleading the consumer.

2.3.5 Individual containers shall contain only one form of presentation from only one species of fish.

**3. ESSENTIAL COMPOSITION AND QUALITY FACTORS**

**3.1 FISH**

Salted fish shall be prepared from sound and wholesome fish, fit for human consumption.

**3.2 SALT**

Salt used to produce salted fish shall be clean, free from foreign matter and foreign crystals, show no visible signs of contamination with dirt, oil, bilge or other extraneous materials and comply with the requirements laid down in the *Code of Practice for Fish and Fishery Products* (CAC/RCP 52-2003)

**3.3 FINAL PRODUCT**

Products shall meet the requirements of this standard when lots examined in accordance with Section 9. comply with the provisions set out in Section 8. Products shall be examined by the methods given in Section 7.

**4. FOOD ADDITIVES**

Only the use of following additives is permitted.

| **Additives** | | **Maximum level in the Final Product** |
|---|---|---|
| Preservatives | | |
| 200 | Sorbic acid | 200 mg/kg, singly or in combination expressed as sorbic acid |
| 201 | Sodium sorbate | |
| 202 | Potassium sorbate | |

**5. HYGIENE**

5.1 It is recommended that the products covered by the provisions of this Standard be prepared and handled in accordance with the appropriate sections of the *General Principles of Food Hygiene* (CAC/RCP 1-1969), the *Code of Practice for Fish and Fishery Products* (CAC/RCP 52-2003), and other relevant Codex Codes of Hygienic Practice and Codes of Practice.

5.2 The products should comply with any microbiological criteria established in accordance with the *Principles and Guidelines for the Establishment and Application of Microbiological Criteria Related to Foods* (CAC/GL 21-1997).

**6. LABELLING**

In addition to the provisions of the *General Standard for the Labelling of Prepackaged Foods* (CODEX STAN 1-1985), the following specific provisions apply:

**6.1 NAME OF THE FOOD**

6.1.1 The name of the food to be declared on the label shall be "salted fish", "wet salted fish" or "salted fillet" "dried salted fish" or "klippfish" or other designations according to the law, custom or practice in the country in which the product is to be distributed. In addition, there shall appear on the label in conjunction with the name of the product, the name of the species of fish from which the product is derived.

6.1.2 For forms of presentation other than those described in 2.3.1 "split fish", the form of presentation shall be declared in conjunction with the name of the product in accordance with sub-section 2.3.2 as appropriate. If the product is produced in accordance with sub-section 2.3.3, the label shall contain in close proximity to the name of the food, such additional words or phrases that will avoid misleading or confusing the consumer.

6.1.3 The term "klippfish" can only be used for dried salted fish which has been prepared from fish which has reached 95% salt saturation prior to drying.

6.1.4 The term "wet salted fish" can only be used for fish fully saturated with salt.

**6.2 LABELLING OF NON-RETAIL CONTAINERS**

Information specified above shall be given either on the container or in accompanying documents, except that the name of the food, lot identification, and the name and address of the manufacturer or packer shall always appear on the container.

However, lot identification, and the name and address may be replaced by an identification mark, provided that such a mark is clearly identifiable with the accompanying documents.

- Standard for Boiled Dried Salted Anchovies – CODEX STAN 236-2003 - http://www.fao.org/3/w9253e/w9253e0m.htm

## Indian Standard (IS 14950:2001)

| Sl. No. | Product | Moisture, percent by mass, Max | Sodium chloride (on moisture free basis), percent by mass | Acid insoluble ash (on moisture free basis), percent by mass, Max |
|---|---|---|---|---|
| 1. | Dry-Salted Cat Fish | 35 | 25 (min) | 1.5 |
| 2. | Dry-Salted Dhoma | 35 | 10-15 | 2 |
| 3. | Dry-Salted Horse Mackerel | 35 | 25-30 | 1.5 |
| 4. | Dry-Salted Threadfin (Dara) | 40 | 25 (min) | 1.5 |
| 5. | Dry-Salted Leather Jacket | 35 | 25-30 | 1.5 |
| 6. | Dry-Salted Mackerel | 30 | 25-30 | 1.5 |
| 7. | Dry-Salted Jew Fish (Ghol) | 40 | 25 (min) | 1.5 |
| 8. | Dry-Salted Seer Fish | 35 | 25-30 | 1.5 |
| 9. | Dry-Salted Shark | 35 | 25-30 | 1.5 |
| 10. | Dry–Salted Surai (Tuna) | 35 | 20-25 | 1.5 |
| | **Dried products** | | | |
| 11. | Dried Bombay Duck | 15 | 7.5 (max) | 1.0 |
| 12. | Laminated Bombay Duck | 15 | 6 (max) | 1.0 |
| 13. | Dried Fish Maws | 8 | - | 1.5 |
| 14. | Dried Prawns | 20 | 5 (max) | 1.0 |
| 15. | Dried Shark Fins | 10 | - | 1.5 |
| 16. | Dried White Baits | 15 | 2.5 (max) | 1.5 |

| Parameter | Requirement |
|---|---|
| Total plate count, A/ox/g | 1 00 000 |
| *E.coli*, Max/g | 20 |
| *Salmonella*, per 25 g | Absent |
| *Heavy metals:* | |
| Mercury, mg/kg | 0.5 |
| Zinc, mg/kg, Max | 50 |
| Copper, mg/kg | 10.0 |
| Arsenic, mg/kg | 1.0 |
| Lead, mg/kg, Max | 1.0 |
| Tin, mg/kg. Max | 50.0 (product packed in tin plate)<br>250.0 (other packaging containers) |

# QUALITY ISSUES IN FISH MINCE AND MINCED BASED PRODUCTS

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## Introduction

Fish mince is finely ground paste of fish meat / mechanically deboned fish meat. It is a commercially important commodity locally referred as 'keema'. The most common way of separating edible flesh from waste is by filleting, but a greater amount of flesh can be recovered in the form of a coarse mince by putting either the unfilleted fish, or the waste left after filleting, through a bone separator. Minced meat is generally stored at – 18 ℃ as frozen blocks. The minced meat can be further processed to produce many products like surimi, sausages, fish ball, fish cutlet, fish burgers, fish fingers, nuggets etc. .Imitated analogue products like crab stick, shrimp analogue etc were also developed from mince which have commercial importance.

'Surimi' is a commercially important intermediate product prepared from fish mince. Surimi is stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is washed with water and blended with cryoprotectants. It has received considerable attention in development of imitation / analogue products. The main species utilized for the production of surimi is Alaska pollock (*Theragra chalcogramma*). Many varieties fishes like croakers, ribbon fish, threadfin bream, lizard fish, Big eye, snapper etc are available from the Indian waters are commercially utilized for surimi production. Fishes namely Alaska pollack, Pacific whiting, blue whiting, threadfin bream, menhaden etc are mainly used for surimi production in temperate region. Surimi products are manufactured by manipulating the gel forming capacity of fish myofibrillar protein myosin. Hence, the suitability to be raw material for surimi production is determined by the functionality of fish myofibrillar protein called 'gelation' which are generally greater in white-fleshed fish than in dark fleshed fish. Fatty fishes such as sardines, herrings and mackerels are difficult to process due to higher content of dark muscle, high lipid content and poor gel forming ability. Surimi is graded depending upon gelation property, whiteness of meat and moisture level.

**Preparation of fish mince**

The steps involved in the preparation of fish mince are pre-processing, meat picking / bone separation and storage. During pre-processing fishes are gutted, beheaded or filleted and the temperature should be maintained between 0 to 4 ˚C. Minced meat is added with cryoprotectants and antioxidants, frozen in plate freezers and stored at -18℃. Headless gutted fish has higher yield than with filleting alone. When the fish are first filleted, an additional 8-12 per cent flesh can be separated from the filleting waste.

**Surimi processing**

Surimi is Fish meat that has been washed of lipids, water-soluble or sarcoplasmic proteins, and other impurities. The various steps involved are

- Beheading and gutting
- Mincing / deboning
- Water washing and dewatering – enhances gel forming ability
- Refining – remove connective tissue, scales (Cylindrical screen)
- Dehydration using screw press
- Addition of cryoprotectants – polyphosphates, sugar, salt (Silent cutters)
- Packing, freezing and storage

Raw material should be kept below 4℃ and process as early as possible. Wash water should be maintained 10 °C or below for adequate separation of water-soluble proteins. pH of wash water should be near 7.0 and total hardness of 100 mg/kg or below, de-watering aids can be added (less than 0.3% salt) in the final stage. Food grade enzyme inhibitors (e.g. egg white, beef protein plasma) should be used for species that exhibit high levels of proteolytic enzyme activity such as Pacific Whiting. Cryoprotectants are effective in protecting physical, functional and structural properties of myofibrillar proteins during frozen storage of surimi. Sucrose, sorbitol, polydextrose, lactitol, maltodextrin, sodium lactate, trehalose and phosphates are among the most-studied cryoprotectants used in the storage of surimi. Sucrose, sorbitol and polyphosphate

are used at 4, 4, and 0.3% respectively for extended frozen storage. These antifreezing agents reduce viscosity, improve moisture retention and enhance the protein stability during frozen storage.

**Quality categories of surimi**

- High quality surimi has high gel strength, low impurities and white color
- There are mainly **three quality categories** of surimi with various grades
- First category has four grades – **SA, FA, A, AA** (Most common category)
- Second category – two grades, **KA, KB** (produced from meat recovered after second refining)
- Third category – two grades, **RA, RB** (produced from meat recovered after third refining step, include meat from collar cuts and frames)

**Quality problems in fish mince and surimi**

Major quality problems in fish mince are dehydration, presence of foreign matter, parasites, bones, odour and flavour, flesh abnormalities and bacteriological hazards.

Major quality problems in surimi are parasites, scombrotoxin, heavy metals, foreign matter, decomposition, residual water-soluble protein, misuse or erroneous quantity of food additives and denaturation of surimi protein.

**Quality attributes of frozen surimi**

- **Moisture content** - Best quality surimi has moisture of 77 -79%
- **Gel strength and deformability** - The gel forming ability of surimi is an important attribute in quality evaluation which can be tested by two methods depending upon the buyer namely puncture test and torsion test.
- **Colour** - Whiteness using colourimeter
- **Impurities** - Presence of scales, fins, bones, foreign matter (hair, filth, metal pieces).

As per Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011 in regulation 2.6 relating to "Fish and Fish Products" (Version 8 9 2020) has given the requirements of frozen fish mince

- The products shall conform to the requirements specified in the table below

**Table 1. Requirements for Frozen minced fish meat**

| S.No. | Characteristics | Requirement |
|---|---|---|
| 1 | Colour of minced fish meat | Characteristic of the species |
| 2 | Texture of the minced meat | Characteristic of the species |
| 3 | Odour | Characteristic of the species, free from rancid, putrid of foreign odour |
| 4 | Flavour | Characteristic of the species, sweetish and pleasant, free from spoilt or foreign flavor |
| 5 | Bone content, % by weight, Max | 1.0 |

**Table 2. Microbiological Requirements for Fish Mince/Surimi and Analogues**

| Sl. No. | Parameter | n | c | m | M |
|---|---|---|---|---|---|
| 1 | **Aerobic Plate Count** | 5 | 2 | $1 \times 10^5$ | $1 \times 10^6$ |
| 2 | **Coagulase positive *Staphylococci*** | 5 | 2 | $1 \times 10^2$ | $1 \times 10^3$ |
| 3 | ***Escherichia coli*** | 5 | 0 | 20 | |
| 4 | ***Salmonella*** | 5 | 0 | Absent in 25 g | |
| 5 | ***Vibrio cholera*** | 5 | 0 | Absent in 25 g | |
| 6 | ***Listeria monocytogenus*** | | | Absent in 25 g | |

**Sampling Plan:**

The terms n, c, m and M used in this standard have the following meaning:

n = Number of units comprising a sample.

c = Maximum allowable number of units having microbiological counts above m.

m = Microbiological limit that may be exceeded number of units c.

M = Microbiological limit that no sample unit may exceed.

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# QUALITY AND SAFETY ISSUES IN SMOKED FISHERY PRODUCTS

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## Introduction

Smoking is an age-old fish preservation technique. Smoking is a process of exposing fish to smoke from smouldering wood or plant materials to introduce flavour, taste and preservative ingredients into the fish. This process is characterised by the combination of salting, drying, heating and smoking. Due to drying effects and antioxidant and bacteriostatic effects of the smoke, the smoked products have extended shelf-life. The fish is subjected to smoke produced through the incomplete combustion of wood in the form of sawdust or woodchips. Smoke is a highly complex mixture of chemicals like organic acids, alcohols, ammonia, carbon dioxide, carbon monoxide, carbonyls, esters, furans, hydrocarbons, lactones, nitrogen oxides, particulates, phenols and the interaction between smoke and the flesh surface is responsible for characteristic golden-yellow colour, odour, flavour and preservative effect.

Depending upon how the smoke is delivered into the food and smoking temperature, four basic types of smoking can be defined: hot smoking, cold smoking, liquid smoking, and electrostatic smoking. Hot smoking is the traditional smoking method using both heat and smoke, which usually occurs at temperatures above 70 °C. For hot smoked fish and fish products, a minimum thermal process of 30 min at or above 62.8 °C is required by USFDA. Therefore, after hot smoking, products are fully cooked and ready for consumption. In cold smoking the core temperatures do not exceed 30 °C. Thus, cold smoked products are not cooked and typically heavily salted. Compared to hot smoking, cold smoking runs longer, has a higher yield and retains the original textural properties much better than the hot-smoked ones. Liquid smoke is smoke condensate that is dissolved in a solvent, such as water or oil. Liquid smoke can be used directly on products by dipping or spraying. It is rapid and much easier to achieve a uniform smoke flavour than traditional cold and hot smoking processes, although the flavour and colour from the traditional smoking cannot be exactly duplicated. However, the application of liquid smoking may be expensive compared to other methods. Electrostatic smoking is another rapid way to smoke fish where fish are sent into a tunnel where an electrostatic field is created. Smoke particles are given a positive charge and deposit onto the surface of the fish which are negatively charged.

The codex standard deals with smoked, smoke-flavoured and smoke-dried fish prepared from fresh, chilled or frozen raw material. Smoked fish is prepared from fish that has undergone a hot or cold smoking process. The smoke must be applied either through smoking or smoking by regenerated smoke or application of smoke condensates. The end product must have smoked sensory characteristics. It allows for the use of spices and other optional ingredients. Whereas smoke-flavoured fish is prepared from fish that has been treated with smoke flavours, without undergoing a smoking process. It must have a smoked taste. For smoke-flavoured fish the spices and other optional ingredients can be used. On the other hand, smoke-dried fish is prepared from fish that has undergone a combined smoking and drying process and may include a salting process. The smoke must be applied through a smoke-drying process traditional for the respective country or an industrial smoke-drying process and the end product must have smoke-dried sensory characteristics. Spices and other optional ingredients can be used.

**Potential major hazards associated with smoking of fish and fishery products**

***Biological hazards***

*1) Listeria monocytogenes:* Typical temperature used for cold smoking is 22-28˚C. However, this temperature is not sufficient to eliminate the risk from *Listeria monocytogenes*, a gram positive, facultative anaerobic, psychrotropic bacteria causing deadly septicaemia, meningitis, spontaneous abortion, and foetal death in adult human beings. Comparatively high temperature used in hot-smoking process and long-time of exposure to that temperature (60-70˚C for 2-3 h) can inactivate the *L. monocytogenes* effectively. At the same time listericidal process should be validated to ensure that the treatments are effective and can be applied continuously. But the hot smoked products are susceptible to post-process contaminations from many of the micro-organisms due to improper handling and storage of the products. Sufficient heat treatment, proper hygienic handling and cold chain maintenance during distribution can reduce the risk of biological hazards in smoked fish and fishery products.

*2) Clostridium botulinum:* The toxin produced by *Clostridium botulinum* can lead to botulism, serious illness and death to the consumer. Even a few micrograms of intoxication can lead to ill-health with symptoms like weakness, vertigo, double vision, difficulty in speaking, swallowing and breathing, abdominal swelling, constipation, paralysis and death. By achieving proper salt concentration in processed fish, proper refrigeration during storage and reduced oxygen packaging like Modified Atmosphere Packaging (MAP) of the products can prevent

the occurrence of *C. botulinum* in smoked fish and fishery products. Salt along with smoke effectively prevents the toxin formation. In cold smoked fish and fishery products, which undergoes mild heat processing, the presence of spoilage organisms prevents the growth of *C. botulinum* and toxin production. Whereas in hot-smoked products, high temperature application causes damages to spores of *C. botulinum* thus prevents the toxin formation. Same process also prevents the prevalence of spoilage organisms and thus extends the shelf life of the product. Thus, the time-temperature combination for smoking, along with salt concentration plays critical roles in safety and quality aspects of the smoked fish and fishery products.

*3) Parasites:* Presence of parasites like nematodes, cestodes, trematodes and any other extraneous matter can be considered as hazard. Particular 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.

***Chemical hazards***

*1) Polycyclic Aromatic Hydrocarbons (PAHs):* PAHs are large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. Incomplete combustion(pyrolysis), during smoking can lead to formation and release of PAHs into the smoked product. Some of them are carcinogenic and mutagenic substances causing serious health issues to the consumers. Processing procedures such as smoking, drying, roasting, baking, frying and barbecuing/grilling can lead to formation of PAHs in food items. Many reports indicate that individual PAHs in smoked fish can go up to a level of 200µg/Kg. Among the 33 PAHs evaluated by the Scientific committee on Food (SCF, 2002) of EU, 15 were found to be having mutagenicity/Geno toxicity in somatic cells of experimental animal in-vivo. They are benzo[a]anthracene, benzo[b]-, benzo[j]- and benzo[k]fluoranthene, benzo[ghi]perylene, benzo[a]pyrene, chrysene, cyclopenta[cd]pyrene, dibenz[a,h]anthracene, dibenzo[a,e]-, dibenzo[a,h]-, dibenzo[a,i]-, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene and 5-methylchrysene. The carcinogenic and genotoxic potentials of PAH are largest among the high molecular weight PAH, i.e., compounds with 4 rings or more. Among that benzo[a]pyrene regarded as potentially genotoxic and carcinogenic to humans. They can cause long-term adverse health effects following dietary intake of PAH.

The PAH contamination in smoked products can be significantly reduced by using indirect smoking process instead of direct smoking of the fish. In indirect smoking, the smoke generated

in an external smoking kiln, under controlled conditions, is used for smoking process. The smoke produced can be even, washed before coming into contact with the food material processed. In addition to that, use of lean fish for smoking, and cooking at lower temperature for longer time can also reduce the PAH contamination significantly. If the smoke condensate is used for smoking, usage of smoke condensate from reputed reliable resources approved by competent authority can effectively reduce the occurrence of PAH contamination in the final product. The formation of PAH in smoked fish can be minimised by following Code of Practice for the Reduction of Contamination of Food with Polycyclic Hydrocarbons (PAH) from Smoking and Direct Drying Processes (CAC/RCP 68-2009) given by Codex Alimentarius Commission.

*2) Histamine*: Histamine poisoning is associated with Scombroid fishes and other dark meat fishes. These fishes having high content of free histidine, which during spoilage are converted to histamine by bacteria like *Morganella morgani*, *Klebsiella pnuemoniae* and *Hafnia alvei*. Histamine is heat stable, even cooking or canning cannot destroy it. Presence of other biogenic amines like cadaverine and putrescine will act as potentiators for histamine production. As per Codex standards, the maximum allowable histamine content in smoked fishes is 200 mg/Kg for species like Scombridae, Clupeidae, Engraulidae, Coryphaenidae, Pomatomidae, and Scomberesocidae. Low temperature storage (< 4°C) of fishes right from catch can effectively reduce the production of histamine in fishes.

*3) Biotoxins*: Biotoxins causing a number of food-borne diseases. The poisoning due to biotoxins are caused by consuming finfish/shell fish containing poisonous tissues with accumulated toxins from plankton they consumed. Paralytic shellfish poisoning (PSP), diarrheic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), and neurotoxic shellfish poisoning (NSP) are mostly associated with shellfish species such as oysters, clam and mussels. The control of biotoxin is very difficult. They cannot be destroyed by any of the processing methods like cooking, smoking, drying or salting. Environmental monitoring of plankton and proper depuration process of the bivalves only can reduce the occurrence significantly.

**The safety and quality issues encountered during the production of smoked products during various steps of its production**:

*1) Selection of raw material*

Top-quality fish are needed to produce a top-quality smoked product. The important step in proper fish handling is to quickly bleed, clean and chill the fish. Generally, the fish shouldn't be accepted if it is known to contain parasites, undesirable microorganisms, pesticides, veterinary drugs or toxic, decomposed or extraneous substances harmful to human health. When fish and shellfish is found to be unfit for human consumption it should be appropriately handled- processed/ disposed. Temperature is the most important factor affecting the rate of fish and shellfish deterioration and multiplication of microorganisms. For species prone to scombrotoxin production, temperature should be effectively controlled to prevent the formation of histamine.

*2) Salting/Brining*

Proper salting is the key step for the flavour and safety of the smoked fish product. It brings the taste and also reduces the water activity ($a_w$) in the product, so that bacterial growth can be inhibited in the smoked fish. Salting is done commonly as dry salting or brining. Efficiency of salt penetration into the fish tissue is affected by several factors, such as species, physiological state of fish (rigor), fish quality (fresh/frozen) fish dimension (thickness), brine concentration, brine time, brine to fish ratio, brine temperature, fat content, texture, etc.

The major hazards and defects include microbiological, chemical and physical contamination, scombrotoxin, undesired texture, decomposition/ physical damage. While salting, it should be taken care that fish size is uniform. To ensure a uniform salt distribution throughout the fish, it should be equilibrated under refrigeration conditions. The time-temperature selected for salting/ brining should not allow for the development of histamine in relevant fishes. The fish should not be subjected to temperature abuse. Brine should be prepared from food grade salt and water of potable quality. Monitoring of salt content of brine and it should be replaced at regular interval. Reuse of brine should be avoided and if it is to be recycled brine must be appropriately processed to minimize microbiological hazards. The vats and other equipments used during the process should be corrosion resistant.

*3) Pre-drying, Hanging and Racking*

After brining fish is rinsed and air dried before smoking. Drying will help smoke deposit evenly on the fish surface during smoking since smoke does not deposit well on a wet surface. Drying process, gives a nice coating on fillets to help seal in moisture, natural juices, flavours and provides a better-looking finished product like glossy skin forms on the cut surface pellicle. In cold smoking, a certain amount of drying prior to smoking helps in producing the pellicle.

The hot smoking can be carried out immediately after racking because pellicle is destroyed by heat. Racking aids in the formation of the pellicle and reduces drying time during the smoking. It provides maximum exposure of the fish to the smoke. For this purpose, racks are more widely used today, but some products still require the use of speats, or spits, to be threaded through the neck, eyes or the gill and mouth of the hanging fish.

The main hazards/ defects encountered are microbiological contamination by *Staphylococcus aureus*, fungal contamination, scombrotoxin, physical contamination, physical damage and decomposition. During the racking and hanging operation, fish should be hung in manner that allows for adequate and smooth flow of air/ smoke. Since, pathogen *S. aureus* gets competitive advantage via brining, it is essential to adhere to strict time-temperature and sanitation controls to avoid risk of contamination and microbial growth. The drying should ensure that loss of water makes it stable during smoking, further excess loss of waters should be avoided for good texture. The drying should be carried out under controlled conditions of air flow, temperature and humidity. To avoid microbial growth and scombrotoxin formation, prolonged exposure to ambient temperature should be avoided.

*4) Smoking and smoke flavouring*

The main hazards and defects encountered during cold and hot smoking are natural toxins, impregnated material in wood, paints, chemicals, undesirable flavour, parasites and microbiological contamination, chemical contamination from smoke, tar and ash, poor colour, flavour and texture, growth of *C. botulinum, L. monocytogenes*, scombrotoxin and decomposition.

It should be taken care that the plant material/ wood used for smoking should be free from natural toxins, paints, chemicals, fungal growth and should be stored in dry environment. The guidelines regarding PAH reduction i.e., Code of Practice for the Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAHs) from Smoking and Direct Drying Processes (CXC 68-2009) should be carefully followed. It is essential to control and monitor the smoking process time and temperature so as to avoid microbial contamination and scombrotoxin formation in susceptible species. This helps in effective control of *L. monocytogenes* and damage to the spores of non-proteolytic *C. botulinum*. Hot smoking temperature should reach at thermal centre of the product. In the cold smoking process the temperature of the products is kept below the coagulation temperature for the proteins of the flesh of the fish. The process should be monitored to achieve the desired colour, taste and

texture. The smoking process should be carried out under hygienic conditions and smoking time should be validated for enough reduction in water content of the product.

The smoke-dried fish is either ready-to-eat or rehydrated before consumption. The fish for smoke-drying should be sufficiently dried to reduce the water content of the skin and flesh for uniform distribution of smoke over product surfaces. Time and temperature of the smoke-drying process should be monitored to achieve the desired texture, water activity and reduce the generation of PAHs. The smoking and drying process should be carried out until final moisture content of the product is less than 10 % or water activity is below 0.75. The cooling of the smoke-dried product should be done under controlled conditions. the packaging should give sufficient protection to the product from moisture absorption. The package should be clearly labelled regarding storage and preparation before consumption.

The smoke flavour can be applied in many ways and at different stages. The heating is optional for this type of product. The smoke flavour treatment should be given in a manner which prevents the formation of scombrotoxin as well as controls the microbial growth. Smoke flavour should not be used to mask poor quality fish. The smoke condensate should be obtained from a reliable and approved source and applied with regards to regulatory approval. Dilutions done, if any, must be with food grade material or potable water as per regulatory approval.

*Cooling/ Slicing/ Packaging/ Storage*

The major hazards and defects during the steps are- microbiological contamination, scombrotoxin formation, survival of parasites, poor taste and texture, decomposition, freezer burn and undeclared allergens. Cooling should be done in a controlled environment to avoid cross contamination and rapid enough so as to minimize the microbial growth. Before slicing the smoked fillets should be cold tempered to facilitate mechanical slicing. The hygiene and sanitation of cutters/ blades and belts is critical to avoid contamination. All smoked products, whether hot or cold smoked, require slow cooling to room temperature, immediately followed by chilling to 4 °C, and if required, quick freezing and storing at -18°C. Smoked products can be vacuum packed, shrink packed, canned/retorted, packed in wooden boxes, MAP/ CAP packed. Smoked products may be chilled or frozen prior to packaging. Reduced oxygen packed products should have additional hurdles like freezing, refrigeration, lower water activity to avoid growth of *C. botulinum*. In case of MAP/ CAP regular monitoring is deemed suitable. The time-temperature required for freezing should be enough to kill the parasites. During the storage regular monitoring of the storage temperature is essential. It also aids in controlling the

microbiological growth i.e., *L. monocytogenes, C. botulinum* and other pathogens like *S. aureus*. The label should include the storage temperature, shelf-life, other handling and storage conditions for safety and quality. The label should also have instructions regarding thawing conditions and usage.

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# QUALITY ISSUES IN THERMALLY PROCESSED FISHERY PRODUCTS

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## Thermal processing

Thermal processing can be subdivided into several more or less overlapping groups based on temperature regime, method or equipment for thermal processing, fish species, packaging method or the microbial target of the process. The number of micro-organisms (either vegetative cells, bacteria or spores) present in the food is reduced by subjecting it to heat for sufficient time to ensure food safety or to reduce spoilage and increase shelf life. Sterilization is the classical method. The temperature regime during processing may vary from 110 to 135 $^{o}$C. Much of the analysis of thermal processing has been developed for foods placed in metal containers, usually the cylindrical metal can made from thin tin-plated steel or aluminium, and heated by steam. This process is often referred to as 'canning'. Pasteurisation is a form of thermal processing. Pasteurisation is a milder treatment than commercial sterilisation and therefore does not give a safe shelf-stable product without subsequent storage at refrigerated temperature.

## Low acid canned foods and *Clostridium botulinum*

*Clostridium botulinum* is a highly heat resistant mesophilic, Gram-positive, rod-shaped spore-forming anaerobic pathogen, which produces the toxin botulin. Growth of *C. botulinum* is a risk in 'low acid canned foods' (LACF) having a pH above 4.6 including fishery products, where it is necessary to apply a time–temperature regime sufficient to inactivate spores of *C. botulinum*.

## Commercial sterility

Canned fishery products are packed in hermetically sealed containers and shall have received a processing treatment sufficient to ensure commercial sterility. Commercial sterility is a 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 (FAO/WHO Codex Alimentarius Commission, 1983).

**Spoiled cans-Types**

1. **Flipper**: The can may be normal in appearance. But, when such a can is hit on the table, can end flips out and becomes convex. When the convex end is pressed, it becomes flat again. A flipper is the initial stage of a swell, but may also be caused by overfilling or lack of vacuum/under exhausting.
2. **Springer:** One end of the can remains permanently convex and if this end is pressed down, the other end flips out.
3. **Soft swell**: Permanently convex can ends and get depressed due to pressure by fingers.
4. **Hard swell**: Permanently convex can ends and do not get depressed due to pressure by fingers.

**Causes of spoilage**

1. **Pre-spoilage or incipient spoilage**: Takes place before the product or the ingredients are thermally processed. Caused by microbial or enzymatic action resulting in gas accumulation, development of off-odours and the presence of excessive numbers of dead microbial cells in the end product.
2. **Under processing**: The product did not receive sufficient heat treatment to become commercially sterile.
3. **Thermophilic spoilage**: Occurs, when the time-temperature conditions are conducive to the growth of thermophilic bacteria. Prevention of thermophilic spoilage can be achieved by cooling the retorted cans rapidly to reach a temperature <40 $^{o}$C and storing finished products at less than 35 $^{o}$C to inhibit the growth of any surviving thermophiles.
4. **Post-process spoilage**: Post-process contamination or leaker spoilage takes place, when microbial contaminants leak into the can after heat sterilization, due to failure of the container to maintain hermetic seal.

**Types of spoilage**

1. Microbial spoilage: It is caused almost entirely by heat resistant microorganisms.
    a. **Gaseous spoilage:** Swelled or bulging can end is the common indication of gaseous decomposition. Gas-forming heat-resistant organisms belong to *Clostridium sp*.
    b. **Non-gaseous spoilage/flat souring:** Some bacteria (e.g., *Geobacillus stearothermophilus*) do not produce gas, when it spoils food. There is no external indication of non-gaseous spoilage, but the product is sour in taste.

2. Chemical spoilage: Hydrogen produced due to internal corrosion leads to can swell
3. Physical Spoilage: Occurs due to faulty retort operation, under exhausting, over filling & high vacuum in tall cans leads to panelling

**Common quality problems/defects in canned fishery products**

1. **Struvite formation:** Struvite is Magnesium ammonium phosphate hexahydrate [$MgNH_4PO_4.6H_2O$]. It appears as glass-like crystals in some canned fishery products such as brine packed shrimp, crab or tuna, particularly when the storage temperature is low. Use of hard water, stale raw material and presence of magnesium in salt/sea water used in canning are responsible for the formation of these crystals.
2. **Sulphide blackening:** The natural compounds in food can react with the metal in the lid to form black deposits**.** It is usually seen in canned shrimp, lobster, crab etc.
3. **Curd and adhesion:** Curd is salt soluble coagulated or precipitated protein, which is often found at the top of canned Salmon or Mackerel. The curd may adhere to can surface and the lacquer may get peeled off when the curd is removed**.** Use of less fresh fish & inadequate brining or precooking lead to curd formation.
4. **Copper sulfide/Blue discoloration:** This is commonly associated with canned crab meat. Copper in the haemocyanin of crab haemolymph react with sulfides formed in heat processing resulting in the formation of blue copper sulfide.
5. **Honey combing:** The meat in the can resembles a honey comb, when stale raw materials are used for canning. This was originally found in canned salmon but occurs in other products such as tuna and sardines.
6. **Retort burn:** This is usually associated with canned shell fishes like clam, mussel or oyster. This occurs due to insufficient filling medium to cover the solid food completely and the top is left dry.
7. **Case hardening:** Surface of fish meat gets dehydrated and hard cover is formed on the surface of meat. This is caused by high heat process and too quick heating process.
8. **Softening in shrimp:** The canned shrimp becomes soft due to decomposition of protein to soluble non-protein components. This can be avoiding by using fresh raw material and maintenance of high level of sanitation.
9. **Mush:** This is flabby condition seen in some species of pilchards caught at the end of its spawning season. This is caused by the invasion of the parasitic protozoan *Chloromyxum.*
10. **Miscellaneous aspects**: Externally rusted cans, damaged cans & cans with severe dents on the body.

**Undesirable changes in canned fish/Influence of canning on the quality of fish**

1. Colour changes
2. Degrades flavour and many fishes become soft in texture.
3. Denaturation, coagulation and precipitation of protein affects its digestibility & nutritive value.
4. Degradation of carbohydrates, which undergo caramelization at high temperatures.
5. Appreciable loss of fat-soluble vitamins A and D, if heated in the presence of oxygen.
6. Fat oxidation/rancidity, if there is no sufficient vacuum in the can.

**Codex Alimentarius International Food Standards** (FAO and WHO. 2020. Code of Practice for Fish and Fishery Products. CODE OF PRACTICE, CXC 52-2003)

**Processing of canned fish, shellfish and other aquatic invertebrates- Identification of hazards:**

1. Biological hazards
   -Naturally occurring marine toxins
   -Scombrotoxin/Histamine
   -Microbiological toxins: *Clostridium botulinum, Staphylococcus aureus*
2. Chemical hazards: components of the containers (e.g., lead) and chemical products (e.g., lubricants, sanitizers, detergents)
3. Physical hazards: Materials such as metal or glass fragments

**Critical Control Points (CCPs)**

According to ICMSF, a CCP may be a location, procedure or processing step at which hazards can be controlled. Two types of CCPs may be identified: CCP-1 that will ensure full control of a hazard and CCP-2 that will minimize but not assure full control. Within the context of HACCP, the meaning of "control" at a CCP means to minimize or prevent the risk of one or more hazards by taking specific preventative measures (PM).

**Hazards and preventive measures in production of low–acid canned fish**

| **Product flow** | **Hazard** | **Preventive measure** | **Degree of control** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Reception of raw material at factory (fish and cans) | Substandard quality entering processing | Ensure reliable source<br>Sensory evaluation | CCP–2 |
| Primary processing | | | |
| Filling of cans | Uncontrolled heat penetration during thermal processing | Avoid inclusion of air, control weights of solids, liquids, product density and headspace | CCP–2 |
| Evacuation, seaming | Recontamination | Standards of closures must be checked at regular intervals. | CCP–2 |
| Thermal processing | Survival of pathogens | Time x temperature (Txt) control. | CCP–1 |
| Cooling | Recontamination | Quality of cooling water. Chlorine level > 1–2 ppm. | CCP–2 |
| Handling of filled (wet) cans | Recontamination | Handling of warm, wet cans must be avoided.<br>Can handling should be designed to minimize mechanical shock. | CCP–2 |
| Storage and distribution | | | |

**Technical guidance for quality maintenance**

Quality of fish should be ensured by purchasing them from a reliable source and also by sensory evaluation. Quality of the cans should be confirmed by a documented quality assurance system by the can manufacturer. Correct filling is important to ensure proper heat penetration during thermal processing. Avoid inclusion of air, control weights of solids, liquids, product density and headspace. Standards of can closures must be checked at regular intervals to prevent recontamination during evacuation and seaming. The thermal processing is a Critical Control Point (CCP)–1 for eliminating all pathogenic organisms. Time x temperature (Txt) control is a measure to prevent survival of pathogens. Rapid cooling of canned fish and shellfish avoids the formation of struvite crystals. Struvite formation may also be prevented by the addition of chelating agent such as sodium hexametaphosphate or EDTA. Quality of water used for can cooling is very important to avoid recontamination. It must be chlorinated (Chlorine level > 1–2 ppm). Handling of warm, wet cans must be avoided. Sulphide blackening

can be minimized by uniform lacquering of can and its careful handling for avoiding exposing of iron. Blue discolouration can be reduced by thorough bleeding of crab while dressing and use of chelating agent in the brine. Maintenance of proper acidity inside the can and use of parchment lining inside the can may also minimize sulphide blackening and blue discolouration. Curd formation can be avoided by cold blanching of fish in 10-15 % brine for 20-30 minutes and subsequent washing. Use of fresh raw material and slow thawing of frozen tuna without rough handling can reduce honey combing. Retort burn can be prevented by using sufficient filling medium to cover the solid food in the can. Case hardening can be prevented by adopting proper thermal processing process. Softening of shrimps can be avoided by using fresh raw material and maintenance of high level of sanitation.

**Food Safety and Standards Authority of India (FSSAI)**

Food Safety and Standards Authority of India (FSSAI) is an autonomous body established by the Government of India under the Ministry of Health & Family Welfare. It usually sets standards for food so that there is no chaos in the minds of consumers, traders, manufacturers and investors. As per Section 31(1) & 31(2) of FSS Act, 2006, every Food Business Operator in the country is required to be licensed/registered under the Food Safety & Standards Authority of India.

**FSSAI (2011) standards for canned fishery products-Decomposition**

The raw material (fish) shall not contain more than **100 mg/Kg of histamine** based on the average of the sample unit tested. This shall apply only to species of fish with potential to form hazardous level of histamine as mentioned in Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011.

**FSSAI (2011) standards for canned fishery products-Final product**

| **Sr. No.** | **Characteristics** | **Finfish** | | | | **Crustaceans** | | **Molluscs** | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Tuna** | **Mackerel** | **Sardine** | **Pomfret/ Seer fish** | **Shrimp / Prawn** | **Crab** | **Mussel** | **Squid** |
| 1. | Medium | Oil | Oil Brine Curry Tomato Sauce | Oil Brine Curry | Oil | Brine | Brine | Oil | Brine |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 2. | Drained wt. as % of water capacity* | 70 | 65 | 70 | 66 | 64 | 65 | 65 | 64 |
| 3. | % of water in the drained liquid** | 5 | 10 | 10 | 10 | | | 5 | - |
| 4. | Disintegrated portion as % of drained weight (max) | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 5. | Vacuum (Minimum) | For round cans 100 mm and negative pressure in flat cans | | | | | | | |
| 6. | Head Space | 5-10 mm | | | | | | | |
| 7. | Can Exterior | shall not be rusted, dented or bulged | | | | | | | |

*A tolerance of ±5 percent is permitted, ** Only applicable for oil medium.

The percentage of sodium chloride in the final product of sardine and mackerel shall be 3.5 percent in the case of brine treated cans. The acidity of brine as citric acid anhydrous shall be between 0.06 and 0.20 percent (m/v).

**Microbiological Requirements for Thermally Processed Fishery Products**

**-Hygiene Indicator Organisms**

| **Product Category*** | **Aerobic Plate Count** | | | | **Stage where criterion applies** | **Action in case of unsatisfactory results** |
|---|---|---|---|---|---|---|
| | Sampling Plan | | Limits (cfu/g) | | | |
| | n | c | m | M | | |
| Thermally Processed Fishery Products | Commercially Sterile** | | | | End of Manufacturing process | Revalidation of thermal process |
| **Test method** | IS: 5402/ISO 4833 | | | | | |

**Commercial sterility should be established as per APHA (2015). Canned Foods—Tests for Commercial Sterility. Compendium of Methods for the Microbiological Examination of Food

**Microbiological Requirements for Thermally Processed Fishery Products – Safety Indicator Organisms**

| **Product Category*** | ***Clostridium botulinum*** | | | |
|---|---|---|---|---|
| | Sampling Plan | | Limits (cfu/g) | |
| | n | c | m | M |
| Thermally Processed Fishery Products | Absence of viable spores or vegetative cells of *Clostridium botulinum* | | | |
| **Test method** | IS: 5887, Part 4 or ISO 17919 | | | |

**Sampling Plan**: The terms n, c, m and M used in this standard have the following meaning: n = Number of units comprising a sample. c = Maximum allowable number of units having microbiological counts above m. m = Microbiological limit that may be exceeded number of units c. M = Microbiological limit that no sample unit may exceed.

**FSSAI standards for Ready–to-Eat Finfish or Shell Fish Curry in Retortable Pouches**

**Decomposition**

The total volatile base nitrogen (TVBN) level of raw material (fin fish or shell fish) should not exceed 35 mg/100g.

**Final Product**

The finished product shall have the odour, flavour and colour characteristic of the product. The bones shall be soft and yielding. The contents of the pouch on opening shall not display any appreciable disintegration. Pieces from which portions have separated out would be treated as disintegrated units. The percentage disintegrated portions of the fish, calculated on the basis of the drained mass shall not exceed 5 % based on the average of five pouches. The product shall be free from foreign materials such as sand, dirt and insects, objectionable odour or flavour. The residual air in the pouch after processing shall be less than 2 % of the volume of the pouch contents. The average proportion of fish to curry in retort pouch shall be in the ratio of 60: 40. The percentage of salt in the product shall be 1 % to 2 %, maximum.

**Processing**

The material shall be packed in retortable pouches, exhausted or vacuumized and heat-sealed. Exhausting can be done either by steam injection or hot filling to achieve residual air level of less than 2 %. Processing (Retorting) shall be done in over pressure autoclave till the product reaches a $F_0$ value of 8-10 minutes at the slowest heating point. The water used for cooling of retort pouches shall be as per IS 10500:2012 standards and chlorinated to maintain free residual chlorine of less than 2 mg/l.

**Packaging and Labelling**

The retort pouches shall be packed in suitable retail containers to prevent physical impact during transportation. Retort pouch materials of food grade quality having the configuration of polyester/aluminium foil/cast polypropylene or four layers consisting of polyester/aluminium foil or aluminium oxide/nylon and cast polypropylene may be used. Other suitable packaging materials which can withstand high temperature and pressure can also be used. The pouches shall be of food grade quality. The retort pouch shall have the mechanical properties as under:

| Sr. No. | Characteristics | Requirement |
|---|---|---|
| 1. | Tensile strength (Kgf/15 mm) machine direction | 3.0-5.25 |
| 2. | Bond Strength (Kgf/15 mm) | 0.225 – 0.750 |
| 3. | Heat seal strength (Kgf/15 mm), Min | 4.60 |
| 4. | Bursting strength ($Kg/cm^2$), Min | 1.74 |

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# QUALITY ISSUES WITH CONVENIENCE FISHERY PRODUCTS

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**Introduction:**

Convenient foods are becoming popular among all classes and ages of people across world. Convenience foods has reduced the preparation time which can be eaten directly or using some heating process. Some of the popular convenient foods include Masala Oats, Corn flakes, canned soup, frozen foods, baked products etc. Most of the convenience foods take very less time to cook. They are often prepared and packaged well for quick and easy thawing or heating the food.

Fish and shellfish convenient products are becoming more popular because of added advantages of human health significant factors. Although this is perishable commodity but they are generally designed so that their tastes remain with long shelf-life. Most of the convenience foods have become very popular because they can be served as a quickie snack or meal. Convenience foods availability on the shelves in super markets has reduced time in the kitchen with less preparation time, fewer leftovers and easy clean up.

The seafood market size was valued at $159,311.9 million in 2019. The fish segment was the highest contributor to the market, with $101,526.2 million in 2019. Based on the application, the retail segment was the leading segment in the seafood market. Asia-Pacific holds the maximum share of the seafood market. The growth of the seafood market can be attributed awareness of the health benefits of seafood and change in lifestyle of consumers. Worldwide per capita fish consumption is 20.5 kilograms per year.

**Types of Convenient foods:**

The convenient products are generally classified into following 04 types of products

1) Convenience products
2) Shopping products
3) Specialty products

4) Unsought products

**Advantages of Convenience foods:**

- Less preparation time and easy presentation and
- Easy cleaning up and hardly get any leftovers remains
- No storing, buying or planning of ingredients.
- Various types of food items especially for inexperienced cooks can be relished.
- Less spoilage and waste occur with packaged convenience foods products.
- Transportation of packaged foods is cheaper especially in concentrated form.
- Cost efficient for mass production and distribution.

**Disadvantages of Convenience foods:**

- Specific need of individual as of homemade may be missed.
- Cooking time is sometimes increased for thawing or longer baking time.
- Control fat, salt and sugar content may be difficult.
- Cost per serving is generally higher than homemade.
- Convenience may lack freshness of fish
- They tend to lack fibres.

**Driving factor for Convenience Foods**

A busy lifestyle due to work, people doesn't have a lot of time to prepare food at home. As there are greater time constraints from work, commitments, and commutation, an individual often prefer for convenience foods. Convenience foods are defined as types of foods that save time in procurement, preparation, and cleanup. Although these convenience foods save time, they tend to have lower nutritional values and can be more expensive.

There are few factors which results people for convenience foods include

- time constraints due to work pressure
- Increased purchase power

- Better food preparation environment, and
- Better healthy options availability on shelves

**Convenience Fishery Products**

As per FSSAI (India), Convenience Fishery Products are tertiary food products made of fish, which are in ready to eat form and also includes snack based items prepared from fish and fishery products meant for direct human consumption such as extruded fishery products, fried items namely fish wafers, crackers, fish cutlets, fish burgers and other such products. These products can be consumed directly after minimal handling and processing.

This category includes Sous-vide cooked products, surimi-based products cooked (in-pack), pasteurized crab meat, pasteurized molluscs which are distributed as refrigerated, but meant for direct human consumption with minimal or no cooking.

Ready to eat form and also includes snack based items prepared from fish and fishery products meant for direct human consumption such as extruded fishery products, fried items namely fish wafers, crackers, fish cutlets, fish burgers and other such products.

**Changes in Physicochemical Properties and Sensory Quality in Seafood Products**

- Color changes that may occur during cooking are mainly attributed to protein denaturation
- Textural changes occurred during sous-vide cooking and non-optimized process

**Changes in Nutrients and Phytochemicals Seafood Products**

- Loss of macro and micronutrients along with other significant nutritional factors like antioxidants.
- Loss of aromatic volatile compounds
- Loss to juiciness and tenderness which may affect the overall sensory attributes of seafood.

**Microbiological Concerns of Sous-Vide Seafood Product**

Microbiological deterioration in perishable products such as seafood occurs rapidly due to neutral pH, high water activity, and nutritional composition. Considering seafood safety pathogenic bacteria can be classified into three groups.

(i) Natural inhabitants of the consumed species, such as *Vibrio* spp., *Clostridium botulinum* and *Aeromonas* spp.

(ii) Environment bacteria such as *Listeria monocytogenes*, *Clostridium botulinum* and *Clostridium perfringens*

(iii) Inhabitant of man or animals such as *Salmonella* spp., *Shigella* spp., *Escherichia coli*, and *Staphylococcus aureus*.

**Mitigation measures to prevent quality issues:**

Protein denaturation in cooking results in color changes can be prevented by optimizing time and temperature. High pressure processing (HPP) can be an alternative use of moderate pressures significantly influenced the texture and color of seafood products.

Plastic foil can prevent the loss of aromatic volatile compounds and water that may retain juiciness and tenderness of the products, and hence sensory attributes enhanced.

Heat is known to be lethal to microorganisms, but different species has its own particular heat tolerance, and there are many factors affecting their thermal resistance. The process is dependent both on the exposure time and on temperature required to achieve the desired death rate. Therefore, it is essential to determine the thermal death kinetics (D and z-values) of target bacteria in different food substrates and to characterize the time durations. Insufficient heat treatment is the major problem which can be combined with the use of natural antioxidants to improve the efficiency of cooking process in terms of food safety during storage.

Fresh or minimally processed foods of high quality with the minimum amount of additives, nutritious healthy and microbiologically safe, are in demand among consumers. Hurdle technology advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors (hurdles) that microorganisms are unable to overcome. The most important hurdles used in food preservation are temperature (high or low), water activity

(aw), acidity (pH), redox potential (Eh), preservatives (e.g. nitrite, sorbate etc.) and competitive microorganisms (e.g. lactic acid bacteria).

**Regulatory requirement for Convenience Fishery Products (FSSAI, 2021)**

Microbiological specification for Convenience Fishery Products have been mentioned in Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011. Item No. 15 of Microbiological Requirements for fish and fishery products need to be considered which are mentioned as follows:

**a. Hygiene Indicator Organisms**

| **Aerobic Plate Count** | | **Coagulase positive Staphylococci** | | **Yeast & mold count** | | **Stage where criterion applies** | **Action in case of unsatisfactory results** |
|---|---|---|---|---|---|---|---|
| Sampling Plan | Limits | Sampling Plan | Limits | Sampling Plan | Limits | | |
| 5/2 | $1x10^3$/ $1x10^4$ | 5/2 | $1x10^2$/ $1x10^3$ | - | - | End of Manufacturing process | Improvement in hygiene; Time Temperature control of batter mix |
| IS: 5402/ISO 4833 | | IS 5887 : Part 2 or IS 5887 Part 8 (Sec 1)/ | | | | | |

**b. Safety Indicator Organisms**

| ***E. coli*** | | ***Salmonella*** | | ***V. Cholerae* (O1 and O139)** | | ***L. monocytogenes*** | | ***C. botulinum*** | |
|---|---|---|---|---|---|---|---|---|---|
| Sampling Plan | Limits | Sampling Plan | Limits | Sampling Plan | Limits | Sampling Plan | Limits | Sampling Plan | Limits |
| 5/2 | 1/10 | 5/0 | Absent/ 25 g | 5/ 0 | Absent/ 25 g | 5/ 0 | Absent/ 25 g | - | - |

| | | | | | |
|---|---|---|---|---|---|
| IS: 5887 Part 1 or ISO 16649-2 | IS: 5887 Part 3/ ISO 6579 | *Vibrio*, Bacteriological Analytical Manual, Chapter 9. USFDA BAM Online, May | IS: 14988, Part 1&2/ISO 11290-1 &2 | | |

## European Chilled Food Federation (ECFF)

ECFF Recommendations provide guidance on process design and hygienic principles related to the manufacture of chilled prepared foods (hereafter referred to as chilled foods), with emphasis on those procedures designed to control the risks associated with bacteria that cause food-borne diseases.

The safety, with respect to *Clostridium botulinum*, of chilled foods that have been mildly heated in hermetically sealed packages or heated and packed without recontamination can be assured by:

- A minimum heat process and strict limitation of chill shelf life or, for longer life products, by storage below 3°C,
- Heat treatment sufficient to deliver a 6 log reduction in numbers of spores of psychrotrophic strains of *C. botulinum* and storage below 10°C, or
- Intrinsic preservation factors shown to be effective in modeling or inoculated pack/challenge tests.

**Cooking and Pasteurization (Cooking Model)**

| | | | |
|---|---|---|---|
| **Cooking** | Pathogenic bacteria survival | Minimum cook time: 2.5 minutes<br>Minimum cook temperature: 210°F (98.9°C)<br>Note: To achieve a 6D reduction of *L. monocytogenes*<br>Maximum shrimp size: 40 count/pound | • Scientific study establishing the thermal process (process validation)<br>• Check the data logger for accuracy and damage and to ensure that it is operational before putting into operation; check it daily, at the beginning of operations; and calibrate it once per year |

| | | | |
|---|---|---|---|
| | | | • Calibrate the scale monthly<br>• Review monitoring, corrective action and verification, records within1 week of preparation |

Source:

**COOKING AND PASTEURIZATION (PASTEURIZATION MODEL)**

| | | | |
|---|---|---|---|
| **Batch pasteurization** | Pathogenic bacteria survival | Minimum initial product temperature: 37°F<br>Minimum length of pasteurization cycle: 120 minutes<br>Minimum water bath temperature: 189°F | • Process establishment<br>• Check the temperature- recording device and dial thermometer for accuracy and damage and to ensure that they are operational before putting into operation; check it daily, at the beginning of operations; and calibrate it once per year<br>• Review monitoring, verification, and corrective action records within 1 week of preparation |

## EU regulations (COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs)

| **Food category** | **Micro-organisms/their toxins, metabolites** | **Sampling-plan (1)** | | **Limits (2)** | | **Reference methods** | **Stage where the criterion applies** |
|---|---|---|---|---|---|---|---|
| | | n | c | m | M | | |
| **Ready-to-eat foods able to support the growth of L.** | *Listeria monocytogenes* | 5 | 0 | 100 cfu/g | | EN/ISO 11290-2 | Products placed on the market during their shelf-life |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **monocytogenes, other than those intended for infants and for special medical purposes** | | 5 | 0 | Absence in 25 g | | EN/ISO 11290-1 | Before the food has left the immediate control of the food business operator, who has produced it |
| **Cooked crustaceans and molluscan shellfish** | *Salmonella* | 5 | 0 | Absence in 25 g | | EN/ISO 6579 | Products placed on the market during their shelf-life |
| **Live bivalve molluscs and live echinoderms, tunicates and gastropods** | *Salmonella* | 5 | 0 | Absence in 25 g | | EN/ISO 6579 | Products placed on the market during their shelf-life |
| **Live bivalve molluscs and live echinoderms, tunicates and gastropods** | **E. coli** | 1 | 0 | 230 MPN/100g of flesh and intra-valvular liquid | | ISO TS 16649-3 | Products placed on the market during their shelf-life |
| **Fishery products from fish species associated with a high amount of histidine** | Histamine | 9 | 2 | 100 mg/kg | 200 mg/kg | HPLC | Products placed on the market during their shelf-life |
| **Fishery products which have undergone enzyme maturation treatment in** | Histamine | 9 | 2 | 200 mg/kg | 400 mg/kg | HPLC | Products placed on the market during their shelf-life |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **brine, manufactured from fish species associated with a high amount of histidine** | | | | | | | |

**EU regulations (COMMISSION REGULATION (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs)**

**2.4. Fishery products**

| **Food category** | **Micro-organisms/their toxins, metabolites** | **Sampling-plan (1)** | | **Limits (2** | | **Reference methods** | **Stage where the criterion applies** | **Action in case of unsatisfactory results** |
|---|---|---|---|---|---|---|---|---|
| | | n | c | m | M | | | |
| **2.4.1. Shelled and shucked products of cooked crustaceans and molluscan shellfish** | E. coli | 5 | 2 | 1 cfu/g | 10 cfu/g | ISO TS 16649-3 | End of the manufacturing process | Improvements in production hygiene |
| | Coagulase-positive staphylococci | 5 | 2 | 100 cfu/g | 1000 cfu/g | EN/ISO 6888-1 or 2 | End of the manufacturing process | Improvements in production hygiene |

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# QUALITY AND SAFETY ISSUES IN COATED FISH PRODUCTS: INDUSTRY PERSPECTIVE

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Coated food industry particularly based on fish is highly sophisticated so as to produce convenience foods such as ready to eat or ready to use products meeting international quality standards. Coated products viz., fish fingers, squid rings, cuttlefish balls, fish balls and prawn burgers form one of the major fish and shellfish based items of trade by the ASEAN countries (Chang *et al.*,1996). A coated food product, also known as enrobed product, is one, which is coated with another foodstuff. Two types of coatings are in common use, the batter and, the crumb or the breading. A batter may be defined as a liquid mixture composed of water, flour, starch and seasonings in to which food products are dipped prior to breading or frying. The breading is normally a bread-based crumb, but other coatings like crumbled potato chips or puffed and coarsely powdered rice grain are also popular. Several varieties of batters and breading in different colours and mesh size are available and are being used in the industry. The coating will impart the desired characteristics to the product when fried and offered for consumption.

The demand for 'ready to eat' or 'ready to use' products has led to the development of several products diverse in taste, texture and appearance based on fish. A major group among them commanding high consumer appeal is the battered and breaded products commonly known as coated or enrobed products. The first commercially successful coated product is 'fish finger; or 'fish stick'. Later several other products particularly the coated fish fillet, fish portions, fish cakes, fish medallions, fish nuggets, breaded oysters and scallops, crab balls, fish balls, coated shrimp products, coated squid rings etc. became prominent in most of the developed countries with the advent of the fast food trade. The present day production of coated seafood items involve fully automated batter and breading lines which start from portioning and end with appropriate packaging of the product (Suderman & Cunningham, 1983; Dikhoof, 1990; Hutchison *et al.*, 1992 ; Joseph, 2003, Ninan ,2012).

**Edible coatings used in fish products**

Coating by battering and breading enhances a food product's characteristics such as appearance, flavour and texture. In some cases a predust is applied on the surface before coating. Battering and breading which form an integral part of the formulation of coated fish products make varieties of coated fish products. The several functions of coatings can be summarized as follows( Kester and Fennema, 1986; Gennadios et al., 1997).

- Edible coatings with good moisture barrier properties could help alleviate the problem of moisture loss from the product.
- Could hold in juices, prevent dripping and enhance product' appearance.
- The rate of rancidity-causing lipid oxidation and brown coloration-causing myoglobin oxidation could be reduced by using edible coatings of low oxygen permeability.
- Edible coating solutions, which have been heated just prior to application, could reduce the load of spoilage and pathogenic microorganisms and partially inactivate deteriorative proteolytic enzymes at the surface of coated fish products.
- Volatile flavor loss from, and foreign odor pick-up by seafoods could be restricted with edible coatings.
- Edible coatings carrying antioxidants and/or antimicrobials can be used for direct treatment of meat surfaces, thereby delaying meat rancidity and discoloration, and reducing microbial loads.
- Coatings applied on the surface of fish portions prior to battering, breading, and frying, could improve the products' nutritional value by reducing oil uptake during frying.
- Provides structural reinforcement to the substrate.
- Acts as appetizing medium.
- Increases the bulk of the substrate thereby reducing cost of the finished product.

**Pre-dust**

Pre-dust is a dry material that is sprinkled on the moist surface of the frozen or fresh food substrate before any other coating is applied. It improves the adhesion of the batter because it absorbs part of the water on the surface of the substrate. If the batter is applied to a surface that is too moist, it can slip, leaving some areas uncovered. Also, the use of predust tends to increase pickup. The predust most commonly used is wheat flour. Starches, gums and proteins, alone or in combination, can also absorb moisture and help to form a structure (Kuntz, 1997; Zhang, 2001). A more sophisticated and expensive pre-dust may contain salt, spices, seasonings and

flavourants for functional and flavouring purposes. Before a fish portion is battered it usually undergoes a pre-dusting step.

**Batter**

The word 'batter' comes from the old French word 'battre' which means 'to beat" as many batters require vigorous beating or whisking in their preparation. A batter is defined as "a liquid mixture comprised of water, flour, starch and seasonings into which food products are dipped prior to cooking". Egg is also a common component of batter. Often a leavening agent is included in the mixture to aerate and fluff up the batter as it cooks or the mixture may be naturally fermented for this purpose as well as to add flavour.

**Types of Batter**

Batters are broadly classified as leavened or unleavened. Leavening means adding a substance to make the dough puffed before it is used. Unleavened batters are also termed as traditional batters. The traditional adhesive batter is a fluid, basically consisting of flour and water, into which the product is dipped before it is cooked or fried. A bond between the product and the coating is formed. The proportion of batter and water is generally in the ratio of 1:2. The desired viscosity and pick up decide the ratio of components in the batter mix.

Leavened batters are also known as Tempura batters and they have their origin in Japan. It is the puff-type specialty batter. Corn flour is important in tempura batters. This batter forms a crisp, continuous, uniform layer over the food, constituting its final coating. Tempura batters provide crust coatings of exceptionally high volume, which are also light in texture. The tempuras are used at very high viscosity levels and always contain leavening agents. Leavened batters require special application equipment, mixing and handling procedures.

**Ingredients (of batter) and their functions**

The commonly used ingredients of batter may be grouped under five categories or classes viz., polysaccharides, proteins, fat/hydrogenated oils, seasonings, leavening agents, gums and water (Table 1). Most of the batters are based on wheat flour, which determines its fundamental characteristics. Gluten in wheat flour with its good elastic properties, can expand during frying, providing a desirable, spongy coating and facilitating the passage of water and oil (Mukprasirt et al., 2001). The moisture content - protein functionality and the quantity of amylase and amylopectin in wheat flour base have found to be well correlated with the texture

characteristics, oil absorption, good appearance and overall acceptability of the coated product. Substitution of wheat flour with rice flour will influence the rheological properties of batter (Mukprasirt et al., 2000). A corn starch-based batter requires continuous mixing during processing because the solids have a tendency to settle out easily, which may result in continuously

changing viscosity and irregular batter pick up (Suderman et al., 1993).

**Table 1. Major Ingredients of Batter and their Functions in Coated Products**

| Class of ingredients | Components | Function in the product |
|---|---|---|
| Polysaccharides | Wheat flour, corn flour, starch and gums | Improves viscosity, emulsifying and foaming capacity, texture and shelf life of the product |
| Proteins | Milk powder, milk protein fraction, egg albumin, seed protein | Improve the water absorption capacity of the flour and thus increase the viscosity of the system |
| Fat/hydrogenated oils | Triglycerides, fatty acids | Texture, flavour imparting |
| Seasonings | Sugar, salt , spices | Enhance plasticizing effect, flavour and impart antioxidant and antimicrobial properties |
| Leavening agents | Sodium bicarbonate, tartaric acid | Release carbon dioxide in tempura batters. |
| Gums | Xanthan, gum Arabic | Impart viscosity and enhance water binding capacity |
| Water | | Provide gelatinization of starch, hydration of proteins, Improves batter viscosity |

*Source - Venugopal, 2006

Modified starches with a high amylose content have good film forming properties which, alone or in combination with other ingredients such as rice flour or flour from other cereals help to reduce oil absorption by creating an effective barrier against oil in fried, battered products. These starches normally have a higher gelatinization temperature (Van Beirendonck, 1998; Higgins et al., 1999; Bertram, 2001).

The gelling ability of hydrocolloids, together with their usual hydrophilic nature makes them suitable for reducing oil uptake during frying in battered products (Annapure et al., 1999). The hydrocolloids most commonly used as a barrier are methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) (Lee and Han, 1988; Ang, 1989; Stypula, and Buckholz, 1989; Meyers and Conklin, 1990). Guar gum based batter showed superior functional properties

when compared to batters based on other hydrocolloids viz. carboxy methyl cellulose and carboxy methyl chitosan (Abbas et al., 2009).

Egg albumen is useful in binding the batter to the product; the lecithin in the yolk can act as an emulsifier, which contributes to its stability (Loewe, 1993). The use of dextrins in batter formulations is associated with an improvement in the crispness of the fried product (Shinsato et al., 1999).

**Batter Preparation**

It may be noted that no exact recipes exist for the batter system. Depending on the food substrate and the desired coating appearance, formulae can be extremely flexible to allow for maximum adaptability in the development of coated products. However batter ingredients can be classified as critical and optional ingredients based on the functions (Table 2). The addition ranges cited in the table are relatively wide, which gives a flexible formulation to suit the final product.

**Table 2 Ingredients for Batter Formulation**

| Ingredient | Addition Range (%) |
|---|---|
| Critical | |
| Wheat Flour | 30-50 |
| Corn flour | 30-50 |
| Sodium bicarbonate | Upto 3 |
| Acid phosphate | Adjust, based on neutralizing value |
| Optional | |
| Flours from rice, soy, barley | 0-5 |
| Oil | 0-10 |
| Dairy powders | 0-3 |
| Starches | 0-5 |
| Gums, emulsifiers, colours | < 1 |
| Salt | Upto 5 |
| Sugars | 0-3 |
| Flavourings seasonings | Depends on taste , flavour |

*Source – Loewe 1992

**Critical Quality Factors of Batter**

Viscosity is the most important rheological property in batter formulations since batter is to be coated over the product in a liquid form and is recognized as one of the most important factors in determining its performance during frying (Shih and Daigle, 1999). The viscosity affects the

pick up and quality of the adhering batter, handling properties of the batter, its appearance and the final texture. While reconstituting the batter with water care should be taken to incorporate the correct quantity of water. Too much water can produce thin batter. Thin batter during frying release a large quantity of water and produce a porous coating that absorbs a lot of oil during frying. Insufficient water can result in a thick batter. A thick batter layer can lead to an incompletely cooked final product, lack of crispness, and a generally hard lumpy appearance. It has an adverse influence on oil uptake during flash frying.

The other factors that affect the rheological properties of batters are the composition and proportion of the ingredients, the solids-water relationship and temperature. Also, like the rheological properties of any fluid system, they depend on factors such as shear rate, duration of shearing, and previous thermal and shear histories (Steffe, 1996). As the batters exhibit a shear-thinning behaviour, an increase in shear rate produced a lower viscosity. Also, an increase in temperature resulted in lower consistency index values in several tempura batter formulations (Baixauli et al., 2003; Salvador et al., 2003)

The solids–water relationship is fundamental in order to achieve an optimum water content and distribution. The volume fraction of water is very critical in terms of oil absorption—a linear increase with percentage moisture—during deep-frying (Shukla, 1993). A thin, not very viscous batter capable of releasing a large quantity of water produces a porous coating that absorbs a lot of oil. There is a strong relationship between oil uptake and removal of water (Gamble et al., 1987). Moreover, a layer that is too thin is difficult to handle and has a poor barrier effect before and during frying. A layer that is too thick can lead to an incompletely cooked final product, lack of crispness and a generally hard, lumpy appearance.

Temperature plays an important role during the reconstitution of batter, since it determines the batter viscosity. Once reconstituted, it should be kept at a temperature low enough to maintain the viscosity and also to control the growth of microorganisms. However very low temperature should be avoided which will result in freezing of the batter on the conveyer line of production (Garthwaite, 1998). The ideal water temperature for batter reconstitution is suggested to be between 10 and 15 $^0$C. At temperatures below 10 $^0$C, the viscosity of water could become too high impeding proper handling and at a temperature above 18 $^0$C, the viscosity could become too low.

Water content can have a direct effect on batter adhesion. Excess water content results in "ice glaze" of the fish portions, which result in poor batter adhesion to fish portions. This will result in a problem called "blow off" which means that batter will blow off or leaving the fish portion's surface when it enters the frying oil. Addition of phosphates in fish blocks will increase the cook time of the product. This will result in darkened and overcooked batters which may give an adverse consumer appeal to the product.

If a prepared batter is not used, it is vital that all dry ingredients should be blended sufficiently so that optimum ingredient distribution occurs. Hydration (mixing with water) of batter before the application should be done slowly by adding a predetermined amount of water to a prescribed quantity of batter mix as the mixing action proceeds. Mixing of batter should continue until no unwetted lumps remain in batter solution. A shortened hydration time of batter results in a partially hydrated batter that may have a chewy texture and contain lumps of dry batter.

**Breading**

The word breading is a general term that encompasses a large group of flour based , ground coatings. It can be defined as "a flour based bread crumb or cracker meal that is applied to a food in a dry form primarily to create a desired coating texture " .Breadings were used by the sections of the food industry as long as the foods have been fried: however the use of breadings to manufacture prefried convenience foods began only in the middle of 1950's. one of earliest commercial applications of breadings was in the formulation of fish sticks.

Breadings for commercial use are prepared by thermal processing of cereals i.e, by subjecting the cereal particles to heat treatment. Based on this process there are four broad groups of breadings which are outlined below:

**Cracker Meal (Traditional Breading):**

This type of breading is widely used in coated fish products. The preparation of the cracker meal is as flollows: the flour,with sugars,salt and any other colour are intensively blended and mixed with water in a continuous running mixer to form a dough. The dough is then forced through a series of paired rollers which make the dough into a thin sheet which is then conveyed over a moving steel belt for rapid baking. The baked sheet of dough which contains approximately 30% moisture is then crumbled through a granulating mill and dried to a final moisture content of approximately 8%. This moisture level ensures the shelf life of the breading and also contribute

to its absorptive capacity. The dried coarse particles are then roller milled, sifted and blended to achieve the desired particle size(mesh) specifications.

**Home- Style Breadcrumbs**

These breadings are prepared in many methods by the traditional bakers. The flour is formed into dough with water, yeast, sugar and salt as required to meet the specifications of the final processor who use the breadings for coating. The dough is then blended and mixed thoroughly using either continuous or batch mixing systems. The dough is then divided, proofed and baked into loaves. These loaves are allowed to cool, and then shredded, dried and sifted to meet mesh specifications.

**Japanese Style Crumbs**

This crumb is also known as 'Oriental Style' or 'Panko' type. These crumbs are made by standard dough ingredients and mixing methods as described in the previous crumb preparations. However, the dough after mixing is proofed in special baking pans that permit unique heat treatment during baking. This will result in a baked product free of brown crust. The baked loaves are then converted to crumbs as in the previous preparations. The crumbs will be crust free, white in colour and has a very porous nature with a splintered appearance.

**Extruded Crumbs**

Breadings can also be made on a wide variety of continuous mixers or extruders. In this process, the flour is continuously mixed under highly turbulent and intensive conditions, steam is injected and the resulting slurry of cooked flour is pumped through an orifice. The slurry will be extruded in the form of a cooked 'rope', which then shredded, dried and sifted to achieve the desired mesh size.

**Breading characteristics**

Breadings may be identified by their functional characteristics when applied to a substrate. The major functional characteristics of breading are mesh size, area to volume relationship, browning rate, moisture absorption, oil absorption, colour and texture.

*Mesh:* Typical breadings have particle size between No.5 U.S. sieve and No.80 U.S. sieve. The proportion of these various mesh fractions governs the final appearance of the food. Based on

the mesh size industry divides the breading into three broad ranges-courses, medium and fine. The larger particles provide visual interest and textural impact while the finest mesh portion rapidly absorbs the moisture in a very few seconds from any batter.

*Area to volume relationship:* The area to volume relationship (shape) of food to be breaded is another important factor. A high area to volume ratio permits a good coverage to be applied without any unfavorable effects on appearance and texture. In cube shaped foods coatings are very difficult to apply.

*Browning rate*: Browning rates of breadings depend largely on the proportion of reducing sugars used in their manufacture. Fast browning rates permit high processing speeds, reduced frying times and lower fry temperatures.

*Moisture absorption:* The rate at which a particle of breading absorbs moisture is a function of its particle size, porosity and gelation. The production rate can be increased if a breading of a smaller average mesh is used. It is porosity, together with mesh size, that determines the rate of absorption and texture of coating.

*Oil absorption:* The absorption of oil and the effective rate of heat transfer in porous granules are higher than in dense granules. The absorption of oil and the exchange of the oil for moisture during frying stage have an important advantage in texture development.

*Colour:* The final fried colour of the product is not solely dependent on the content of reducing sugars in the breading. It also depends on the other colours which are added to the breadings (paprika extracts, tomato pigment, synthesized carotene, annatto etc).

*Texture:* Mesh, porosity and absorption are the major crumb factors that contribute to texture. Coarse, dense crumbs may be very acceptable when the food is oven heated and non-oil appearances desired. Dense crumbs tend to absorb less oil when pre-fried; however, this same type of crumb when fully fried may have an unacceptably hard texture. The appropriate action is to select a coating with a medium particle size.

**Table 3. Characteristics of Processed Breadings**

| Characteristics | Breading Type | | | |
|---|---|---|---|---|
| | Cracker meal | Home-Style | Japanese | Extruded |

| Granule type | Flat/Spherical | Spherical / Crumb like | Splintered | Shredded/Dense |
|---|---|---|---|---|
| Presence of crust | Minimal | High | Minimal | Minimal |
| Granulation range | Wide | Wide | Wide | Medium/Fine |
| Mesh | 4-140 | 4-140 | 4-140 | 20-140 |
| Colour | Variable | Variable | Variable | Variable |
| Browning Rate | Slow / rapid | Moderate / rapid | Slow except when toasted | Slow |
| Density | High | Medium | Low | Low |
| Texture | Firm | Crisp | Tender to crisp | Firm to hard |
| Water Absorption rate | Variable | Rapid | Rapid | Rapid |
| Oil Absorption | Low | Medium | Variable | Low |
| Process suitability | Prefry, full fry | Prefry, sometimes full fry applications | Full fry | Prefry, sometimes full fry applications |

*Source - Dyson, 1992

**Frying medium**

Fat is the frying medium. Some fats may have specific flavour, which may be carried over to the product. Fat, besides being the heat transfer medium, is also a food ingredient and will influence the eating quality. Usually bleached and refined vegetable oils are used for frying.

The fat, because of the high temperature it is exposed to, may become degraded due to oxidation, polymerisation and contamination by food particles. Therefore, the fat used should be tested for evaluation of quality by determination of its free fatty acids, smoke point, peroxide value as well as colour for all of which there are prescribed standard limits.

**Steps in the production of coated products**

The production of coated fish products involves several process stages and steps and uses a subtle combination of art and technology. In most cases it involves the following steps.

**Portioning/forming**

Portioning is an important stage in the production of coated fishery products. Cutting loss and surface area of the portions are the two important points, which determine the economics of coated products. Cutting loss is negligible when manually done with a band saw, whereas with automatic block cutting machines it is in the range of 5-10 %. Skinless and boneless fish fillets are nowadays converted into predetermined shape and size using specially designed forming machines. There are forming/moulding machines available for other applications.

**Pre-dusting**

Before a fish portion is battered it usually undergoes a pre-dusting step. The purpose of pre-dusting is to prepare the surface of the portion so that batter can adhere uniformly. Pre-dusting also improves the adhesion of batters to frozen or greasy food surfaces. Pre-dust normally consists of a very fine raw flour type material. A more sophisticated and expensive pre-dust may contain spices and seasonings for both functional and flavouring purposes.

**Application of batter**

Conventional batters are of low to medium viscosity and hence can be applied with total submersion or overflow batter applicators. Low viscosity batters are normally applied in an overflow configuration. Medium viscosity batters may require a total submersion system depending on the product requirements.

The pre-dusted product is conveyed to the batter applicator and transferred to the next conveyor, which will draw it through the batter. The fish portion is totally submersed in the batter as it is drawn through it. Other applicators may use a pour-on application in addition to the submersion method. Irregular shaped products should be placed on the line with any concave surface upward to prevent air pockets from inhibiting batter pickup.

Line speed is a very critical factor affecting batter pickup. An excessively fast line speed will reduce the batter pickup. Too low a line speed also can result in excessive batter adherence. Excess batter, if carried over to the breading section, will cause formation of lumps and this can cause blockages in the breading machine. This will also cause formation of shoulders and tails on the edges of the product and contaminate subsequent breading application. Therefore, to overcome the problems the excess batter is removed by blowing air over the product. The position of the air blower should be as close to the product as possible to control the airflow across the product. Carry over from the pre-dusting operation also is critical. Where pre-dust is carried over, the viscosity of subsequent batter will increase leading to an increase in pickup.

**Application of breadings**

Breadings are applied to the battered food products using breading applicators. The belt speed of the breading machine is so adjusted to closely match the belt speed of the batter applicator. For soft products the crumb depth should be maintained as thin as possible to avoid product

damage in the breading machine; however, frozen or hard products should have a deep bed of crumbs. Pressure rollers are used to apply sufficient force to press crumbs onto the battered product.

Japanese style crumbs with their low bulk density and larger granule sizes make the crumb pickup difficult by the normal batter systems. Special batter formulations, sometimes containing raising agents, may have to be used at medium viscosity for a desired level of pickup of crumbs. Specially designed breading machines are used to apply uniform particle size distribution or granulation to both top and bottom of the product with minimum crumb breakdown. Air blowers are used to remove excess crumb from the product after breading. Excess crumb carried into the fryer can cause unsightly black specks on the product. Filters are used to remove small particles from the oil to prevent this phenomenon.

**Pre-frying or flash frying**

After coating with batter/bread crumbs many products are often flash fried prior to freezing. The purpose of pre-frying is primarily to set the batter/bread coating on the fish portion. Flash frying develops a characteristics crust and gives the product a characteristic fried (oily) appearance and taste. Therefore, the temperature of frying oil and the time of frying are critical. The normal frying temperature is between 180–200°C and the frying time 20-30 seconds. The term pre-frying is used because the final product frying is completed by the consumer for duration of 4-6 minutes depending on the portion size and thickness. The battered/breaded fish portions enter the frying medium through a conveyor system, the speed of which is adjusted so as to keep the fish portion in the hot vegetable oil for the required time.

**Freezing**

The fish portion leaves the frying oil with a coating temperature equivalent to that of the oil but still frozen in its center. Although the fish flesh center is frozen the surface flesh may be partially thawed. Hence, a quick and efficient freezing method is very essential to keep the quality of the coated product.

The first step in preparing the fried fish portion for freezing is air-cooling. This is usually accomplished with the use of a fan or a series of fans. This allows the coating temperature to drop, while at the same time allowing the batter coating to recover from the frying shock and

also to stabilize itself. The coated fish portions are then fed to the freezer through conveyor belts. Freezing is usually carried out in air blast freezers at -40°C.

**Packing and storage**

The coated product may undergo desiccation, discolouration and become rancid during storage. Use of proper packaging can prevent/retard these changes and enhance shelf life. Thermoformed containers are commonly used for packing coated products. The packaged products are usually stored at -20°C in master carton.

**Coating systems**

The varied and complex systems, which are termed batters and breadings, are merely components of the finished product. Using these components products are custom designed in terms of texture, flavour and visual attributes. The four basic coating systems are single line, Tandem line, Tempura or batter fry line and Tempura Japanese.

The single batter breading system, even one with a pre-dust rarely involves a pickup greater than 30%. Tandem lines consist of two batter breading machines and occasionally a pre-duster. Pickup with in this system is greater than 30%. The single and Tandem lines are commonly used for shrimp and fish sticks. Tempura lines are used for products that are coated with a leavened batter and immediately fried. These batters must be applied and processed evenly because they are the outermost coatings in the finished product. In this method the coated products should not touch each other before the batter is set in frying. Pickup in this system is normally between 30-50%. In Tempura Japanese system a leavened batter in conjunction with Japanese style or porous breadcrumbs are used. Pickup in this case also varies from 30-55%.

**Equipment in Battering and Breading process**

Prior to the introduction of machines breading lines in food processing plants consisted of a conveyor surrounded by a personnel who battered and breaded by hand. The process was slow, tedious, low production rates and difficult to maintain the hygienic standards. Today a large number of automatic and highly sophisticated processing equipment of varying capacities are available. Commonly used equipment in the production of coated products are grading equipment, peeling and deveining equipment, cooking equipment, meat bone separator, fish meat strainer, automatic band saw, forming machine, kneading machine, pre-duster, battering and breading machine, fryer, freezing equipment such as air blast freezer, cryogenic thermal

freezer, modular spiral belt freezer, fill and seal machine, vacuum packing machine with gas fleshing capability etc.

**Processing of some coated fish and fishery products**

**Fish finger or fish portion**

Fish finger is the first commercially successful coated fish product. Fish fingers are regular sized portions cut from rectangular frozen blocks of fish fillet or fish mince. A standard fish block in commercial practice in Europe is 47.9cm long, 25.4 cm wide and 6 cm thick weighing 7.5 Kg. On the production line the blocks are subdivided by a series of band saws and subsequently cut into the desired width and shape. Fish fingers are made into different shapes such as rectangular, square, wedge and French cuts. A typical British fish finger normally weighs about 28 g (1 oz) of which up to 50% of the total weight is contributed by the batter and crumbs. Accordingly, a rectangular piece of 7.5x2.0x1.5 cm weighing about 15 g may give a final weight of 28 g

The frozen fish block is prepared by mixing fish fillet/mince with 0.6% sodium tripolyphosphate and 1% sodium chloride, placing in a frame of convenient size, pressing slightly and frozen to form a solid block of fixed dimension. The frozen block is cut into suitable uniform sizes. These pieces are given a coating of pre-dust, batter and breading. The battered and breaded fish fingers are flash fried in oil at 180-200 $^0$C for 30 seconds. After cooling, the fingers are frozen preferably in an IQF freezer. The frozen fish fingers are packed in thermoformed trays or pouches and stored at –20$^0$C. Commercial and retail distribution is in frozen state.

The fish fingers when fried in vegetable oil develop a golden brown colour with attractive appearance and odour. It has been observed that the sensory quality of fish finger developed from the frozen block of fish fillets is superior to that developed from the block of mince.

**Coated fish fillets**

A fish fillet is a skinless, boneless fish loin cut along the central bone frame and trimmed free of loose or hanging meat. Fish fillets can be prepared manually as well as using filleting machines. Manual filleting gives better yield compared to machine filleting.

Big fillets are cut into the desired size and cold blanched in 3% brine containing 0.1% citric acid for 3-5 minutes. The drained fillets are then pre-dusted, battered with an adhesive batter and further coated with bread crumbs. Generally medium size porous crumbs having a relatively large granulation are used. The battered and breaded fillets are then subjected to pre-frying, freezing and packaging as in the case of fish finger.

**Coated Shrimp products**

Coated shrimp in different forms and styles can be prepared from wild and farmed varieties. The most important among them are butterfly, peeled and deveined, round tail-on, nobashi etc. Generally, shrimp based coated products are expensive. The products from farmed shrimp have indicated longer frozen storage shelf life (16-18 months) compared to those from wild variety (12-14months) at $-20^0$C.The important steps in the production process are preparation of raw material, cold blanching in 1% brine (optional), pre-dusting, battering, breading, flash frying, packing, freezing and frozen storage .Black tiger shrimp or white shrimp of 26/30 to 31/40 counts /kg are generally used.

**Coated Butterfly Shrimp**

*Process*: Wash the whole shrimp in potable water and remove the head. Remove the telson by gently raising upwards. Peel the shrimp leaving the shell intact on the last segment and the tail fans. De-vein the shrimp and trim the tail fans using a pair of scissors. Cut through the dorsal side length-wise using a sharp scalpel or knife (Butterfly cut) to partially separate the lateral muscle block. Gently open the cut surface to reveal the butterfly shape. Wash in chilled potable water and drain.

Coat the butterfly shrimp with a thin layer of pre-dust followed by coating with a conventional (adhesive) batter or a tempura batter depending upon the market requirement. Coat the battered shrimp with breading (Japanese style light coloured coarse crumbs for Japan markets and darker coloured crumbs (yellow-orange) for European and US markets.

Arrange the coated shrimp in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Freeze at -40°C in an air blast freezer and store below -20°C in master carton.

**Breaded "Peeled and deveined shrimp"**

*Process*: Wash the whole shrimp in potable water. Peel off the shell and devein. Thoroughly wash in chilled potable water and drain.

Pre-dust the shrimps with a thin layer of flour. Coat the pre-dusted shrimp with the conventional (adhesive) batter followed by breading with Japanese style light coloured coarse crumbs for Japan markets and darker coloured crumbs (yellow-orange) for European and US markets.

Arrange the coated shrimp in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Freeze at -40°C in an air blast freezer and store below -20°C in master carton.

**Coated fantail round**

*Process:* Wash the whole shrimp in potable water and remove the head. Remove the telson by gently raising upwards. Peel the shrimp leaving the shell intact on the last segment and the tail fans. De-vein the shrimp and trim the tail fans using a pair of scissors. Wash in chilled potable water and drain.

Pre-dust the shrimps with a thin layer of flour. Coat the pre-dusted shrimp with the conventional (adhesive) batter followed by breading with Japanese style light coloured coarse crumbs for Japan markets and darker coloured crumbs (yellow-orange) for European and US markets.

Arrange the coated shrimp in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Freeze at -40°C in an air blast freezer and store below -20°C in master carton.

**Breaded "Nobashi" (Stretched shrimp)**

*Process:* Wash the whole shrimp in potable water and remove the head. Remove the telson by gently raising upwards. Peel the shrimp leaving the shell intact on the last segment and the tail fans. Wash the prawns in chilled water and drain. Make three or four parallel cuts, across or diagonally on the ventral side using a sharp razor. Keep the prawn on a cutting board with

bottom side down. Stretch the shrimp to the desired length by gently pressing it using a stainless steel mould.

Pre-dust the stretched shrimp with a thin layer of flour. Coat the pre-dusted shrimp with the conventional (adhesive) batter followed by breading with Japanese style light coloured coarse crumbs for Japan markets and darker coloured crumbs (yellow-orange) for European and US markets.

Arrange the coated shrimp in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Freeze at -40°C in an air blast freezer and store below -20°C in master carton.

**Coated products from squid**

Squid rings and stuffed squid are popular coated products processed out of squid. Stuffed squid is generally processed out of small size animals. Both the products have good demand in the export market.

**Squid Rings**

*Process*: Prepare squid tubes from fresh whole squids by carefully removing the ink sac, squid pane, tentacles and viscera. Peel off the skin and wash tubes in potable water. Cut the cleaned tubes in the form of rings of uniform size (1 cm). Cook the rings in 3 % brine containing 0.1% citric acid for 1-2 minutes and cool under a fan. Coat the rings with a suitable pre-dust, batter and bread crumbs. Flash fry the coated rings for 20 seconds at 180°C and freeze in an IQF freezer. Arrange in PVC/polystyrene trays and vacuum pack in laminated pouches. Store the products in master cartons at -20°C.

**Stuffed Squid**

*Process*: Prepare cleaned squid tubes as explained earlier. Prepare a stuffing mixture containing cooked squid tentacles, cooked potato, fried onion, spices etc. Fill the cleaned tubes with the stuffing mixture. Give a coating of pre-dust, batter followed by breading with the preferred crumbs. Flash fry the coated stuffed squid tubes at 180°C for 30 seconds and freeze the stuffed squid in IQF freezer. Arrange in PVC/polystyrene trays, preferably in "well" trays and vacuum pack in laminated pouches. Store the products in master cartons at -20°C.

**Coated products from bivalves**

The most important bivalves which are suitable for the production of delicious coated products are mussels, clams and edible oysters. The glycogens present in their muscle in appreciable amounts gives them a characteristic flavour and taste and make them delicious. Coated products from mussels, clams and oysters have become commercially important in the export as well as domestic market because of their delicacy. Since majority of bivalves are filter feeders, living attached to the bottom of their habitat they accumulate large number of microorganisms in their gut. Hence great care and hygienic handling practices are required for preparing consumer safe ready to eat products out of these animals. The first step in the preparation of products from these animals is a cleansing process called depuration. This is a biological purification process intended for making the bivalve meat fit for human consumption with respect to microbial contamination. The process removes the microbial load from the gut and body of the animals by subjecting them to starvation and facilitating to discharge contaminants.

**Coated clams and mussels**

The clams and mussels after depuration are washed well by spraying potable water over the animals. Collect the depurated bivalves in a large vat and heat to boil. On heating the animals will start opening their shells. Stop heating when all the animals have opened their shells. Transfer the boiled animals to a table top and allow to cool. After cooling, shuck the meat either by hand or using a sieve that is traditionally used. Blanch the shucked meat in 3% boiling brine containing 0.1% citric acid for 3 - 5 minutes, drain and allow to cool. Pre-dust the meat with fine flour followed by battering and breading with suitable crumbs. Flash fry the coated bivalve meat at 180°C for 20 to 30 seconds depending on the size and freeze in an IQF machine. Arrange in PVC/polystyrene trays and vacuum pack in laminated pouches. Store the products in master cartons at -20°C.

**Coated Edible Oyster**

Since the shell of edible oyster is covered with mud and dirt, depuration is not always effective in reducing the microbial load from the gut and body of the animals to the desired level. Because of this a slightly different method is followed for the processing of this bivalve unlike clams and mussels.

In this case the meat is shucked out manually using a pair of pliers and then cooked in 3% brine solution containing 0.1% citric acid for 10 minutes and cooled. The gut contents including hepatopancreas are then removed manually. Since it is a post blanching operation utmost care should be taken to avoid any type of contamination. The meat is then pre-dusted with fine flour followed by battering and breading with suitable crumbs. The coated oyster meat is then flash fried at 180°C for 20 to 30 seconds depending on the size and frozen in an IQF machine. The frozen product is then arranged in PVC/polystyrene trays, preferably in "well" trays and vacuum packed in laminated pouches. Trays are packed in master cartons and stored at -20°C.

**Fish Mince based coated products**

Fish mince from marine as well as freshwater fish can be used for processing a variety of coated products such as fish cutlets, fish balls, burgers, loaves, patties etc.(Regenstein, 2004; Grantham, 1981; Venugopal and Shahidi, 1995; Venugopal et al.,1992; Joseph et al.,1984) .The mince from different species could be combined to prepare composite fillets (Venugopal, 2006).The method of preparation of these products is briefly outlined below:

*Fish cutlets:* Fish cutlets are prepared using cooked fish mince, which is mixed with cooked potato, fried onion, spices and herbs. It is then formed into the desired shape, each weighing approx. 40 g. The formed cutlets are pre-dusted with a fine flour (optional) battered with a medium thick batter and then coated with medium bread crumbs and flash fried for 30 seconds at 180°C(optional). The pre-fried cutlets are then frozen in an IQF machine and then arranged in PVC/polystyrene trays, preferably in "well" trays and vacuum packed in laminated pouches. Trays are packed in master cartons and stored at -20°C.

*Fish balls:* Fish balls are generally prepared from mince of low cost fish. Balls can be prepared by different ways. The simplest method is by mixing the fish mince with starch, salt and spices. This mix is then made into balls, cooked in boiling 1 % brine. The cooked balls are then battered and breaded.

*Fish burgers (Fish patties):* Fish burgers are more or less similar to fish cutlets. Fish mince from lean white meat fish is used. Cooked mince is mixed with cooked potato, fried onion, flour, mild spices and formed into round shapes. Generally, the starch content is to be kept below 15% and the meat content must not be less than 30% for ensuring a meaty flavour. Burgers are battered, breaded and flash fried before packing and freezing.

**Crab Claw Balls**

Crab claw ball is a highly delicious high value moulded and coated product. Crabs of Portunus / Charybdis species are generally used.

*Process:* Crab claws are severed from the body and washed well in chilled potable water. The last (distal) segment carrying the pincers is cut open using a pair of scissors or cracked open using a cracker and shell removed leaving the cartilaginous septa and muscle intact. The meat from the legs and claws is separated by using a cracker and washed in chilled potable water. The shell on the claw is removed keeping the claws unbroken and the meat removed exposing the claw ligament. The meat is mixed with 2% starch based commercial batter mix to the required consistency. This is then stuffed in ball shape on the exposed claw ligament. Alternatively the body meat mixed with the batter mix also can be used for stuffing. The stuffed claw balls are then frozen in an air blast freezer. The frozen stuffed claw balls are then pre-dusted with fine flour, battered with medium viscous batter and breaded with medium coarse bread crumbs. The coated claw balls are then flash fried for 30 seconds at 180 0C, arranged in PVC/polystyrene trays, preferably in "well" trays and vacuum packed in laminated pouches, frozen at -40°C in an air blast freezer and stored at -20°C in master carton.

**Common quality problems encountered during the coating of Seafood**

*Voids*

Presence of voids is a common quality problem that occurs during the application of batter to fish portion. Voids are bare areas on a fish portion that do not accept the batter. This is caused by many factors such as excessive line speed, shape of the fish portion, absence of predusting material, a non-adhesive surface, ice glaze, and air pockets formed during the application (Suderman, 1992). Once the void is formed, it is difficult to remove it from the fish portion due to the thick consistency of the batter. Hence the portion has to be removed from the line.

*Blow off*

Blow off can be observed when some or all of the batter is blown off or removed during frying. This problem is accelerated if the portions contained voids. The lingering portions of batter will be fried excessively and give the product a dark unacceptable appearance.

*Pillowing*

Pillowing will appear as an elevated dome of batter on the product with a large air pocket beneath it. It is caused by the formation of steam pocket due to water vapourization which is trapped under the batter during the frying process. Once the product is cooled, the puffed dome collapses and create an undesirable wrinkled appearance. Pillowing is mainly caused due to the improper blending of the batter mix and also in some cases, due to the very high leavening levels of batter mix (Suderman, 1992).

*Tailings*

Batter extends beyond the product like a tail or stringer. This is caused due to the excessive thick batter which results in inadequate blow off during the production (Johnson and Hutchison, 1983). The batter will accumulate behind the product as the name suggests.

**Standards for Quick Frozen Fish Sticks (Fish Fingers), Fish Portions and Fish Fillets - Breaded or in Batter (Codex Standards 166-1989)**

This standard applies to quick frozen fish sticks (fish fingers) and fish portions cut from quick frozen fish flesh blocks, or formed from fish flesh, and to natural fish fillets, breaded or batter coatings, singly or in combination, raw or partially cooked and offered for direct human consumption without further industrial processing.

***Product Definition*** A fish stick (fish finger) is the product including the coating weighing not less than 20 g and not more than 50 g shaped so that the length is not less than three times the greatest width. Each stick shall be not less than 10 mm thick. A fish portion including the coating, may be of any shape, weight or size. Fish sticks or portions may be prepared from a single species of fish or from a mixture of species with similar sensory properties. Fillets are slices of fish of irregular size and shape which are removed from the carcass by cuts made parallel to the back bone and pieces of such fillets, with or without the skin.

***Process Definition*** The product after any suitable preparation shall be subjected to a freezing process and shall comply with the conditions laid down hereafter. The freezing process shall be carried out in appropriate equipment in such a way that the range of temperature of maximum crystallization is passed quickly. The quick freezing process shall not be regarded as complete unless and until the product temperature has reached -18°C or colder at the thermal centre after thermal stabilization. The product shall be kept deep frozen so as to maintain the quality during transportation, storage and distribution. Industrial repacking or further industrial

processing of intermediate quick frozen material under controlled conditions which maintains the quality of the product, followed by the re-application of the quick freezing process, is permitted.

***Presentation*** Any presentation of the product shall be permitted provided that it:

- Meets all the requirements of the standard, and
- Is adequately described on the label to avoid confusing or misleading the consumer

**Essential Composition and Quality Factors**

*Raw Material:* Fish Quick frozen breaded or battered fish sticks (fish fingers) breaded or battered fish portions and breaded or battered fillets shall be prepared from fish fillets or minced fish flesh, or mixtures thereof, of edible species which are of a quality such as to be sold fresh for human consumption.

*Coating*: The coating and all ingredients used therein shall be of food grade quality and conform to all applicable Codex standards.

*Frying fat (oil)*: A fat (oil) used in the cooking operation shall be suitable for human consumption and for the desired final product characteristic.

*Final Product:* Products shall meet the requirements of this standard when lots examined in accordance with provisions outlined in Codex standards for lot acceptance and comply with the provisions set out in the definitions for defectives. Products shall be examined by the standard methods of sampling, examination and analysis.

*Decomposition:* The products shall not contain more than 10 mg/100 g of histamine based on the average of the sample unit tested. This shall apply only to species of Clupeidae, Scombridae, Scombresocidae, Pomatomidae and Coryphaenedae families.

**Hygiene**

It is recommended that the products covered by the provisions of this Standard be prepared and handled in accordance with the appropriate sections of the General Principles of Food Hygiene (CAC/RCP 1-1969), the Code of Practice for Fish and Fishery Products (CAC/RCP 52-2003), the Code of Practice for the Processing and Handling of Quick Frozen Foods (CAC/RCP 8-1976) and other relevant Codex Codes of Hygienic Practice and Codes of Practice: The products should comply with any microbiological criteria established in accordance with the Principles and Guidelines for the Establishment and Application of Microbiological Criteria

Related to Foods (CAC/GL 21-1997). The final product shall be free from any foreign material that poses a threat to human health.

When tested by appropriate methods of sampling and examination prescribed by the Codex Alimentarius Commission, the product:

(i) shall be free from microorganisms or substances originating from microorganisms in amounts which may present a hazard to health in accordance with standards established by the Codex Alimentarius Commission;

(ii) shall not contain histamine that exceeds 20 mg/100 g. This applies only to species of Clupeidae, Scombridae, Scombresocidae, Pomatomidae and Coryphaenedae families;

(iii) shall not contain any other substance in amounts which may present a hazard to health in accordance with standards established by the Codex Alimentarius Commission.

**Labelling**

In addition to provisions outlined in Codex General Standard for the Labelling of Pre-packaged foods, the following specific provisions apply:

- The Name of the Food The name of the food to be declared on the label shall be "breaded" and/or "battered", "fish sticks" (fish fingers), "fish portions", or "fillets" as appropriate or other specific names used in accordance with the law and custom of the country in which the food is sold and in a manner so as not to confuse or mislead the consumer.
- The label shall include reference to the species or mixture of species
- The proportion of fish content should be declared on the label.
- In addition, there shall appear on the label either the term "quick frozen" or the term "frozen" whichever is customarily used in the country in which the food is sold, to describe a product subjected to the freezing processes.
- The label shall show whether the products are prepared from minced fish flesh, fish fillets or a mixture of both in accordance with the law and custom of the country in which the food is sold and in a manner so as not to confuse or mislead the consumer.
- The label shall state that the product should be maintained under conditions that will maintain the quality during transportation, storage and distribution.

**Definition of Defectives**

The sample unit shall be considered defective when it exhibits any of the properties defined below:

*Foreign Matter (Cooked State)* The presence in the sample unit of any matter which has not been derived from fish (excluding packing material), does not pose a threat to human health, and is readily recognized without magnification or is present at a level determined by any method including magnification that indicates non-compliance with good manufacturing and sanitation practices.

*Bones (Cooked State)* (In packs designated boneless) More than one bone per kg greater or equal to 10 mm in length, or greater or equal to 1 mm in diameter; a bone less than or equal to 5 mm in length, is not considered a defect if its diameter is not more than 2 mm. The foot of a bone (where it has been attached to the vertebra) shall be disregarded if its width is less than or equal to 2 mm, or if it can easily be stripped off with a fingernail.

*Odour and Flavour (Cooked State)* A sample unit affected by persistent and distinct objectionable odour and flavours indicative of decomposition, or rancidity or of feed.

*Flesh abnormalities* Objectionable textural characteristics such as gelatinous conditions of the fish core together with greater than 86% moisture found in any individual fillet or sample unit with pasty texture resulting from parasites affecting more than 5% of the sample unit by weight.

**Lot Acceptance**

A lot shall be considered as meeting the requirements of this standard when:

(i) the total number of defectives as classified according to Section 8 does not exceed the acceptance number (c) of an appropriate sampling plan with an AQL of 6.5;

(ii) the average percent fish flesh of all sample units is not less than 50% of the frozen weight;

(iii) the average net weight of all sample units is not less than the declared weight, provided there is no unreasonable shortage in any container; and

(iv) the Food Additives, Hygiene and Labelling requirements of Sections 4, 5 and 6 are met.

**Conclusion**

Coated fishery products are an important category of ready–to-cook products which command a high consumer appeal and replaces the conventionally processed fish products in many

market segments, particularly the urban and semi urban sectors. The production and marketing of these products offer greater scope for the utilization of low value fishes. Besides, many of the cultured freshwater species can be potential raw material sources for the production of coated items which can significantly improve the prospects for value addition and income generation in the fast growing freshwater aquaculture sector. The coating process can also modify the flavour and enhance the acceptability of freshwater species. Coating is the best option for value addition in fisheries which can ensure the total utilization of resources.

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# QUALITY ISSUES IN FISH PICKLE

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## Introduction

Pickling is one of the oldest and safest methods of fish preservation. Pickling preserves fish by keeping it in brine and or vinegar over a period without refrigeration (Fellow, 1997). Pickling suppresses the growth of spoilage and pathogenic bacteria and provides desirable and characteristic changes in flavour, texture and colour in fish. Recently, fish pickles have been gaining popularity like other pickles and demand for these products is also increasing. The quality, safety and mass production concern were the challenging factors faced by the food producers.

According to FAO and WHO 2020, Pickling is the process whereby primary fatty fish is mixed with suitable salt (which may contain vinegar and spices) and stored in watertight containers under the resultant pickle that forms by the solution of salt in the water extracted from the fish tissue. They may be added to the container, and pickled products remain in a brine solution. Pickles are classified into two types based on their fermentation aspects: unfermented pickles and fermented pickles. Unfermented pickles are made either by using concentrated brine (up to 16% salt) and or vinegar followed by pasteurization or refrigeration. Pasteurization essentially destroys spoilage bacteria and inhibits enzymatic activity, preventing pickle softening. The average shelf life of pasteurized fish pickles is 1-2 years. In fermented pickles, the raw materials are kept immersed in dilute brine (2-5% salt) for 1-2 weeks. Naturally occurring bacteria will grow and produce lactic acid, which preserves the pickle for an extended period. The shelf life depends on the proper acidity, salt concentration, temperature and sanitary conditions.

**Method of preparation**

The best fish pickles are usually made from high fatty fishes. The species used for fish preparation differs according to places: shad, salmon, herring, shrimp, shellfish, tuna, anchovies, sardines, striped bass, and black cod are some of the examples. The different species require different preparation techniques, but the basic steps to be followed are outlined below:

Requirements:

- Raw material;
- Edible fish/ shrimp/mollusc
- Spices and condiments such as ginger garlic, chillies, curry powder;
- Edible common salt;
- Preservation media;Vinegar (4 % acetic acid); and
- Edible vegetable oils.

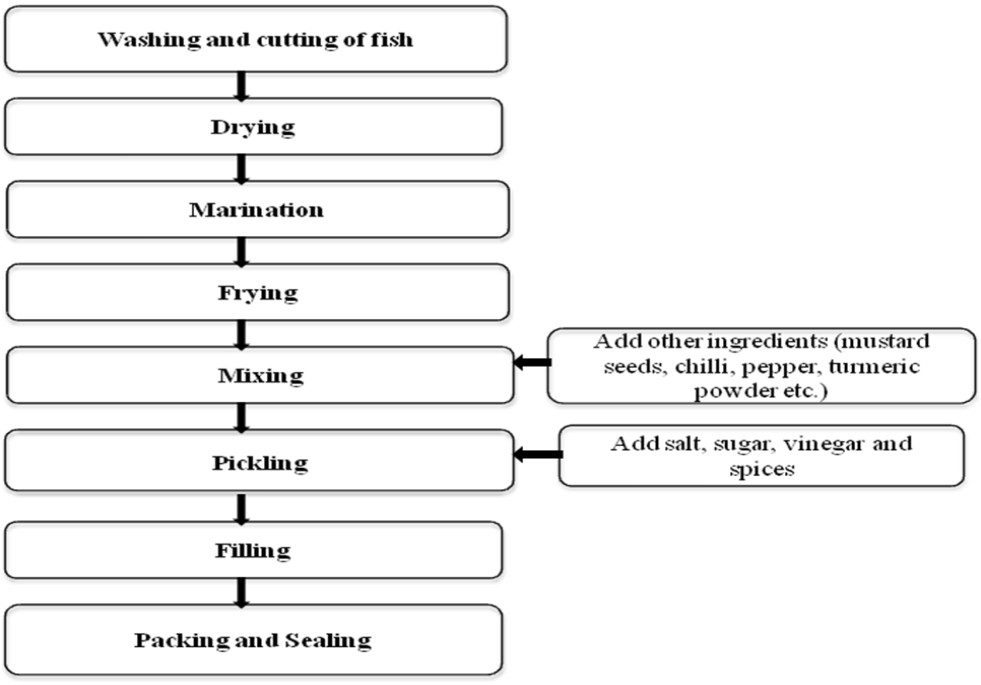


**Fig: 1. General flowchart for fish pickle preparation**

**Quality criteria for fish pickle.**

Pickle is the major forms of fish preservation in many countries because of its desirable organoleptic qualities and requires less mechanical energy input. Pickling if done under hygienic conditions by adding an adequate quantity of salt, spices and preservatives like acids, would have a extended shelf life. The raw materials and other ingredients used to prepare pickles should be fresh and free from contaminants. The required quality criteria for fish pickle are given below:

- Fish pickle should have desirable and characteristic colour, flavor, texture, and appearance free of defects and visible fungal growth.
- Meat particles should be well integrated, not too hard, and have no indications of softening.
- No artificial colouring matter and firming agents other than edible common salt and vinegar in fish pickle
- The pickle should have characteristic pleasant aroma and should be free from any objectionable off -taste smell or odour
- Sodium chloride in the pickle should be of
- 12-16% on weight basis.
- The pH of a fish pickle should be maintained in the range of 4-4.5
- The fluid portion of the fish pickle should be a maximum of 40% by weight.
- Acidity as acetic acid of fluid portion in fish pickle should have a maximum of 2.5 to 3 by weight.
- The amount of salt, sugar and spices should be standardized according to the quality of fish. It should be measured or weighed and well distributed in the container.
- All fish should be well immersed in the resulting pickle before the container is closed.
- During curing, fatty fish should always be covered with a pickle.

**Quality problems in fish pickle**

Pickling imparts unique and characteristic taste, flavor and texture to fish, but the changes occurs during storage should be carefully monitored. The selected problems which affect the quality of pickle are listed in table.1

| Problem | Causes |
|---|---|
| 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 |
| Shriveled, tough pickles | Pickles overcooked, syrup too heavy, too strong a brine or vinegar solution |
| Dark, discolored pickles | Iron utensils used, copper, brass, or zinc cookware used, Hardwater, 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,<br>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 |

Source: Behera (2020)

**Other quality issues in fish pickle**

Another quality and safety concern in a fish pickle is the synthesis of biogenic amines by microbial decarboxylation of amino acids. The biogenic amines mainly reported in fish pickles are histamine, tyramine, tryptamine, putrescine, cadaverine, spermidine and spermine. Some of the histamine forming bacteria is halotolerant (salt-tolerant) or halophilic (salt-loving) and they can produce histamine in pickled fish products. Histamine forming bacteria can also form histamine even at elevated acidity (low pH). Therefore, histamine formation can be found in

pickled products. Refrigeration or low-temperature preservation should be necessary to inhibit histamine formation during processing and storage. The limits of histamine are shown in the table.2. 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. The outbreaks of foodborne pathogens such as *E. coli* O157:H7 and Salmonella sp. in acidified foods (pH < 4.5) were also reported recently. In pickled products, *E. coli* O157:H7 is the greatest pathogen concern because of its low infectious dose and high acid tolerance (Medina Pradas, *et al.*, 2017).

*Clostridum botulinum* is another food born pathogen of concern in pickled products. Botulism caused by *Clostridum botulinum* is a serious disease and an essential apprehension in all food preservation processes. By controlling the pH level to 5 or below, salt to 5% wps(water phase salt) or more, moisture (water activity) to 0.97 or below, or some combination of these barriers, in the finished product sufficiently to prevent the growth of *C. botulinum.* Other microbiological requirements are listed in table 2.

The raw fish used for pickle preparation should be ensured that there is no live parasite 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).

| **Parameters** | **n** | **c** | **m** | **M** |
|---|---|---|---|---|
| **Histamine Level ( mg/kg)** | **9** | **2** | **200** | **400** |
| **Microbiological Requirements** | | | | |
| **Aerobic plate count (cfu/g)** | **5** | **0** | **$10^3$** | |
| **Coagulase positive Staphylococci(cfu/g)** | **5** | **1** | **$10^2$** | **$10^3$** |
| **Yeast &mold count (cfu/g)** | **5** | **0** | **100** | |

| | | | |
|---|---|---|---|
| ***E coli*** | **5** | **0** | **20** |
| ***Salmonella*** | **5** | **0** | **Absent/25g** |
| ***Vibrio cholerae*** | **5** | **0** | **-** |

Table 2. Histamine limits and microbiological requirements of fish pickle.

**Packaging and Labeling**

Fish pickles shall ordinarily be packed in glass containers or in food grade polyethylene pouches as may be found suitable so as to protect it from deterioration.

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# QUALITY ISSUES IN POWDERED FISH-BASED PRODUCTS

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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$^oC$ 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.<br>Methods for detection of bacteria responsible for food poisoning:<br>Part I Isolation and identification of enteropathogenic *Escherichia coli* and the enumeration of *Escherichia coli*<br>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.

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# TUNA PROCESSING: QUALITY AND SAFETY REQUIREMENTS

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## Introduction

The Indian seafood export trade is a sunrise sector with tremendous opportunities for future growth and further expansion. The industry had a modest beginning in early nineteen sixties when a couple of enterprising entrepreneurs from Cochin exported prawn pulp to Rangoon in Burma, the present-day Myanmar. Since then, the export trade has grown in leaps and bounds. Today it is a multimillion-dollar industry catering to the requirements of various overseas markets like the EU, the US, Japan, China, Vietnam, Thailand and a number other countries.

## Fishery resources of India.

The maritime states of India are blessed with oceans around them which are home to varied and valuable flora and fauna. The unpolluted waters of our oceans are rich repository of a number of commercially important fin fishes and shell fishes.

Length of our coastline:8118 Km.

Excuse Economic Zone :2.02 million sq.Km

Continental shelf area: 0.53 million sq. km.

Fish production

2019-20. growth rate

Marine fish production :3.72 MMT. -3.2%

Inland fish production: 10.43 MMT. 4.35%

A comparison of marine and Inland fish production shows that the former is stagnant while the latter has registered an impressive growth since 2000. In 2000-01 the marine and inland fish production were 2.81 MMT and 2.84 MMT respectively, that is both sectors contributed more or less equally. But by 2020-21 their contributions to total fish production were changed to 26.3% for marine fish and 77.7% inland fish. The dwindling of marine sector is due to the over exploitation of marine fisheries while a sudden surge in aquaculture has contributed to the growth of the inland sector. The introduction of Pacific white shrimp ( Vannamei) has led to a substantial

increase in aquaculture production. Major share of aquaculture shrimp is by Andhra Pradesh and West Bengal.

Contribution of fisheries sector to our economy is 7.28%.

**Export scenario:**

Exporters as on 26/09/2021

Manufacturer exporters. :640

Merchant exporters : 548

Route through exporters. : 104

Ornamental fish exporters. : 45

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Total. :1337

**Seafood categories**.

Today a wide variety of seafood are exported from India which can be broadly classified into the following categories:

1. Frozen seafoods
2. Fresh/chilled seafoods
3. Canned seafoods
4. Dried seafoods
5. Freeze Dried seafooods
6. Live seafoods and
7. Ornamental fishes.

**Tuna export.**

The trade is dominated by frozen shrimp and there are other items like frozen fishes and frozen cephalopods. Tunas is a comparatively new item of export from India. Targeted tuna fishing, it's processing and export started only after 2000. The tuna fishery of India is contributed by two species namely yellow fin tuna *(Thunnus albacares)* and skipjack tuna (*Katsuwonus pelamis)*. Tunas are predominantly exported as frozen product either as whole round or Gilled and Gutted with not much value addition. Frozen tuna are chiefly canning grade and buyers are mainly canaries in countries like Thailand, Tunisia, Turkey and Algeria. Countries such as Vietnam and Thailand are reprocessing hubs and from there the value-added tuna product like canned tuna and

tuna loins are re-exported to other lucrative markets like EU countries. A minor quantity is also exported as fresh/chilled by air. Fresh chilled tuna export is a small but growing segment with better value realization.

The main landing season for tuna is from September to April. The main landing centres are vaizhinjam and Kochi in Kerala, Nagapattinam, Chennai, Thengapattinam and Thoothukudi in Tamil Nadu, Vizag and Kakinada in Andhra and Veravel in Gujarat. Yellow fin tuna offers great potential for value addition. Diversified and value added product such as tuna loins, steaks, blocks, saku and poke cubes are only a few among them. Tuna contributes a number of by product like tuna belly, eye, head, cut meat, scooped meat ( Nakauchi) and dark meat which are generated during the processing of value added products. In short, practically every part of tuna can be used commercially.

**Quality and safety requirements**

The harvesting and processing of yellow fin tuna poses several challenges. Some are related to quality and safety while others are related to sustainability issues.

**A. Quality and safety related issues**

**1. High histamine content:**

A serious problem in frozen tuna exported from India. This is due to temperature abuse of raw tuna during harvesting and post-harvest handling. Either onboard fishing vessels, or during transportation or during processing stages. When exposed to elevated temperature histamine formation takes place in tuna meat at rapid rates. The amino acid histidine present in tuna muscles is converted into histamine by the action an enzyme histidine decarboxylase by the action of the bacteria *Morganella morganii* which is accelerated at high temperature. Onboard handling of yellow fin tuna requires specialized techniques. Unlike other fishes tunas are warm blooded animals and when ice is put on the body surface of tuna, the CNS tries to maintain the temperature constant and as a result the internal temperature increases favouring histamine formation. As a result, the quality and colour of the meat at the centre will be impaired.

Raw material meant for value addition requires special handling. The fish immediately after catch is stunned by blowing the head between the eyes using a hammer so as to inactivate the CNS. Then a spike is inserted into the soft spot on the head to destroy the brain. This is followed by evisceration and gilling. The Gilled and Gutted fish after cleaning is stuffed with crushed ice in the belly cavity and the fish is then kept immersed in ice or ice- water slurry. This enables rapid

chilling of the entire fish to below +2 degree Celsius. The tuna handled this way can be used for further processing into value added product.

**2. Honeycomb formation:** A related phenomenon that occur due to temperature abuse is honeycomb formation, a concern in canned tuna. It severely affects consumer acceptance. It is a condition that affects the connective tissue and is correlated to the amount of collagen solubilised during decomposition. It appears as irregular sponge like holes and pits on the surface of loins which resembles honeycomb. This is also due to time- temperature abuse. Fish exposed to prolonged times can become honeycombed. Rapid chilling immediately after capture will prevent honeycomb formation.

**3. Mushy tuna syndrome ( MTS): i**s a condition where the texture of tuna muscles turns pasty or mushy after cooking. This happens due to autolytic degradation of skipjack tuna during cooking which is influenced by initial quality and processing conditions. MTS makes the raw material unfit for canning. The exact reasons for MTS are still not fully understood.

**4. Microbial spoilage**: proliferation of microorganisms happens at elevated temperatures leading to decomposition. Proper icing of fish and keeping the cold chain throughout the supply chain will prevent microbial spoilage.

.**5. Heavy metals**: presence of heavy metals like mercury, lead and cadmium is a quality issue in tuna. Since tunas are long lived, high predators they tend to absorb and concentrate heavy metals in the muscles. This is an environmentally induces hazard. As a precautionary measure it is better to avoid the consumption of large sized tunas, for example above 50 Kg.

**Value added tuna products**

**1. Tuna loins**: the fish is cut longitudinally from head end towards the tail end close to the bones. From each fish four loins are cut; two upper loins and two lower loins. The skin and dark meat is removed and is then trimmed to improve the appearance.

**2. CO treated loins**: in this case the raw loins is treated with food grade CO. It improves colour and the appearance of the product. US FDA has accepted CO treatment as a safe method. However, the EU and Japan have not recognised CO treatment as an acceptable method.

**3.Tuna steaks**: the loins are sliced into steaks of appropriate size.

**4. Saku:** a high value product which is made from CO treated loins. These are rectangular pieces having a dimension of 15*5*2.5 cm.

**5**.**Tuna cubes**: small cubes obtained from the cut pieces generated during steak production.

**6.Tuna belly**: a byproduct obtained during loining.

**7.Nakauchi:** the meat recovers from the skeletal frame after cutting loins.

**8. Dark meat**: a byproduct obtained during the trimming of loins.

**Conclusion**.

The seas around India are a rich repository of tunas. If these valuable resources properly harvested and hygienically handled employing scientific methods India can emerge as a major processing hub of various value added tuna products.

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# QUALITY ISSUES ASSOCIATED WITH FISHERY BY-PRODUCTS

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While cutting and pre processing of fish, parts such as head, bones, frames, tails, skin and viscera, are considered as waste and of low value. Such parts of the fish which are considered to be by-products which may constitute as much as 70 percent of the fish depending on the species of fish and the final product envisaged. Most of the time accumulation of fish waste is an environmental problem, but scientific intervention on the use of these by-products for human/animal consumption would improve the nutritional value of the diet, could reduce waste generated by fish processing and provide greater economic sustainability for fish processors.

The shortage of fishery resources calls for the development and adoption of new technological processes for better utilization of waste and by-products from fish processing activities. Some quantity these by-products are currently used as raw materials for animal feed, as fish meal or as fertilizer. It is estimated that their utilization in human foodstuffs, nutraceuticals, pharmaceuticals, or cosmetics would increase their value many folds. It has been estimated that apart from the quality losses in the supply chain, worldwide, around 130 million tonnes of fish waste is produced each year. Globally around 17.9 to 39.5 million tonnes of whole fish is discarded each year by commercial fishing operations. The value of loss through wastage could be as large as US$ 50 billion each year due to poor management of seafood resources. The strict environmental regulations for the disposal of fish processing waste add to the operational cost of seafood industry. The recovery of compounds from fish processing wastes would serve the dual purpose of obtaining these valuable biomolecules as well as controlling the environmental pollution problems associated with the disposal.

The remains of the fish are commonly called by-products and if treated correctly, classified as category 3 by-products according to EU regulation, meaning parts of animals that are fit for, but not intended for human consumption (EC No 1774/2002). An essential step in up-grading by-products to co-products for human consumption is that systems such as Good Manufacturing Practice (GMP) and the Hazard Analysis and Critical Control Point (HACCP) used in food production, are applied.

The diversity of species processed and the heterogeneity of the waste generated during the cutting operations has manifold implications. Low value realisation of fish waste due to non adaption of various technologies is the key factor on quality issues of fish waste as a raw material. In fact, the quality issues of any product derived from fish waste relate to the quality of raw material. Diversity and mixing up of waste, unscientific handling leading to oxidation and bacterial degradation are the major issues related to raw material.

By products derived from fishery discards

| Product Category | Potential Products |
|---|---|
| Edible products | Fish bone broth/soup, Fish head stock, Products from recovered meat from waste |
| Industrial products | Fish oil, Fish meal |
| Bio-functional/ health products | Chitin derivatives, Collagen derivatives, Fish protein hydrolysate, Glucosamine, Chondroitin sulphate, Squalene, Sulphated polysaccharides, Hyaluronic acid, Fish bone calcium, Collagen surgical sutures |
| Feed and fertiliser | Fish silage, fish meal, fish manure, compost, foliar spray |

**Fish meal**

In most animal diets, protein is the most expensive portion and is usually the first nutrient that is computed in diet formulation. Fish meal is produced by removal of 90 to 95% of water and fat present in the raw material which is highly concentrated nutritious feed supplement consisting of high quality protein, minerals, vitamins of B group and other vitamins, essential minerals, namely phosphorus, calcium and iron and other unknown growth factors. When fatty fish is used for making fish meal; which is carried out normally by wet rendering process, fish oil is also produced simultaneously.

**Table Specifications for different grade fish meal**

| | | Grade 1 | Grade 2 |
|---|---|---|---|
| 1 | Moisture% by mass maximum | 10 | 10 |

| | | | |
|---|---|---|---|
| 2 | Crude Protein% by mass minimum | 60 | 50 |
| 3 | Ammoniacal nitrogen% by mass Maximum | 0.5 | 0.5 |
| 4 | Crude Fat or Petroleum Ether Extract% by mass maximum | 10 | 10 |
| 5 | Acid Insoluble Ash% by mass maximum | 3 | 3 |
| 6 | Chloride (as NaCl)% by mass max. | 4 | 5 |

Presence oil in excessive level in the fish meal is considered as disadvantage due to a possible danger of imparting off-flavour to animal products. However, a total content of fish oil of 1% or less in the feed mix, no harm will be off to poultry products. In excess level, the oily residue of the meal gradually oxidizes on storage. During this reaction the iodine value decreases from 130 to 90-95 units, the oil becomes less readily soluble

During this oxidation, a certain amount of fatty acids, peroxides and hydroperoxides are formed, which may be considered harmful. Freshly produced meal contains about 75 units of peroxides. The value decreases rapidly on storage and becomes the peroxide value insignificant.

Oxidation of fat present in the meal has also been identified as the cause of spontaneous heating which may cause danger during shipments and/or storage. Even when no direct fire hazard existed, the quality of the meal is seriously jeopardized. And such oxidative heating is responsible for much inferior quality meal. Fish meal can be stabilised by means of incorporation of antioxidants ethoxyquin or BHT immediately after manufacture.

**Fish oil**

Fish oil is a by-product obtained during fish meal production and then subjected through various steps in order to yield the final product. The oils contain mainly triglycerides of fatty acids (glycerol combined with three similar or different acid molecules) with variable amounts of phospholipids, glycerol ethers and wax esters. They are imparting positive effect in powerful metabolic and physiological regulators, which also influence the excessive fat deposition in the arteries. Fish body oil of fish is more important as an industrial product besides its limited use

in human consumption. It Contains poly unsaturated fatty acids (PUFA), particularly n-3 PUFA n-3 PUFA which has beneficial effect in controlling heart ailments in humans. Fish oil is has use as carriers of fat soluble vitamins A and D.

Sardine oil is prepared from fresh or well preserved or frozen sound wholesome sardine fish either whole or dressed body portion. It is prepared by cooking, pressing and separating oil from press liquor by centrifugation or by any other suitable means. The requirements sardine oil includes: shall be free from foreign matters in settled or suspended condition, and separated water. The product shall be a bright and clear liquid when heated to a temperature of 40°C. (ii) it shall be free from any other kind of oil including mineral oils. It shall be free from foul and offensive putrefactive odour and should have only characteristic fish- oil odour. (iii) it shall be of greenish straw light golden yellow or light brown colour. (iv) product shall also conform to the requirement given in table below:

FSSAI requirements for sardine oil

| Sr. No. | Characteristics | Requirements |
|---|---|---|
| 1. | Free faty acids as percent oleic acid, w/w, max | 1.0 |
| 2. | Moisture, percent by weight, max | 0.5 |
| 3. | Iodine Value | 145-180 |
| 4. | Saponification value | 185-205 |
| 5. | Unsaponifiable matter, percent, w/w, max | 2.0 |
| 6. | Refractive Index (40°C) | 1.4739-1.47 |

Oxidative deterioration of fish oil

Due to its high content of polyunsaturated fatty acids, including EPA and DHA, fish oils are highly susceptible to oxidative spoilage and the rate of fish oil oxidation is significantly different from that of other oils. Various factors govern the oxidative reactions that occur at centres of unsaturation. Certain metals, visible light and light of shorter wavelengths, some oxidative enzymes, and other biological substances, accelerate oxidative deterioration. During the autoxidation of fish oils, undesirable flavours and odours develop at very low peroxide values at an early stage of oxidation, even during the induction period. A large number of saturated and unsaturated aldehydes, ketones, acids, and other products have been isolated from oxidised oils. Oxidation of lipids not only produces rancid odours and flavours, but also can

decrease nutritional quality and safety by the formation of secondary products The products of lipid oxidation are known to be health hazards since they are associated with aging, membrane damage, heart disease and cancer

In recent days there are quality concerns on fish oils, especially omega 3 supplements meant for human consumption. Excess amount of Polychlorinated Biphenyls (PCBs), dioxins, mercury, mislabelling, lower or higher claimed amount of EPA/DHA are considered some of the emerging quality concerns in fish oil.

**Limit of undesirable substances in feeding stuff prepared from fish/aquatic products (Directive 2002/32/EC)**

| **Undesirable substance** | **Product** | **Maximum content in mg/ kg (ppm) relative to a feeding stuff with a moisture content of 12 %** |
|---|---|---|
| Arsenic | Feeding stuffs obtained from the processing of fish or other marine animals | 15 |
| Lead | Feeding stuffs obtained from the processing of fish or other marine animals | 10 |
| Fluorine | Feeding stuffs of animal origin with the exception of marine crustaceans such as marine krill | 500 |
| | marine crustaceans such as marine krill | 3000 |
| | calcareaous marine algae | 1000 |
| Mercury | Feeding stuffs produced by the processing of fish or other marine animals | 0.5 |
| Nitrite | Fishmeal | 60 (expressed as sodium nitrite) |

| | | |
|---|---|---|
| Cadmium | Feed materials of animal origin | 2 |
| Aflatoxin | complete feeding stuff | 0.01 |
| Dieldrin | Fish Feed | 0.02 |
| Camphechlor (toxaphene) —sumof indicator congeners CHB 26, 50 and 62 | Fish, other aquatic animals, their products and by-products with the exception of fish oil | 0.02 |
| | Fish oil | 0.2 |
| | Feedingstuffs for fish | 0.05 |
| Chlordane | All feedingstuff | 0.02 |
| DDT (sum of DDT-, TDE- and DDE isomers, expressed as DDT) | All feedingstuff | 0.05 |
| Endosulfan | complete feedingstuffs for fish | 0.005 |
| Endrin | All feedingstuff | 0.01 |
| Heptachlor | All feedingstuff | 0.01 |
| HCB | All feedingstuff | 0.01 |
| HCH<br>Alpha isomer<br>Beta isomer<br>gamma isomer | All feedingstuff | <br>0.02<br>0.01<br>0.2 |

**Limit of Dioxin and PCBs in Fish oil as per EC regulation 1259/2011 amending Regulation (EC) No 1881/2006**

| **Foodstuffs** | **Maximum level** | | |
|---|---|---|---|
| | Sum of dioxins (WHO-PCDD/F-TEQ) | Sum of dioxins and dioxin-like PCBS (WHO-PCDD/F-PCB-TEQ) | Sum of PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6) |
| Marine oils (fish body oil, fish liver oil and oils of other marine organisms | 1.75 pg/g fat | 6.0 pg/g fat | 200 ng/g fat |

| | | | |
|---|---|---|---|
| intended for human consumption) | | | |
| Fish liver and derived products thereof with the exception of marine oils | | 20.0 pg/g wet weight | 200 ng/g wet weight |

Presence of ethoxyquin in crustaceans has been a major cause of concern for export of seafood to Japan. Ethoxyquin is commonly used as antioxidant in fishmeal production to prevent rancidity. Japan has amended its requirement from earlier 0.01 ppm to 0.2 ppm (parts per million) in crustaceans, including the farmed shrimp. The SPS notification issued by European Union (G/SPS/N/EU/61/Add.1) in July 2014 has classified Ethoxyquin as pesticide and a limit of 0.01 ppm has been fixed for aquatic products.

**Chitin and chitosan**

Chitin is the second most abundant natural polymer available next to cellulose. It is composed of units of the amino sugar N acetyl glucosamine. It is the main source for the production of chitosan. In India, the major sources for chitin and chitosan is processing wastes of crustaceans like, shrimp, crab, lobster and squilla. Shrimp processing industries around the world turn out enormous quantity of head and shell as industrial waste. The head and shell constitute nearly 60% by weight of the whole prawn depending on the species and size of the prawn. In India its availability is estimated to be 100,000 tons annually and it is the single largest fishery waste of the country. Crab shell is yet another waste thrown out in large quantities from seafood processing centers. At present only a small portion of this finds use as ingredient in shrimp/poultry feed mix. The industry finds it extremely difficult to properly dispose of the same. Many a time this waste poses a problem of environmental pollution also.

**Specification**

In commerce chitin and chitosan with the following characteristics are acceptable to the end users.

| **Characteristics** | **CHITIN** | **CHITOSAN** |
|---|---|---|
| Moisture % | <10 | <7 |
| Ash % | <2 | <1 |
| Protein % | <2 | nil |
| Colour | off white | off white |

| | | |
|---|---|---|
| Particle size | 10-20 mesh | 60-80 mesh |
| Solubility in 1% acetic acid | nil | soluble |
| Insolubles in 1% acetic acid | N.A | <0.5 |
| pH | 7.0-7.5 | 8-9 |
| Nitrogen % | 6.5-6.8 | 7-7.5 |
| Deacetylation % | N.A | >80 |
| Viscosity (m pa s) in 1% acetic acid at 1% level at 28°C | N.A | <100 |

**Silage**

Fish silage may be defined as a liquid product, made from whole or parts of fish, to which no material has been added other than acid and in which liquefaction is carried out by enzymes already present in the fish. Fish silage is a nutritionally balanced diet extensively used in feeds in combination with other ingredients. It is found to be superior to other protein diet of plant or animal origin. The nutritional composition of fish silage is almost similar to fish except a slight increase in moisture content.

Ensiling can be achieved either by treating the fish directly with a mineral acid or organic acid or by lactic acid produced *in situ* by fermentation. The fish is partially digested and preserved by the acid. The most commonly used organic acids are propionic, acetic and formic acids. A 3% by weight of 98% formic acid is added to the well ground fish mince and mixed well ensuring a pH around 4 to prepare acid fish silage using organic acid. The whole fish/waste is comminated in a mechanical mincer and the required quantity of acid or acid mixture is added and the slurry is mixed well. After this process the whole material becomes a good paste that can be stored in tanks with daily stirring. Within 15-20 days the silage is ready for use.

**Lipid Oxidation**

Unsaturated long chain fatty acids released from fish by lipid hydrolysis by lipases absorb oxygen and undergo rapid auto-oxidation, releasing a large number of volatile carbonyls and making the silage rancid. The rate of lipid oxidation is directly related to exposure to sunlight, presence of pro-oxidants, concentration of heavy metals, temperature, and other factors. Oxidised lipids are responsible for the poor nutritional quality of the silage. Hence, silage produced from fatty fish has shorter shelf-life than one produced from lean fish. Addition of

antioxidants like BHA, BHT and ethoxyquin can substantially retard the development of rancidity but are seldom added in silage.

Fish silage has an inherent defect, its liquid consistency, which makes it difficult to transport to distant places and to store. Feeding experiments in India (Anon, 1972-78) showed that it was extremely difficult to convince the farmers who rear poultry, pigs and cows about the efficiency of fish silage as a protein supplement because of this disadvantage. To overcome this problem, a solid feed mix was compounded out of boiled fish silage and rice bran powder in the ratio 21% protein. It is easily transported and has extended shelf-life at ambient temperatures in the tropics. The rice bran contains all vitamins, particularly the B group, and many other micronutrients required for animals.

**Fish Protein Hydrolysate**

Protein hydrolysates are the breakdown products of proteins viz., smaller peptide chains with 2-20 amino acids obtained by hydrolysis either chemically or enzymatically. This process facilitates recovery of essential nutrients viz., amino acids as well as has immense scope in food, nutraceutical and pharmaceutical industry on account of the excellent physicochemical, functional as well as bioactive properties they possess. Based on the extent of hydrolysis that the parent protein undergoes, the properties exhibited by the hydrolysates vary considerably.

Although enzymatic hydrolysis of proteins develops desirable functional properties, it has the disadvantage of generating bitterness which is identified as a major hindrance regarding utilization and commercialization of bioactive. The mechanism of bitterness is not very clear, but it has been documented to be associated with the presence of bitter peptides mainly comprising hydrophobic amino acids. In addition to hydrophobicity of peptides, peptide length, amino acid sequence and spatial structure also influences the perception of bitter taste

Strict control of any hydrolysis experiment and termination at low degree of hydrolysis is a common and desirable method to prevent the development of a bitter taste and the retention of functional properties

As enzymes have different preferences for amino acids, choosing the most appropriate enzyme is the most widespread methodology for reducing bitterness. Enzymes with a high preference for hydrophobic amino acids such as alcalase are often preferred and frequently yield products of low bitterness

Oxidation products play a part in the development of bitter taste. A few suggested methods for bitterness reduction include treating hydrolysates with activated carbon that partly removes bitter peptides with absorption, extracting bitter peptides with solvents and by plastein reaction which is the formation of a gel-like proteinaceous substance from a concentrated protein hydrolysate. Masking can be performed by adding additives or molecules, e.g. cyclodextrin, to the hydrolysate to mask the bitter taste.

Although the specific health benefits from different hydrolysates are mostly supportable scientifically, the consistency of these benefits is debatable on account of the quality changes during storage on account of its hygroscopicity and other biochemical changes. Encapsulation may be adopted as an effective technique in this regard to improve the stability and delivery of these sensitive bioactive components by selection of suitable wall material.

Economic feasibility in upscaling is a major constraint with regard to the hydrolysate production. This can be effectively addressed by following standard protocols by using suitable inputs like raw material, enzymes, other hydrolytic conditions like time, temperature etc.

**Surveillance and Monitoring**

It is essential to conduct periodic surveillance and monitoring of human pathogenic bacteria in seafood to prevent outbreak and spread of the disease. The traditional methods like serotyping and phage typing although useful do not provide information on source of the hazard. The modern genotypic tools like pulsed field gel electrophoresis (PFGE) or multi locus sequence typing (MLST) are useful in source-tracking of pathogens, retrospective population studies and determining clonality of strains. For simultaneous determination of precursors of putative virulence determinants such as pathogenicity islands, pathogenicity loci, antibiotic resistance genes, transposons, plasmids and phages and their spread across different species, specially designed microarray would be quite useful.

Risk assessment of both chemical and microbial hazards in seafood by-products has not been attempted in most parts of the world. In absence of risk assessment data, regulatory agencies are finding it difficult to impose any safety standard.

As manufacture of most of the by-products involve heavy chemical extraction and downstream processing, presence of microbial hazards are mostly due to post-process contamination. On the other hand, many chemical hazards may get concentrated during extraction and may pose severe safety concerns.

**Gelatin**

Recently fish skin, bone and scales has been identified as an important source for collagen and its derivatives. Evnthough there are many uses for gelatin in the food and pharmaceutical industries, apprehensions on the problems like allergenicity and odour development are to be addressed. Compared to bovine and porcine origin, the market share of fish gelatin/collagen/collagen peptide is still considered very small. Some limiting factors that hamper the large-scale development of the fish gelatin and collagen/collagen peptide industry include absence of internal quality assurance system, the difficulty to obtain certification on fish raw material etc. Certification is required for traceability, which has become an essential requirement for food additives, especially from animal sources. The intrinsic quality factors (such as odor, color, bloom strength, and viscosity of fish gelatin and collagen/collagen peptide) and quality variations from batch to batch and from species to species pose another major hindrance to the development of fish gelatin/collagen industry. The technical difficulties associated with producing fish gelatin and collagen/collagen peptide for human consumption generally surround the elimination of the unpleasant fish odor from the product. Persisting residual odor in fish gelatin and collagen can cause problems especially when it is intended for direct use in mildly flavored products and in cosmetic products. In cases, the product is odor free when produced, but when formulated into other products, the odor returns with generation of off-flavors

The allergenic activity resides in the meat of the certain fish, but recently concern has been raised as to whether products such as fish gelatin, which is derived from skin and bones, also may possess an allergenic potential. Recent interest in the labelling of foods derived from allergenic sources necessitates determination of the potential allergenicity of such food ingredients and pharmaceuticals including fish gelatin and collagen. The major allergen parvalbumin was purified from cod muscle tissues, and polyclonal antibodies were raised towards it. Washing of the skins, a common industrial procedure during the manufacturing of fish gelatin, reduced the level of parvalbumin about 1000-fold to 0.5 ppm.

There is an increasing concern about the heavy metal content of gelatin and collagen extracted from fish scale and fish skin. Heavy metals are considered as one of the most critical contaminants of the aquatic ecosystem because of their potential to enter water bodies and also their bioaccumulation and biomagnification in the food chain. Fish are commonly situated at the top of the food chain and are considered as a susceptible aquatic organism to toxics present

in water. As regards heavy metals, fish gelatin/collagen shall not contain Arsenic in excess of 2 ppm, zinc in excess of 20 ppm, copper in exces of 30 ppm, and other heavy metals in excess of 50 ppm, chromium 10 (ppm) and lead 5ppm.

Presence of excess amount of heavy metals has also been reported from gelatin manufactured which needs serious attendtion in selecting the raw material. Even though some washing steps involved in gelatin/collagen manufacture can substantially reduce the pesticide incidence in the final product, there were several export rejections in the country on account of pesticide detection in the final product.

Even though fishery by products are utilised for the production of industrially and pharmaceutically important products, there are quite lot of quality issues specific to each product. Certification of the raw material, following of GMP and HACCP practices etc can reduce many of the quality issues with regard to such products.

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# FREEZE DRYING OF SEAFOOD

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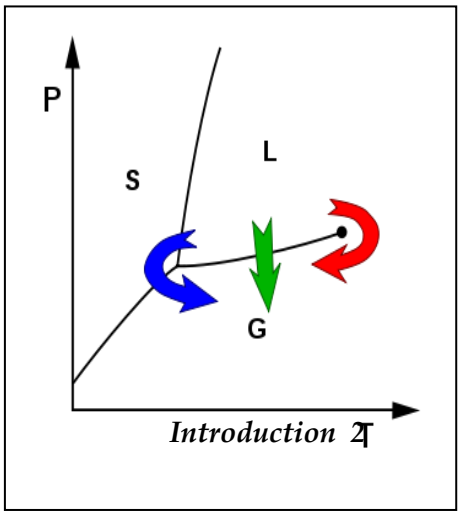
**Freeze-drying** (also known as **lyophilization or cryodesiccation**) is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze-drying works by freezing the material and then reducing the surrounding pressure and adding enough heat to allow the frozen water in the material to sublime directly from the solid phase to gas.

In a typical phase diagram, the boundary between gas & liquid runs from the triple point to the critical point. Freeze-drying (blue arrow) brings the system around the triple point, avoiding the direct liquid-gas transition seen in ordinary drying (green arrow).




**Freeze drying process**

There are three stages in the complete drying process:

a) Freezing
b) Primary drying
c) Secondary drying

***a)*** **Freezing :**

The freezing process consists of freezing the material. In the plant, the freezing process is taken part in the blast freezer. It is important to cool the material below its **eutectic point**, the lowest temperature at which the solid and liquid phases of the material can coexist. Larger crystals are easier to freeze-dry. To produce larger crystals, the product should be frozen slowly or can be cycled up and down in temperature. This cycling process is called **annealing**. Usually, the freezing temperatures are between $-30^0$C and $-40^0$C.

***b*) <u>Primary drying :</u>**

During the primary drying phase, the pressure is lowered (to the range of a few millibars) and enough heat is supplied to the material for the water to sublimate. The amount of heat necessary can be calculated using the sublimating molecules' latent heat of sublimation. In this initial drying phase, about 95% of the water in the material is sublimated. This phase may be slow because, if too much heat is added, the material's structure could be altered.

In this phase, pressure is controlled through the application of partial vacuum. The vacuum speeds up sublimation, making it useful as a deliberate drying process. Furthermore, a cold condenser chamber and/or condenser plates provide a surface(s) for the water vapour to re-solidify on. This condenser plays no role in keeping the material frozen; rather, it prevents water vapor from reaching the vacuum pump, which could degrade the pump's performance. Condenser temperatures are typically below $-30^0$C. It is important to note that, in this range of pressure, the heat is brought mainly by conduction or radiation; the convection effect is considered to be inefficient.

***c)* <u>Secondary *drying :*</u>**

The secondary drying phase aims to remove unfrozen water molecules, since the ice was removed in the primary drying phase. This part of the freeze-drying process is governed by the material's adsorption isotherms. In this phase, the temperature is raised higher than in the primary drying, to break any physico-chemical interactions that have formed between the water molecules and the frozen material. Usually the pressure is also lowered in this stage to encourage desorption. After the freeze-drying process is complete, the vacuum is usually broken with an inert gas, such as nitrogen, before the material is sealed. At the end of the operation, the final residual water content in the product is around 1% to 4%, which is extremely low.

**History of Freeze Drying**

This technology was used during world II to carry Blood plasma and penicillin when needed to treat the wounded in the field, and because of the lack of refrigerated transport, many serum supplies spoiled before reaching their recipients. This was also used by NASA to make freeze dried meals for the astronauts where they can carry very easily.

**Properties of Freeze dried products**

- Light weight – Reduces cost of shipping
- Can store at low temperature so less storage cost
- Physical structure is not altered so no change in shape,colour,flavour, etc
- Reconstitution is much faster by easily adding water.
- Can also be used to increase the shelf life for many years.

**Applications of Freeze Drying**

*Food industry* **–** Seafood, Culinary herbs, fruits, cereal, instant coffee. Freeze dried seafood are used in Instant foods like Noodles, Soups and Pasta

*Pharmaceutical industry and biotechnology* **-** Drying the material to increase the shelf life of products such as vaccines & other injectables and sealed in a vial.

*Technological industry* - In bio-separations & also freeze-drying is often reserved for materials that are heat-sensitive such as proteins, enzymes, microorganisms and blood plasma. The low operating temperature of the process leads to minimal damage of these heat-sensitive products.

*Other uses* - freeze-drying is used to conserve special strains of bacteria, for floral preservation, etc.

**Equipments Involved**

- Refrigeration system comprises of compressor,Condensor,Evaporator etc
- Vacuum System
- Control system
- Freeze drying chamber with condenser

**Major Markets**

- USA
- Japan
- China
- Australia
- Thailand
- Mexico

**Quality Challenges**

- Moisture percentage – Less than 3% to be maintained to reduce water activity thereby bacterial load.
- Microbiology – Total plate count, Total Coliform, Yeast and mould
- Bulk density – Grams/Litre
- Number of pieces/kg
- Extraneous matter
- Reconstitution/ Rehydration - 3 minutes in warm water

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# DEVELOPMENT OF SEAWEED-BASED PRODUCTS AND RELEVANT QUALITY ISSUES

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Seaweeds are photosynthetic aquatic organisms which does not have true roots, stems or leaves but the appearance resembles terrestrial plants. These marine macrophytes are widely distributed from tidal, intertidal regions to deep sea. Seaweeds are known throughout the world but the production is mainly restricted to Eastern and South-eastern Asia. It is well accepted as food and consumed by human in east Asian country. The seaweeds are used as food source in Asian countries since time immemorial. The use of seaweed as food source is traced back to fourth century in Japan and to the sixth century in China. Today along with Japan and China, Republic of Korea are the largest consumer of seaweeds as food. In other parts of the world seaweeds are usually niche or novel foods, mostly consumed in coastal communities as traditional food. Seaweeds are an important constituent of the aquaculture production globally. In the year 2019, seaweed contribute nearly 30% of global aquaculture production on wet weight basis. According to FAO the red and brown seaweeds production was second and third after carps, barbels and cyprinids. During 2019, seaweed contributed almost 5.4% of total aquaculture production value of USD 275, which was higher than so many fish species such as Tilapia and other Cichlids.

Different seaweed groups are used to produce hydrocolloids such as agar, alginate and carrageenan. The seaweed cell walls contain long chain polysaccharides, which are known to provide the flexibility to the seaweeds and helps them to survive in different intensity of water movements where they grow. Hydrocolloids are generally water soluble non crystalline substance with very high molecular weight when dissolved in water gives a viscous solution. Hydrocolloids present in the seaweeds are known as Phycocolloids.

Phycocolloids like agar, alginate and carrageenan are water soluble and gives thick viscous solution in aqueous solution and forms gels and jellies of varying degree of firmness. These properties of these hydrocolloids are used in different food industries for increasing thickness and stabilizing the products.

The use of seaweed as source of hydrocolloid has long history and goes back to year 1658, when the gelling properties of agar was first discovered in Japan, the hot water extract from red seaweed was found to produce colloidal solution. Irish moss, *Chondrus crispus* a red seaweed known to contain carrageenan a popular thickening agent in the food industries. Alginate is hydrocolloid extracted from brown seaweed. Due to its diverse properties seaweeds find uses in food and non-food industries such as food additives, animal feeds, pharmaceuticals, nutraceuticals, cosmetics, textiles, biofertilizers/bio-stimulants, bio-packaging and biofuels among others.

Due to ever increasing population and limited availability of land space, importance of oceanic space is gaining importance as an alternative source food production and seaweed is considered as one of the important food sources.

**Agar yielding seaweed (Agarophytes)**

Agar is the general name for polysaccharides, which are dried amorphous gelatin like non-nitrogenous extract from some red algae. *Gelidum sp* and *Gracilaria sp,* are the most commonly used raw material for the extraction of agar. In terms of gel strength *Gelidium sp* is known to give better quality of agar. Mainly *Gelidium sp* used for production of agar comes from natural resources. *Gelidium* sp is a slow growing and small in size hence cultivation of this species in tanks/ponds seems to be economically challenging.

**Carrageenan yielding seaweeds (Carrageenaophytes)**

Naturally collected *Chondris crispus* commonly known as Irish Moss a red seaweed was the original source of carrageenan. When carrageenan industry has grown and the demand for raw material increased the Chondrus was being supplemented with species like *Iridea sp, Gigartina sp* and *Eucheuma sp*.

**Algin yielding seaweeds (Alginophytes)**

Alginates are also called as algin in short for is present in the cell walls of brown seaweeds. Seaweeds that grow in more turbulent conditions usually have a higher alginate content than those grow in calm waters. Any brown seaweed can be used for alginate production but the chemical structure of alginates varies from one genus to another and hence the properties of alginates vary accordingly. Based on the dry weight a commercial alginophytes contain nearly 20% of alginate in it. The quality of alginate is based on its

viscosity, higher the viscosity better the product is. It is observed that seaweed growing in temperate or cold water produces better quality of alginates than the seaweed growing in warm or tropical water. Some of the commercially important alginophytes are *Ascophyllum sp.*, *Laminaria sp., Ecklonia sp, Macrocystis sp*. *Sargassum sp* etc.

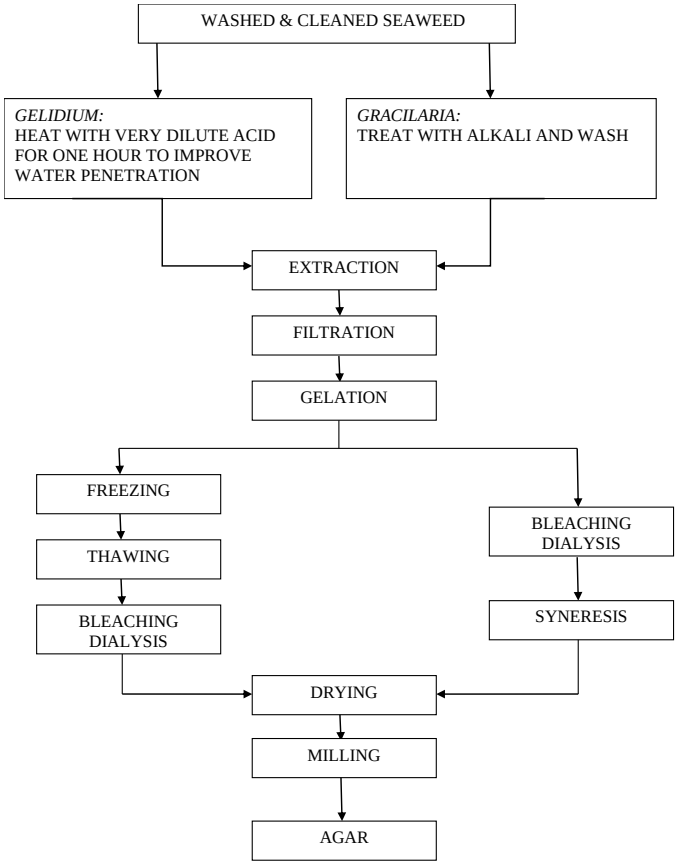
**Method for Agar production**

Agar can be extracted from seaweeds in a very simple way through hot water extraction process. Seaweed should be washed repeatedly with sea water and fresh water to remove sand, dirt, epiphytes etc and then heat with the water for few hours. The agar exudes in the water subsequently it is filtered to remove the residual seaweeds and the hot filtrate is cooled. But in more scientific way extraction of agar involves, cleaning, pre-treatment, extraction, filtration, bleaching and dewatering. Purification can be done by freezing-thawing cycle. The method can vary from one species to other *Gracilaria sp* needs slightly mild processing condition than *Gelidium sp* and *Gelidiella sp*. Pretreatment involves treatment of cleaned seaweed with alkali generally 2-5% sodium hydroxide at 85-90$^0$C for an hour, after removal of alkali seaweed may be treated with mild acid to neutralize the solution. During simple hot water extraction, the cleaned seaweed can be treated with water at 95-100$^0$C for 2-4 hrs.

The quality of extracted agar depends on the following factors

a) Intrinsic factor i.e., species of the seaweed.
b) Environmental factor such as temperature, salinity, water during the growth of the seaweed.
c) Harvesting like degree of mixing of other seaweed species.
d) Post-harvest like extraction protocol followed.

**The schematic representation of the extraction of agar from seaweed**

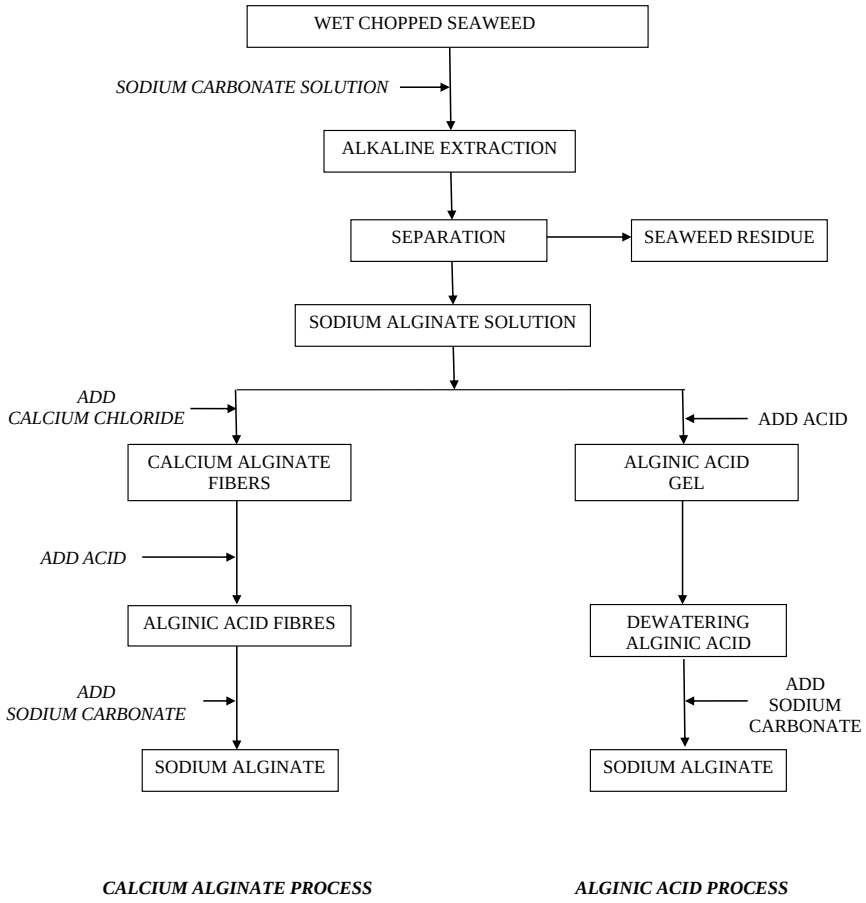



**Source:** FAO Fisheries Technical Paper, No. 441

## Alginate production

The term alginate is used for the salts of alginic acid i.e all the derivatives of alginic acid. Alginate is a cell wall polysaccharide in the brown algae which is present as calcium magnesium and sodium salts of alginic acid. The aim of the extraction process is to extract powdered/ dry sodium alginate as the calcium and magnesium form does not dissolve in water. The extraction process involves cutting and chopping of seaweed into small pieces and stirred with hot alkali, mainly sodium bicarbonate for nearly 2 hours. During the process alginate dissolves as sodium alginate to form a thick slurry. The slurry also contains insoluble part of the seaweed, mainly cellulose. The solution is filtered and then the alginate is precipitated from the filtrate either as alginic acid or calcium alginate.

**The schematic representation of the extraction alginate from seaweed**



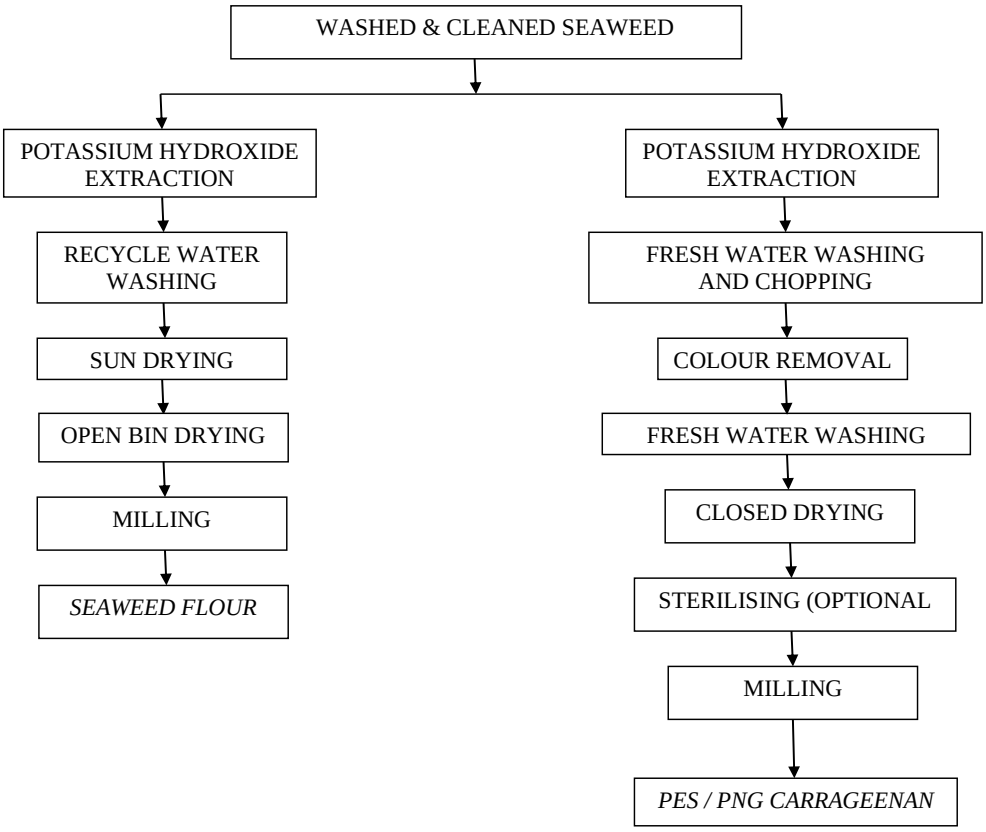

**Source:** FAO Fisheries Technical Paper, No. 441

**Carrageenan production**:

There are two different methods of extraction of carrageenan from seaweeds in first method the carrageenan is extracted in aqueous solution and the seaweed residues are filtered. In second method carrageenan is actually not extracted from seaweed but all other thing which is soluble in water and alkali is washed out of the seaweed leaving behind carrageenan and cellulosic part in the seaweed and the product is sold as semirefined carrageenan. In the second method carrageenan doesn't require to be extracted from aqueous solution hence the process is cheaper than the first method.

The alkali treatment removes the sulphate group from the polysaccharide and helps in increasing the formation of 3,6 Anhydro galactose which in turn enhances the gel strength.

**The schematic representation of the extraction of carrageenan from seaweed**




**Source:** FAO Fisheries Technical Paper, No. 441

**Conclusion:**

Phycocolloids from seaweed have immense potential to be used in food and pharma industries but the potential is still underutilized. Therefore, the use of seaweed extract-based products especially phycocolloid based products should be popularized and awareness should be created for its production and utilization.

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# QUALITY AND SAFETY ISSUES ASSOCIATED WITH FERMENTED FISHERY PRODUCTS

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## Introduction

Fermented fishery products (FFPs) are consumed by people from different parts of the world and are more popular in South-east Asian countries. The FPPs possess a unique blend of aroma and flavour which contributes to achieved its characteristics taste. The FPPs are reported with several health benefits owing to beneficial microbes, nutraceutical and functional molecules (Paredes and Harry, 1988). Food Safety and Standard Regulations, India (FSSR, 2011) defined fermented fishery products as any fishery product that has undergone degradative changes by the action of enzymes or microbes either with or without salt. Non-traditional products manufactured by accelerated fermentation, acid ensilage and chemical hydrolysis also belong to this category. The popular ethnic FFPs in India are *hentak, ngari, tungtap, puthi shidal, lona ilish, phasa shidal, hidal,* etc. (Thapa *et al*., 2004). The categories of various FFPs in South-east Asian countries, African countries, American countries and European countries are presented in Table 1. Some of the popular FFPs in South-east Asia are *nam-pla* and *pla-ra* in Thailand, *phu quoc*, *shiokara* and *narezushi* in Japan, *budu* and *belacan* in Malaysia, *patis* and *buro* in Philippines, *nuoc-mam* and *mams-ca* in Vietnam, *makassar* and *trassi* in Indonesia and *ngapi* in Myanmar. While *feseekh*, *momone*, *lanhouin*, etc. are popular FFPs in African countries. However, it is essential to understand that the health beneficial aspects of FFPs can be limited if potential food safety hazards and other quality issues are not considered. Therefore, in this chapter the quality issues in fermented fishery products and the corresponding mitigation measures were discussed.

## Significance of fermented fishery products

Fermented fish are rich source of essential fatty acids such as omega-3 fatty acids (EPA, DHA) & omega-6 fatty acids (AA, linoleic acid); essential amino acids and micronutrients such as cobalt, chromium, copper, iron, manganese, nickel, zinc, boron, selenium, calcium, magnesium, etc. Fermented fish are potential diet for improving nutritional security particularly in developing countries. Certain FFPs possess probiotic microbes such as lactic acid bacteria (LAB). Commonly isolated LAB from FFPs are *Lactobacillus acidipiscis, L. versmoldensis, L. plantarum, L. Alimentarius, Lactococcus lactis ssp. lactis, Tetragenococcus halophilus, T. muriaticus,*

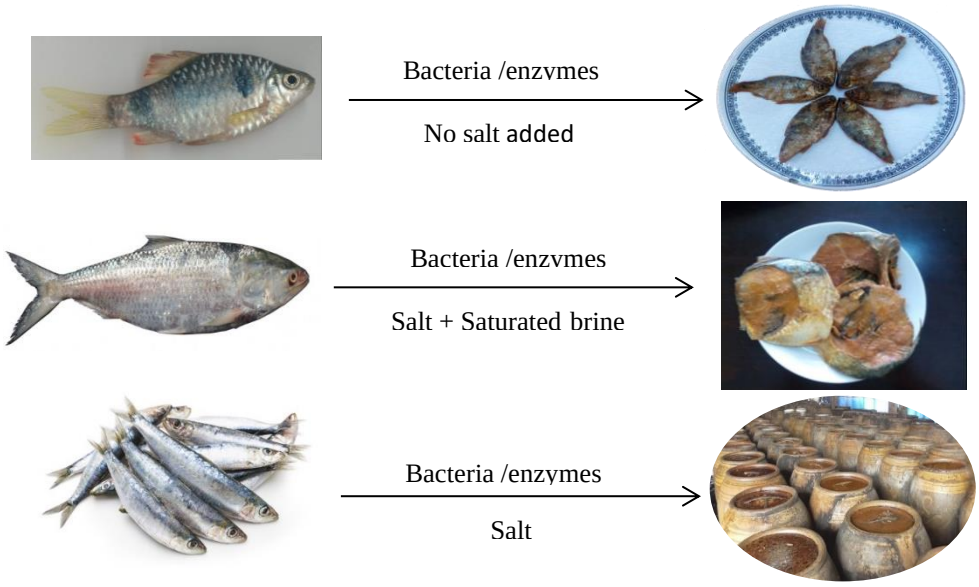
*Pediococcus pentosaceus, P. acidilactici,* etc. Ingestion of probiotic yogurt has been reported to stimulate cytokine production in blood cells and enhance the activities of macrophages (Solis and Lemonnier, 1996). Similar effect can be anticipated from LAB associated with FFPs. Bioactive peptides isolated from FFPs are reported to inhibit Angiotensin-I-converting enzyme (ACE) and control hypertension. Fermented products produced by lactic and bifids have potential anticarcinogenic activity (Goldin and Gorbach, 1977). The consumption of fermented foods containing viable cells of *Lactobacillus acidophilus* decrease β-glucuronidase, azoreductase, and nitroreductase (which catalyze the conversion of procarcinogens to carcinogens), thus possibly removing procarcinogens and activating the immune system of consumers, it is also anti-hypertensive (Goldin and Gorbach, 1984).

**Table 1.** Different categories of fermented fishery products in the world

| **Countries** | **Sauce** | **Paste** | **Whole/slice** |
|---|---|---|---|
| Japan | Phu Quoc | Nukazuke, Shiokara, | Narezushi, Funazushi |
| Thailand | Nam-pla, pla-ra, | | Plaa-som, som-fug |
| Indonesia | Makassar, bakasang, bud | trassi | |
| Malaysia | Budu, pekasam, belacan | | |
| Philippines | Patis, buro, | bagoong ( shrimp) | |
| Vietnam | Nuoc-mam, | Mams-ca | |
| Norway | | | Rakfisk (fermented salmo saithe |
| Ghana | | | Momone |
| Korea | | | Jeotgal (shrimp, oyster, fis Hongeo-hoe |
| Myanmar | | Ngapi | |
| Bangladesh | | | Shutki, Lona ilish |
| India | | | Seedhal, ngari, Hentak, Lo ilish, etc. |
| Greece | Garam | | Lekarda |
| Egypt | | | Feseekh (gray mullet) |
| Iceland | | | Hakarl (shark) |
| Sweden | | | Surstromming (herring) |
| China | | | Fermented silver carp |

| | | | |
|---|---|---|---|
| Sri Lanka | | | Columbo cured mackerel |
| Alaska | | | Igunaq |
| Canada | | | |
| Greenland | | | |

Source: Tamang and Kailasapathy (2010)




Hongeo-hoe: Korea

Igunaq: Greenland, Alaska, Canada

Kusaya: Japan



Lekarda: Greece

Rakfisk: Norway

Feseekh of Egypt



Bakasang and Budu of Indonesia

Momone of Ghana


Nam pla of Thailand

Ngapi of Myanmar

**Advantages of fish fermentation**

- Simplest way of preserving fish during surplus catch
- Overcome scarcity of fishery products in times of fishing off-season
- Imparts unique aroma and characteristics taste to the products
- Nutrients enrichment of the product by the action of fermentative microflora during the process of fermentation
- Production of beneficial microbial enzymes in the products
- Value addition of the products
- Development of varieties in fishery products
- Economic and affordable technology by the fisher folks
- High end equipments are not required
- No high technical subject matters are involved
- Create business or entrepreneurship opportunity
- Provide livelihood to fisher's community

- Utilization of rest raw materials (silage, hydrolysate, etc.)
- Minimization of protein loss from fishery sector

**Chemical changes during fish fermentation**

The pH of fish decrease during fermentation due to production of organic acids, free amino acids and large acidic polypeptides by biochemical and microbial actions. These acids contribute to the flavour development of fermented fishery products. Microbial actions degrade protein which leads to the production of volatile compounds from amino acids and small peptides. Trimethylamineoxide (TMAO) undergoes reduction to produce trimethylamine (TMA) by bacterial action giving rise to fishy odour of fermented fish. However, not all microorganisms play roles in aroma development. In the degradative changes occurring during fermentation, no significant changes were reported in the amino-acids profile particularly the essential ones. The products of fat oxidation take part in further reactions especially with amines and other decomposition products of proteins to produce coloured compounds as well as odorous substances. Lipases present in the fish flesh also hydrolyse the lipids, but the extent is dependent on the level of salting and fermentation. The volatile bases particularly TMA, DMA and $NH_3$ are associated with changes in the organoleptic and textural quality of fish.

**Composition and biochemical qualities of fermented fishery products**

| **Parameter** | **Range of values** |
|---|---|
| Moisture | 35- 69% |
| Protein | 30-40% |
| Lipid | 10-15% |
| Ash | 10-25% |
| pH | 4.25 to 6.0 |
| Salt content | 5- 28% or nil in some products |
| Volatile nitrogen fraction | TVBN: 60- 450 mg/100g<br>NPN: 2-3 g% |
| Lipid by-products | PV: 60-70 meq$O_2$/Kg lipid<br>FFA: 30-50 (as % Oleic acid) |
| Water activity($a_w$) | 0.98 to 0.89 |

**Common quality issues in fermented fishery products**

- Histamine formation in favourable environment
- Mycotoxin formation in poorly stored raw materials and products

- Botulinum toxin production in favourable condition
- Contamination with foodborne pathogens when handled unhygienically
- Growth of parasites
- Production of very high volatile nitrogen compounds
- Development of rancidity
- Dehydration and dryness in poorly stored products
- Occurrence of sand particles
- Discolouration of the products

**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

**Histamine content and associated histamine formers**

Fermented fishery products such as fish sauce and fish paste are reported to possess high content of histamine. Sanceda *et al.* (1996) reported histamine level of 430 ppm in *nampla* and 1380 ppm in Korean anchovy sauce. Kirschbaum *et al*. (2000) also reported histamine at 721 to 757 ppm in anchovy fish sauce. The majority content of histamine in 549 commercial fish sauces in Thailand was in the range of 200-600 ppm (Brilliantes and Samosorn, 2001). Fermented fishery products are usually consumed in small amounts, so higher concentrations of histamine could possibly be tolerated in these products. Yatsunami and Echigo (1993) identified halotolerant *Staphylococcus* spp., *Vibrio* spp., and *Pseudomonas* spp. as histamine formers from fermented salted sardine (nukazuke) products. *Tetragenococcus muriaticus*, a moderate halophilic and lactic acid coccus,

is a histamine-forming bacterium in salted and fermented fish products and it possess histidine decarboxylase gene (Kobayashi *et al*., 2004). These suggest that accumulation of histamine in salted and fermented fishery products may be affected by their histidine content and composition of halophilic histamine forming bacterial flora. Levels of above 200 mg/kg have been associated with human illness. However, levels as low as 50 mg/kg have known to cause illness, but this is uncommon. Most cases of illness caused by histamine in fish and fishery products have been above 200 mg/kg, and often above 500 mg/kg.

**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.

***Clostridium botulinum* and botulinum toxin formation**

Toxins produced by *Clostridium botulinum* under favourable condition in fish before salting can be stable in the salted product. Abdalla (1989) reported that in *fessiekh* processing the pH of about 6.4 to 6.9 and the salt level of 6-7% offers favorable conditions for the growth of *C. botulinum* and other proteolytic bacteria. This could possibly be the reason for fatalities involving the consumption of *fessiekh* in Egypt where the uncooked product is a delicacy among some people. Botulinum toxin have a lethal dose of 1.3–2.1 ng/kg in humans.

**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.

**Foodborne pathogen in fermented fishery products**

Foodborne pathogenic bacteria such as pathogenic *E. coli*, coagulase positive *Staphylococci* and *Salmonella* may be present in fermented fishery products if cross contamination occurs through contaminated water, contaminated surfaces and unhygienic handling.

**Preventive measures**

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.

**Biotoxin (Ciguatera)**

Biotoxin occurs in fishes of tropical and subtropical area, particular in reef fishes such as snapper, barracuda, grouper, etc. The toxins accumulate in fish when they feed on marine algae, where the toxins are present in sub-lethal state. The use of such contaminated fishes as raw material for production of fermented fishery products might cause serious consumer illness. Therefore, selection of appropriate fish species as raw material for preparation of fermented fishery products is very crucial.

**Prevention measures**

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.

**Regulatory guidelines for fermented fishery products (FSSR, 2011)**

| **PARTICULARS** | **ORGANISM & TEST METHOD** | **SAMPLING** | | **LIMITS** | | **STAGE WHERE CRITERION APPLIES** |
|---|---|---|---|---|---|---|
| | | *n* | *c* | *m* | *M* | |
| *Hygiene Indicators (cfu/g)* | *Coagulase positive Staphylococci* **Testing:** | 5 | 1 | $1\times10^2$ | $1\times10^3$ | End of Manufacturing process |

| | | | | | | |
|---|---|---|---|---|---|---|
| | ISO : 6888-1 or ISO : 6888-2 | | | | | |
| | *Yeast &mold count* **Testing:** IS:5403/ISO: 21527 | 5 | 0 | 100 | | End of Manufacturing process |
| *Biogenic amine (mg/kg)* | *Histamine* **Testing:** ISO : 19343: 2017 | 9 | 2 | 200 | | 400 |
| *Safety Indicators* | *Escherichia coli (cfu/g)* **Testing:** IS: 5887 Part 1 or ISO: 16649-2 | 5 | 2 | 4 | 40 | - |
| | *Salmonella* **Testing:** IS: 5887 Part 3/ ISO: 6579 | 10 | 0 | **Absent/25g** | | - |
| | *Clostridium botulinum* **Testing:** IS: 5887, Part 4 or ISO: 17919 | Absence of viable spores or vegetative cells of *Clostridium botulinum* and absence of botulinum toxin. | | | | - |

| *PARTICULARS* | **HAZARDS** | **LIMITS** |
|---|---|---|
| *Toxic Heavy Metals* | *Arsenic (As)* | 76 mg/Kg |
| | *Cadmium (Cd)* | 0.3 mg/Kg |
| | *Mercury (Hg)* | 0.5 mg/Kg |

| | | |
|---|---|---|
| | *Lead (Pb)* | 0.3 mg/Kg |
| | *Chromium (Cr)* | 12 mg/Kg |
| General parameters for fish sauce | pH | 5.0 – 6.5 (Traditional Product)<br>> 4.5 (If ingredients used to assist fermentation) |
| | Total Nitrogen Content | >10g/ L |
| | Amino Acid Nitrogen Content | > 40% of total nitrogen content |
| | NaCl | > 200g/L |

Where,

*n* = Number of units comprising a sample

c = Maximum allowable number of units having microbiological counts above m

m = Microbiological limit that may be exceeded number of units c

M = Microbiological limit that no sample unit may exceed

**Conclusion**

Fermented fishery products can be thus regarded as a nutrients rich diet where essential fatty acid and amino acids, mineral and vitamins are present in significant percentage. Fermented fishery products are potential diet for improving nutritional security in the society. However, the quality of the products differs from region to region. The GMP and SSOP need to be in place for hygienic fermented fishery products. Effort needs to be taken by the processors to comply the quality parameters of the regulatory guidelines. All category of hazard needs to be controlled by elimination or minimizing to an acceptable limit for safe consumption of the products.

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# PRE-REQUISITE PROGRAMMES

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HACCP IS NOT A STAND-ALONE SYSTEM. Effective HACCP system is built on a solid foundation of prerequisite programs. These are very much essential to the successful application and effective implementation of HACCP system. They provide basic environment and operating conditions that are necessary for the production of safe, wholesome food.

Before applying HACCP system in an organization it is necessary to ensure that the pre-requisite programmes are developed established and maintained effectively so as to provide a firm support to the HACCP system. Thus, a carefully developed and properly implemented pre-requisite programme can make HACCP implementation very simple. Thus the following points are to be considered essentially for a successful HACCP plan:

- Management commitment
- Plant design as per GMP
- Insect and pest control
- Hygiene and sanitation
- Trained personnel
- Site selection
- Plant layout
- Work-flow in processing
- Training at defined frequency

Most of these pre-requisite programmes are addressed by the Sanitation Standard Operating Procedures (SSOP) and Current Good Manufacturing Practices (cGMPs) listed in the Code of Federal Regulation (Current Good Manufacturing Practice in manufacturing, packing or holding human food, Code of Federal Regulation No.21 Part 110) and Standard Operating Procedures (SOPs). All food processors are expected to keep a written SSOP based on cGMP. The SSOP developed by the establishment should contain detailed procedures pertaining to daily sanitation procedures used before (pre-operational sanitation) and during (operational sanitation) operations so as to prevent direct product contamination or adulteration.

**Standard Sanitation Operating Procedures**

Each processor should implement a written SSOP focusing on the following eight areas of sanitation.

**Eight Key Areas of SSOP**

1. Safety of water
2. Condition and cleanliness of contact surfaces
3. Prevention of cross contamination
4. Maintenance of hand –washing/sanitization and toilet facilities
5. Protection from adulterant
6. Labelling, storage and use of toxic compounds
7. Pest management
8. Health of food handlers

**1. Safety of process water**

An adequate supply of potable water with appropriate facilitates for its storage, treatment, distribution and temperature control and monitoring should be made available in the establishment. Water that directly comes into contact with food, or food-contact surfaces or water used for ice production should be derived from a safe and sanitary source. It is necessary to chlorinate the pre-treated water to a level as required for the particular food. Potable water should meet the guidelines for drinking water quality stipulated by EU Directive 98/83/EC or Indian National standard IS:4251

The processor should ensure that there are no cross-connections between potable water system and non-potable water system when the latter is used for purposes like refrigeration, steam generation, fire fighting etc. It is always necessary to keep a plumbing diagram of the factory showing potable and non-potable water system separately. The over-head tank should be kept closed so as to avoid external contamination. The tank should be cleaned and disinfected at least once in three months. Water potability should be ensured at least once in six months. However, the bacterial quality of water is to be checked every fortnight.

**2. Condition and cleanliness of Contact Surfaces including utensils, gloves and outer garments**

All food contact surfaces such as plant equipment and utensils, including equipments used for ice production and storage should be made of non-toxic materials. They should be so designed as to facilitate easy cleaning. They should be able to withstand the action of food, ingredients,

and chemicals, cleaning compounds and the environmental conditions (like extremes of temperature, humidity, salinity etc.) under which they operate. Each factory should have a regular cleaning schedule to clean and disinfect the food contact surfaces. The Sanitation Supervisor should ensure that the contact surfaces are cleaned well and that there are no chances for contamination from these contact surfaces. The efficiency of cleaning should be verified once in 3 months by drawing swab samples from the contact surfaces. Gloves and outer garments that can come into contact with food should be made of water-proof material and should always be kept clean.

**3. Prevention of Cross-contamination from insanitary objects to food, food- packaging material and other food contact surfaces including utensils, gloves and outer garments and from raw product to cooked product.**

Employee's hands, gloves, outer garments, utensils and food contact surfaces of equipment that come into contact with unclean objects (like waste, and other insanitary objects) should not come into contact with food before they are cleaned and sanitized.

Care should be taken to ensure that employee's hands, gloves, outer garments utensils and food contact surfaces of equipment that come into contact with raw products should not come in contact with cooked products.

There should be physical separation for cooked, ready-to- eat products and raw food during refrigerated storage.

**4. Maintenance of hand washing, hand sanitizing and toilet facilities**

Sufficient number of hand washing facilities should be provided with sanitizing preparations and single-use towels or hand dryers. It is the responsibility of the sanitation Officer/Hygiene Officer to ensure that everybody entering the processing hall wash and disinfect their hands. Level of chlorine in the hand-dip is to be monitored 2-3 times daily and proper records to this effect are maintained. Adequate toilet facilities, maintained in sanitary conditions, should be provided.

**5. Prevention of food, food packing material and food contact surfaces from adulteration with lubricants, fuel, pesticides, cleaning compounds and other chemical, physical and biological contaminants**

Necessary control should be taken to protect food, food contact surfaces and food packaging materials from adulteration with fuel, lubricants, pesticides, cleaning compounds, sanitizing agents, metal fragments or other chemical or physical contaminants. Care should be taken to

protect food and food contact surfaces from contaminants that may drip, drain or drawn into the food.

Whenever compressed gases are used (such as in Modified Atmospheric Packaging) they should be filtered or treated to ensure that these gases do not contaminate the food with unapproved food additives or other physical, chemical or microbiological contaminants.

**6.Proper labelling, storage and use of toxic compounds**

Toxic products should be identified, held, stored and used under strict control of the on-line QC so as to avoid contamination of food, food- contact surface or food-packaging materials. All such products should be properly labelled and stored away from food processing area.

Ina food-processing establishment only the following toxic materials should be permitted for use:

- Those required for cleaning and sanitizing
- Those required for testing purposes in the laboratory
- Those required for plant and equipment maintenance and operation and
- Those necessary for use in the operation of the plant.

There should be physical separation of dry and wet chemicals.

**7.Control of employee health**

All employees should be subjected to periodic health check-up. If any person has or appears to have an illness, open lesion or any other source of microbial contamination that can contaminate the food, food-contact surface or food packaging materials, such persons should be excluded from doing work till he/she is fully recovered as evidenced by a medical examination. Employees reporting for duty after illness or long absence should be medically examined. The Medical Officer should certify that the individual is medically fit to work in a food industry. This certification is to be obtained at least once in a year.

**8.Exclusion of pests**

Adequate measures should be taken to exclude pests from all areas of the food processing plant and to prevent contamination of food, food-contact surfaces and food packaging materials. Wherever baits are used for controlling rodents, a bait map showing the location of the trap should be kept. Whenever insecticides or rodenticides are used in a food processing area, it should be done only under expert supervision after taking adequate safety precautions to prevent contamination of food, food contact surfaces and food packaging materials. All food

processors should keep a written SSOP with procedures to be followed routinely to maintain a sanitary environment for producing a safe and unadulterated food product. A Hygiene Officer or Sanitation Supervisor should be employed to monitor the SSOP, document it and to take corrective action as and when necessary.

The eight areas described above should be monitored and documented by each food processor during processing at sufficient frequency. In a company working under the HACCP system, if the eight areas of SSOPs are not monitored regularly, it becomes a major non-compliance. For each SSOP a regular system of monitoring as per example given below is to be developed.

**Scheduled for Monitoring and Documentation**

| What | how | Frequency | Who | Records | Verification |
|---|---|---|---|---|---|
| Chlorine level in water | Using test papers | Twice daily | Hygiene Officer or sanitation supervisor | Daily Sanitation Check List | Weekly verification of records by QA Manager |
| Cleanliness of contact surfaces | Visual Observation | Twice daily | Hygiene Officer or sanitation supervisor | Daily Sanitation Check List | 1. Weekly verification of records by QA Manager<br>2. Quarterly assessment of bacterial load on contact surface by swab-tests. |

**CURRENT GOOD MANUFACTURING PRACTICES (cGMP)**

These are measures of general hygiene as well as measures that prevent food from being adulterated due to unhygienic handling under insanitary conditions.

Common cGMP activities include the following:

1. **Environmental hygiene**

   While constructing a food processing plant, care is to be taken to avoid areas leading to contamination of food.

2. **Selection of site for the Factory**

Food processing factories should be selected in a locality where:

- ☞ Road frontage is available
- ☞ Good quality labour is available
- ☞ No chance for contamination from poultry
- ☞ No chance for contamination from butchery
- ☞ No chance for contamination from tannery
- ☞ No contamination from sewage disposal
- ☞ No contamination from municipal/hospital waste

3. **Building exterior**

Premises should be devoid of vegetation, which can provide shelter to pests. The area immediate to the building should be either tarred or concreted to avoid windblown dusts. All debris and garbage should be properly cleaned. Proper drainage is to be ensured. No branches of trees shall touch the building.

**4. Building interior**

Internal layout of the factory should have sanitary design features to facilitate cleaning.

- ❖ The building should be made of durable and easy to clean material.
- ❖ The surface of walls and floors should be made of impervious and nontoxic material.
- ❖ Walls should have a smooth surface and polished up to a minimum height of 5 ft. from floor to facilitate easy cleaning.
- ❖ Floors should have adequate drainage, preferably in the opposite direction of the process flow.
- ❖ Floor-wall joint and wall-wall joint should be rounded to avoid accumulation of dirt.
- ❖ Ceilings and overhead fixtures should be so constructed as to minimize the build-up of dirt and condensation.
- ❖ Windows should be easy to clean with slopping window to minimize the build-up of dirt. Where necessary, the windows should be fitted with removable and cleanable washable insect-proof screens.

5. **Ground level water tanks**

If there are any ground level water tanks, they have to be protected from birds' excreta, falling leaves and rain water. It is ideal to fix ceramic tiles inside the water tank to avoid crevices and subsequent bacterial contamination.

**6. Equipment**

All equipments should be designed and constructed so as to ensure proper cleaning and disinfection. Equipments and containers should be made of non-toxic materials. Only food grade plastic and food-grade steel are to be sued for food contact surfaces.

Equipments used for cooking, cooling and freezing of food should attain the desired temperature as rapidly as possible. Such equipments should have temperature control and monitoring facilities.

Containers used for collecting, holding and storing of waste products and inedible or dangerous substances should be made of impervious materials and should be specifically identifiable Containers for holding dangerous substances should be kept in locked room under strict vigil to prevent accidental contamination.

**7. Drainage and waste disposal**

Adequate drainage and waste disposal facility should be provided. They should be constructed in such a way as to avoid the risk of contamination of food. Liquid waste from the unclean area shall not flow through the clean area. Wherever regulation exists, the food processing factories shall get a proper certification for effluent treatment. A proper system of waste collection and removal should be established.

**8. Personal hygiene facilities**

The plant should have sufficient number of hand wash stations provided with potable hot or cold water, liquid soap and hand sanitizer. Water taps should be of foot operable type. Adequate numbers of toilets hould be provided for male and female workers separately.

9. **Ventilation**

Adiquate means of natural or mechanical ventilation should be provided. Air intakes should preferably be on the roof or at least six feet above the ground, the incoming air should not take in dust, noxious odours or exhaust air from the plant. Ventilation system should be designed and constructed in such a way that air never flows from unclean areas to clean areas.

**10. Lighting**

There should be adequate natural or artificial lighting. Sufficient light will improve the quality of work. It will also be useful to reveal any defect/filth/physical hazard present in the food product. Light fixtures should be properly protected so that broken glass pieces will not contaminate food in the event of accidental breakage. It is advisable not to fix any light bulb just above the processing table.

11. **Traffic flow pattern**

Product flow inside the plant should be uni-directional without any chances of back flow so that raw material is received at one end and finished product is hipped form the opposite end. Movement of employees, equipment and tools form unclean areas to clean area should be controlled so as to prevent cross contamination. It is advised that even air flow from dirty areas to the cleaner area is to be avoided.

**12 Storage**

Adequate storage facilities for food, food-ingredients, packing materials and non-food chemicals like cleaning materials, lubricants, refrigerants and fuels should be provided.

13. **Training**

All food handlers who directly or indirectly come into contact with food should be trained either by outside agencies or by in-house staff. Food handlers should have adequate knowledge and skill to perform their role hygienically. Personnel handling toxic and hazardous chemicals should be properly trained in safe handling techniques. Staff Engaged in hazard analysis, CCP monitoring corrective action or verification should be trained in the HACCP system and they should be competent enough to perform their duties.

14. **Calibration**

A Schedule for calibration of equipments should be established. All CCP monitoring equipments like thermometer, pH meter, moisture meter, electronic

clock, hygrometer etc. should be calibrated at regular frequencies usually once in a year. All weights, pressure gauges and temperature gauges of food processing equipment should also be calibrated and necessary documents generated.

15**. Transport vehicle cleaning**

All vehicles used for transporting raw materials, finished products, packaging material and water and ice (whenever sourced from outside) should be cleaned and sanitized prior to use. For perishable food articles like fishes, meat etc. use refrigerated trucks or reefer containers.

When the same conveyance is used for transporting different food or non-food articles, proper cleaning should be done between loads and the cleaning should be documented.

16. **Personnel hygiene and cleanliness**

All employees who directly come in contact with food, food-contract surfaces and food packaging material should adhere to strict hygiene practices when on duty so as to prevent contamination. These hygiene practices mainly include the following:

a) Employees should wear proper outer garments suitable to the operation to prevent contamination of food, food-contract surfaces or food packaging materials
b) Utmost importance should be given to personnel cleanliness. Habits like biting nails, chewing, Scratching body parts etc should be discouraged.
c) Employees should be instructed to wash their hands thoroughly using sanitizers in a hand washing facility before commencement of work, after each absence from the workstation or whenever the hands become soiled or contaminated.
d) All food handlers should be directed to remove all unsecured jewellery and other objects that can fall into the food, equipment or container. They should also be instructed to remove jewellery like rings, bangles, hair pins, toe rings or anklets.
e) When gloves are used, they should be maintained clean and in sanitary condition. The gloves should be of an impermeable material and shall be replaced by fresh ones at the interval of 2 hrs.
f) Appropriate clothing like hairnet, cap and beard covers should be used to avoid contamination with hair
g) Employee should not be allowed to eat, drink, smoke or chew gum in production areas

From the above, it is clear that the success of HACCP system depends greatly on the effective implementation of pre-requisite programmes like SSOP and CGMP. The HACCP Team. Therefore should give due importance to these pre-requisite programmes while implementing the HACCP system.

**Standard operational Procedures (SOPs)**

**Approach:** Standard operational procedures (SOPs) are written documents of the processor on the operating procedures to be followed in the unit. The processor should say what are the raw material to be used and the quality specification for the raw material.

**Raw Material Sampling:** In the case of non-branded items, quality is to be checked on each arrival. It is better to depend upon branded products and, in such cases, samples are drawn once in three months and tested for quality as per laid down specifications.

**Responsibility:** The Hygiene Officer/Sanitation Officer will be responsible to this and the records will be maintained.

**Approved Vendors/Suppliers:** Each Company shall effect purchase only through approved vendors/suppliers. All new suppliers are evaluated for their capability to supply products as per specification. The assessment of suppliers is done with on-site evaluation of their facilities, verification of track records and evaluation of samples. Records of assessment and a list of approved suppliers are maintained. Production Manager is usually responsible to approve the suppliers as well as to remove them from approved status in cases of poor performance. All suppliers are to be re-evaluated for their performance once in a year.

**Visiting Premises of Vendors:** SOP should specify whether any quality/safety guarantee is to be obtained from the Vendor. It should also spell out the company's schedule to inspect the premises of the Vendors. Proper documentation with recommendations/suggestions for improvement is also to be generated. In cases, where the results of inspection indicate chances for hazards from these premises, the Vendor's name is to be removed from the approved list.

**Receipt of Raw Material:** Raw material shall preferably be received in air-conditioned receiving areas provided with air curtasss and self-closing doors. All items are to be bought from well-reputed suppliers who maintain high standard of food, hygiene and requirement specification. Supplier's premises should also be inspected to know about the packaging and storage conditions. They have to be informedabout standard and quality specifications of the product including the delivery temperature.

All materials received in are to be checked weighed and kept away from floor preferably on stainless steel platforms. The food shall be inspected for its freshness; temperature, colour, odor, contamination infestation, satisfactory packing, expiry date and labelling. The external packing material such as cartons, gunny bags etc. are to be removed before the food item is taken to the store.

The temperature at which raw material is to be received is to be specified. Mode of storage and precautions to be taken are to be spelled out. SOP should explain in detail the various process step involved in the productionof food product including the time temperature conditions at various stages, The names of any preservatives, additives, chelating materials, antioxidants and colouring materials added have to be declared.

SOP should specify the end product quality specifications of the products produced and should specify the quality of tests to be performed, the testing frequency and the parameters to be tested is better to depend upon competent accredited laboratories, the unit may have to insist the source form where the packaging materials are to be purchased and the quality specifications. The unit may have to insist food grade certificate in case, the material comes direct contact with the packaging material. The mode, type and duration of cleaning and disinfection of process machinery, contact surfaces, water tank etc. may have to be specified in the SOP. SOPs should be written in a concise, step-by-step easy to read and easy to understand format. The information presented should be unambiguous and not complicated.

Prerequisite programs deal with the "Good housekeeping" concerns of the establishment, whereas, HACCP manages specifies process hazards. Prereqisite programms are outside the HACCP plan, but still within the HACCP system.

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# GOOD AQUACULTURE PRACTICES (GAPS)

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## Aquaculture

The broad term aquaculture refers to the breeding, rearing, and harvesting of plants and animals in all types of water environments, including ponds, rivers, lakes, and the ocean. The breeding rearing of fish, shellfish, or plants in ponds, enclosures, or other forms of confinement in fresh/marine waters for direct harvest of the product. The propagation and rearing of aquatic organisms (both marine and freshwater) in controlled or selected aquatic environments for any commercial, recreational, or public purpose. Potential purposes of aquaculture include bait production, wild stock enhancement, fish cultures for zoos and aquaria, rebuilding of populations of threatened and endangered species, and populations of threatened and endangered species.

## Good Aquaculture practices (GAqPs)

Good aquacultural practices (GAqPs) are activities, procedures, or considerations that maximize environmental and economic sustainability, product quality and safety, animal health, and worker safety, while also minimizing the likelihood of a disease outbreak on the Farm.

## GAqPs

- Regulatory and non-regulatory compliance
- Facility siting and design
- Source water
- Facility security
- Animal health
- Feed management
- Record keeping

## Global Scenario- Aquaculture

Aquaculture Market is expected to reach USD 54.9 Billion by 2027 growing at the growth rate of 7.3 % in the forecast period 2020 to 2027. India Contributes to approximately 4 % of the

world seafood trade with 5th position in world seafood export. The export of marine product from India has steadily grown over the years from 1.2 Billion US Doll in 2000 to 5.7 billion US Dollar in 2016-2017 in- spite of the changing SPS ecosystem nd stringent importing countries requirements including primary production and traceability. Given the projected population growth over the next two decades, it is estimated that at least an additional 40 million tons of aquatic food will be required by 2030 to maintain the current per capita consumption. Aquaculture, probably the fastest growing food-producing sector, now accounts for almost 50 % of the world's food fish and is perceived as having the greatest potential to meet the growing demand for aquatic food.

**Site selection-Aquaculture Farm**

- Good climatic condition.
- Availability of uninterrupted power supply.
- Supply of clear, good quality sea / fresh water throughout the year.
- Construction of the farm shall not disturb the ecosystem.
- Farm surroundings should not be contaminated by undesirable pollutants / chemicals.
- Avoid predator prevalence in the locality.
- The percolation rate / porosity of soil of the pond shall be low enough to hold the pond water satisfactorily.

**Pond Preparation and Stocking**

- Organic matter is more or in case of disease / medication in previous cycle, the sludge sediments of the pond should be removed suitably.
- Proper sediment management and monitoring shall be implemented to avoid contamination.
- The pond shall be allowed to fully dry and disinfect at least once in a year.
- Pond Preparation and Stocking Probiotics may be applied, if required.
- Water pH and algal bloom shall be allowed to stabilize before stocking.
- Avoid over stocking.

**Bio security Structures and Facilities**

- Reservoirs to facilitate water treatment to maintain water quality and control diseases.
- Crab fencing around each pond and around the farm to eliminate crabs/other carriers in pond.
- Bird fencing on the ponds to prevent birds from spreading diseases to other ponds and farms.
- Fences and barriers to control human and animal entrants into the culture area or the farm.
- Foot and hand washes, wheel baths and net wash in entrances to the farm and the culture areas to avoid contamination.
- Usage of screens and filters for removing insect vectors and eggs.
- Selection and stocking of certified SPE seed.

**Fencing (Both bird and Crab fence)**

- Prevents the birds and crabs from one farm to other farm.
- Gap between two strings
- Should Be finished before treatment

**Farm Input Management**

- Inputs based on legal requirements.
- Maintain proper stock / utilization register of all inputs.
- The quality of inputs shall be checked while receiving.
- Banned chemicals / pharmacologically active substances shall not be received or stored or used.
- Only products approved for use by the farm shall be stored and the chemicals shall always be stored and used according to the instructions given in the lab
- Only post larvae / fingerlings supplied by approved hatcheries accompanied by Test Report shall be accepted.

**Water Management**

- Continuous supply of good quality water
- The physico-chemical quality of water should be monitored
- Suitable filtration system.
- Water should be tested for microbiological and chemical contaminants
- Aeration to maintain sufficient dissolved oxygen level in water through suitable mechanism.
- Generator facility.

**Feed Management**

- Feed from approved Feed Mills.
- Stored in well ventilated, dry store,
- Ensure that banned chemicals / pharmacologically active substance are not used in the feed.
- Feeding of appropriate quantity of right type of feed shall be done at appropriate time.

- Feeding should be stopped before harvest.
- Feeding according to the feeding plan and monitoring required for excess feed.
- The feeding schedule based on monitoring observations where necessary revised.

**Usage of Fertilizers or Other Chemicals**

- The rate and mode of application of fertilizers should be planned.
- The usage of fertilizer containing ammonia or ammonium in water with pH of 8 or above shall be avoided.
- The usage of fertilizer containing ammonia or ammonium in water with pH of 8 or above shall be avoided.
- The rate and mode of application should be planned

**Waste and Effluent Management**

- Waste shall be disposed of actively in a suitable manner to avoid cross contamination.
- Chemical wastes and non-biodegradable wastes shall be disposed of as per legal requirement.
- Waste water shall be treated suitably before discharge.
- The effluent water shall be monitored for pH, suspended solids, soluble phosphorus, total ammonia nitrogen, BOD etc. on a laid down frequency and records maintained.

**Monitoring**

- Continuous monitoring of physico-chemical parameters of water such as salinity, pH, nitrogenous compound concentration, temperature, dissolved oxygen level, suspended solids etc. at regular intervals should be done to ensure optimal environmental conditions for maximum growth and survival.
- Monitoring records shall be maintained.
- The feeding habits, change of colour of water, health condition and size of animal, signs of stress etc. shall be monitored at regular intervals.
- If water quality seems to be bad, feeding may be reduced, aeration increased, water exchanged and or approved inputs (probiotics / lime) added.
- Pond mud shall be monitored for pH regularly. Primary productivity shall also be monitored on a laid down frequency. Monitoring of weather such as wind speed, rain fall, temperature etc. shall also be done.

**Optimum Values for Major Water Quality Parameters (as applicable)**

| Para meter | Optimum level | Ideal frequency of monitoring |
|---|---|---|
| Dissolved oxygen | > 4.0 mg / l | Twice daily in ponds |
| pH | 6.5-8.5 | Twice daily in ponds |
| Alkalinity | Minimum of 50 mg / l, 100-400 mg / l preferred | Several times a year in ponds |
| Hardness | Same as alkalinity | Same as alkalinity |
| Ammonia ($NH^3$) | < 0.15 mg / l | Twice weekly in ponds |
| Nitrate ($NO^3$) | < 50 mg / l | Once daily |
| Nitrite ($NO^2$) | < 0.5 mg / l in low-chloride water | Weekly in ponds |
| Hydrogen sulfide | < 0.15 mg / l | Upon initial use and periodically throughout season |

**Cleaning & Sanitation and Personal Hygiene ϖ Adequate cleaning & sanitation**

Adequate cleaning & sanitation shall be maintained at all areas of the farm, including machineries / equipment to avoid microbial contamination.

Employees shall strictly adhere to good personal hygiene practices.

**Medication**

- Only permitted chemicals / pharmacologically active substance shall be used under the advice of veterinary medical practitioner/Farm technician for treatment of animals.
- Proper withdrawal period shall be followed for the authorised Veterinary Medicinal Products ( VMPs) used in the facility.
- In case withdrawal period of particular VMP is not prescribed, by the VMP manufacturer, then the default withdrawal period of 500 degree days shall be followed.

**Pest control**

Suitable pest control measures shall be adopted to prevent entry pests into the farm

**Harvest and Transportation**

Proper care shall be taken while harvesting to avoid damage to the animals.

The harvested animals shall be hygienically handled and properly iced before dispatch to approved establishments to avoid deterioration and microbial contamination.

Before harvest Chloramphenicol and metabolites of Nitrofuran should be tested at designated lab

**Record Keeping**

The farm shall maintain all records as required to establish traceability of animals reared, input & output records, monitoring records, test reports, cleaning records etc

The traceability record as given below shall be maintained by the farm for verification by the Competent Authority.

**Traceability Record**

1. General information
   Name of the aquaculture farm & location
   Approval Number
   Pond Number
   Pond area
   Production capacity of pond
2. Post Larvae/ fry/fingerlings stocked in the pond
   Hatchery name & approval number
   Stocking date & quantity Type of stocking
   Pre-harvest Test Report from hatchery
3. Details of feed used in the pond
   Name of Feed Mill & approval number
   Type, quantity & lot no of feed utilized
   Test report of banned chemicals from Mill

4. Therapeutic drugs used

a) Drug - 1 Details of drug

Disease treated

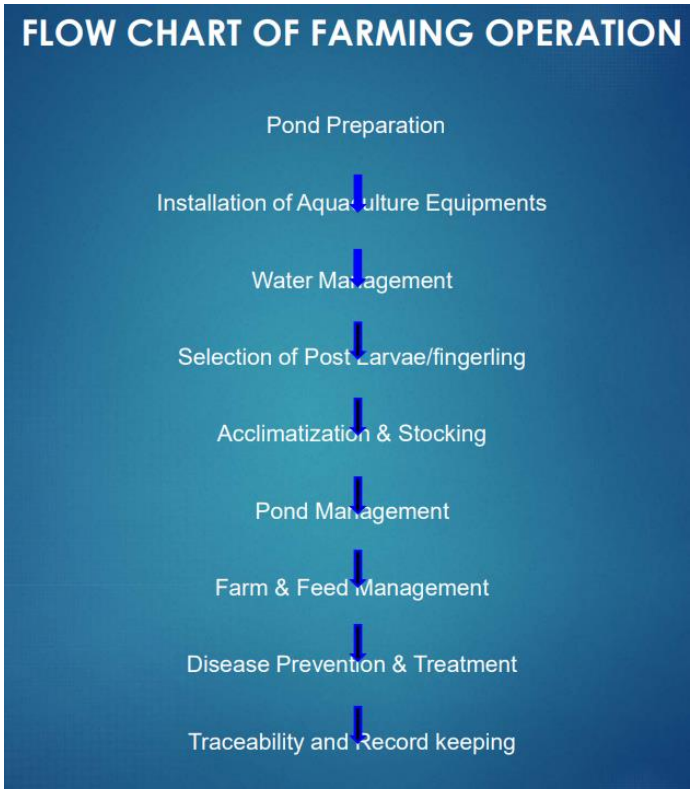
Date of application with quantity

Withdrawal period applied

b) Drug – 2

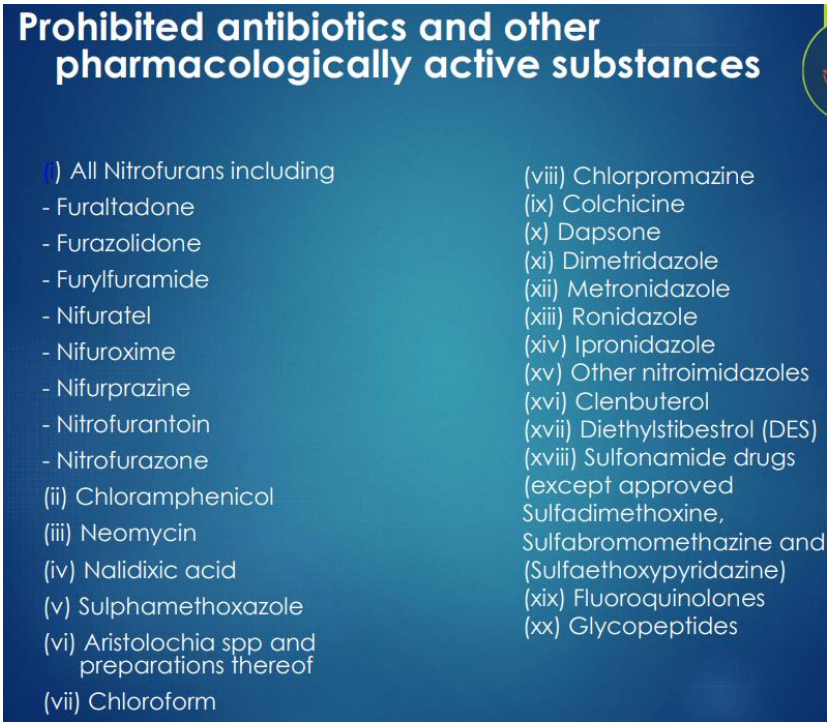
Withdrawal period applied

5. Pesticide used in the pond
   a) Compound - 1 Details of compound Condition treated, Date of application with quantity Application period
   b) b) Compound - 2 Details of compound Condition treated, Date of application with quantity Application period
6. Details of Harvest from the pond
   Date of Harvest Quantity harvested Sodium meta-bisulphite treated or not
7. Details of supply materials harvested from the pond Name and approval no of establishment(s) Quantity supplied to each establishment Mode of transportation Pre-harvest Test Report(s) pertaining to the supply




**Control of Veterinary Drugs at Farm**

- To prevent, diagnose, cure or alleviate a disease, condition or pest infestation.
- To cure or alleviate an injury.




**Prohibited veterinary drugs and contaminants and other substances**

Substances having anabolic effect and unauthorised substances, namely (a) stilbenes, stibene derivatives and their salts and esters; (b) steroids. (ii) Veterinary drugs and contaminants namely:- (a) antibacterial substances, including quinolones; (b) anthelmintics (iii) Other substances and environmental contaminants namely :- (a) organochlorone compounds including PcBs; (b) mycotoxins; (c) dyes. Provided that the use of items at sl. No. (i)(b), (ii) (a) and (b) for therapeutic or zoo-technical purposes may be authorised by qualified Veterinary surgeons or Fishery Scientists.

**Use appropriate registered & permitted veterinary chemical products**

- Purchase the Chemical from Licensed Shop.
- All technicians must use registered veterinary chemical products in accordance with the instructions on the product's approved label
- Identify the problem and make sure you are using the appropriate registered chemical product.
- The instructions on the approved product label show the crop, animal or situation for which the product can be used and the pests and diseases that can be controlled.
- It should be used according to the instructions on the approved label

**Read and understand the material safety data sheet (MSDS)**

- These MSDSs can be obtained from the supplier when products are purchased.
- Read the MSDS carefully before using or storing chemical products
- Follow the instructions carefully when applying, handling or storing products

**Animals need to be under the care of the Farm Technician**

Under the legislation, a veterinary surgeon or fishery Scientist

Farm Technician can only use, prescribe, supply or recommend the use of a veterinary chemical product to treat animals under their care.

This applies whether the veterinary chemical product is registered, unregistered or veterinary chemical product is registered, unregistered or prepared by the veterinary surgeon.

**Discuss your Drug application with neighbours**

Discuss your plans with neighbours and spraying contractors, and keep in touch with what is happening around you, including other people's plans regarding use of veterinary chemical products.

**Chemical residues and withholding periods**

- Information about chemical use and withholding periods prior to harvest appear on the label of registered chemicals.
- If a aqua technician has provided instructions for treating animals with a veterinary chemical, you should follow that advice.
- It is important to follow the correct dose rates and any withholding period advised by your technician before harvesting - aquaculture products for human consumption.
- Harvesting before the withholding period could mean unacceptable chemical residues for the withholding period could mean unacceptable
- Where treated animals are subject to a withholding period, you must take reasonable steps to ensure that the animals can be identified during the period.
- Physical segregation and written records can be used for this purpose.
- Farm technician have certain obligations to inform the owner of a trade-species animal of any withholding periods that apply to the treatments

- Treated trade-species animals must be identified as such during the treatment period and the withholding period.
- Animals may be identified several ways, including tagging, physical segregation and written records.
- They are also obligated to ensure that any withholding period is observed.

**Information to be given by a Farm Technician**

Farm technician are obligated to provide full and appropriate written instructions about the treatment of an animal

**Record Keeping & Storage**

- Farm technician must make detailed records of the treatment of trade species animals.
- These records must be kept for two years or another period prescribed under a regulation.
- The veterinary drugs prescribed by Farm technician shall be separately kept from the unprescribed drugs.
- The handling or disposal of expire

**Training for workers**

- Create awareness of veterinary chemical products.
- Knowledge about banned veterinary drugs as per National & Importing countries requirements.
- Farm management practices.
- Pre-harvest requirements


**Fish quality assurance**

- According to the International Standards Organization (ISO), Quality Assurance (Q.A) consists of all those planned and systematic actions necessary to provide adequate confidence that a product or service will satisfy given requirements for quality.
- Proper handling of fish between capture and delivery to the consumer is a crucial element in assuring final product quality. Standards of sanitation, method of handling and the time/temperature of holding fish are all significant quality factors. With a few exceptions, fish are considered free of pathogenic bacteria of public health significance when first caught.

**Microbiological testing**

A number of microbiological tests of fish and fish products are used by authorities to check that the microbiological status is satisfactory. The purpose of these tests is to detect pathogenic bacteria (Salmonella, Staphylococcus aureus, E. coli) or indicator organisms of fecal pollution (fecal coliforms, fecal streptococci) or other types of general contamination or poor handling practices (coliform bacteria, faecal streptococci, total viable count). Microbiological testing can be costly and time consuming. Estimation of bacterial numbers in fish is frequently used to retrospectively assess microbiological quality or to assess the presumptive safety of the product. The number, size and nature of the samples greatly influence the results and even the

most elaborate sampling cannot guarantee the safety of the product. However, it is still worthwhile; if substandard consignments are found, the psychological effect on the seller is high, especially if the consignment is deemed for export to countries that have established microbiological criteria.

**Microbiological standards to be met Sampling plan and recommended microbiological limits for seafood (ICMSF 1986)**

| Product | Test | Case | Plan Class | no. of samples | no. of positive results | Limit per gram or per cm² | |
|---|---|---|---|---|---|---|---|
| | | | | | | m | M |
| Fresh and frozen fish | APC | 1 | 3 | 5 | 3 | $5 \times 10^5$ | $10^7$ |
| | *E. Coli* | 4 | 3 | 5 | 3 | 11 | 500 |
| Precooked breaded fish | APC | 2 | 3 | 5 | 2 | $5 \times 10^5$ | $10^7$ |
| | *E. Coli* | 5 | 3 | 5 | 2 | 11 | 500 |
| Frozen raw crustaceans | APC | 1 | 3 | 5 | 3 | $10^6$ | $10^7$ |
| | *E. Coli* | 4 | 3 | 5 | 3 | 11 | 500 |
| Frozen cooked crustaceans | APC | 2 | 3 | 5 | 2 | $5 \times 10^5$ | $10^7$ |
| | *E. Coli* | 5 | 3 | 5 | 2 | 11 | 500 |
| | *S. aureus* | 8 | 2 | 5 | 0 | $10^3$ | - |
| Cooked, chilled, and frozen crabmeat | APC | 2 | 3 | 5 | 2 | $10^5$ | $10^6$ |
| | *E. Coli* | 6 | 3 | 5 | 1 | 11 | 500 |
| | *S. aureus* | 9 | 2 | 5 | 0 | $10^3$ | - |
| Fresh and frozen bivalve molluscs | APC | 3 | 2 | 5 | 0 | $5 \times 10^5$ | - |
| | *E. Coli* | 6 | 2 | 5 | 0 | 16 | |

**The HACCP concept**

- The system is based on the recognition that microbiological hazards exist at various points, but measures can be taken to control these hazards. The anticipation of hazards and the identification of control points are therefore key elements of HACCP.
- The system offers a rational and logical approach to control food hazards and avoid the many weaknesses inherent in the inspectional approach. Once established, the main effort of the quality assurance programme will be directed towards the Critical Control Points (CCPs) and away from endless final product testing. This will assure a higher degree of safety and at less cost.

**Main elements of the HACCP system**

- Identify potential hazards. Assess the risk of occurrence.
- Determine the Critical Control Points (CCPs)
- Establish criteria to be met to ensure that each
- CCP is under control.

- Establish a monitoring system.
- Establish corrective action when CCP is not under control.
- Establish procedures for verification.
- Establish documentation and record-keeping.

**Checklist for ensuring seafood safety**

1. Landed fish should not be exposed to the sun and should be iced.

2. Inspect fish for appearance and odour and reject fish of unacceptable quality.

3. Periodically perform bacteriological tests on representative samples.

4. Follow a cleaning schedule for all work areas and surfaces, using water containing 5 to 10 ppm of free chlorine.

5. Remove all fish slime and blood by hosing down with chlorinated water. At the end of the day, rinse all surfaces with clean water having 5 ppm of chlorine.

6. Apply personal hygiene rules strictly to prevent contamination of fish. Smoking and spitting in work areas should not be permitted. Hands must be washed with bactericidal soap prior to handling fish and after a visit to the toilet.

7. Check that water supply and treatment systems are in order. Water and ice samples should be analysed as per testing schedule by ISO certified laboratories for levels of chemical and bacteriological contamination and potability certificates obtained.

8. The harbour should be free from litter and other wastes.

9. Check to ensure that all drainage systems are in good working order.

10. The harbour should be free of animals, rodents and pests.

11. Ensure that there are no bird nests in the fish handling area.

12. Check that wastes are being disposed of sanitarily.

13. Check cold storage equipment to ensure that the right temperature is being maintained.

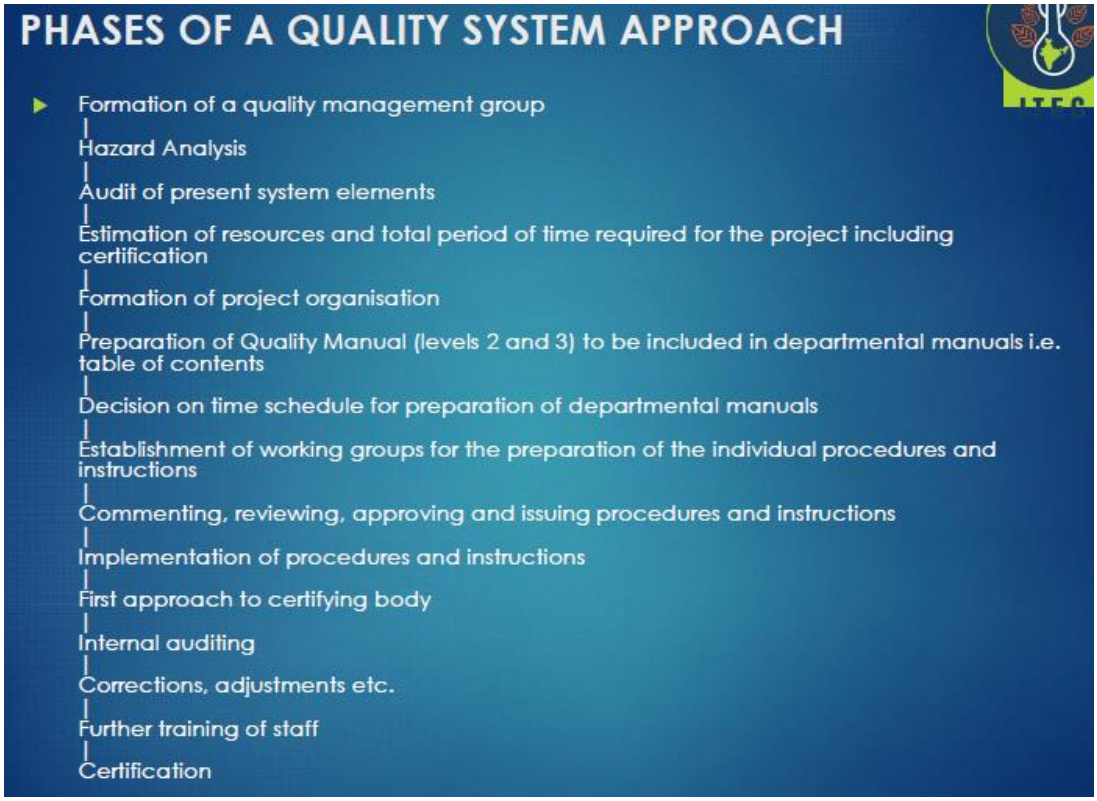
14. Ensure that all precaution and warning signs are readable.

**Advantages of the HACCP system**

- Control is proactive in that remedial action can be taken before a problem occurs. Control is through features that are easy to monitor such as time, temperature and appearance.
- Control is cheap in comparison with detailed chemical and microbiological analysis.
- The operation is controlled by persons directly involved with the fish product.
- It can be used to predict potential hazards.

**The ISO-9000 series certification of the International Standards Organization**

- ISO 9000 standards comprise many elements. Of these, management responsibility and commitment is the first and most important element. The next element is the presence of a documented quality system organized in three levels comprising the Quality Manual, Procedures and Instructions. are a vital part of the ISO 9000 standards with particular reference to the food industry.
- ISO 17025 ,17021, 17020,17065



**Conclusion**

- Good aquaculture practices are a common-sense approach to enhancing animal welfare, product quality and safety, worker safety, and environmental and economic sustainability. The larger and more intense the facility, the more detailed will be the associated GAqPs,
  as well as the record keeping.
- If situations change over time, so should the GAqPs. Good aquacultural practices should be adjusted whenever there are intended or unintended changes. Good aquacultural practices and the documentation that accompanies them will enhance buyer confidence and producer accountability

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# 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, can not 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

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# CHEMICAL HAZARDS IN FISH AND 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

**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.

**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 1-2mg 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.

**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.

**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.

# BIOLOGICAL HAZARDS- BACTERIA OF PUBLIC HEALTH SIGNIFICANCE

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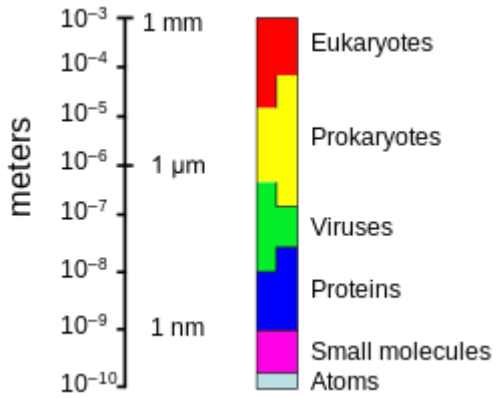
A microorganism, or microbe, is an organism of microscopic size, which may exist in its single-celled form or as a colony of cells. Technically a microorganism or microbe is an organism that is microscopic. The scientific study of microorganisms began with their observation under the microscope in the 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**

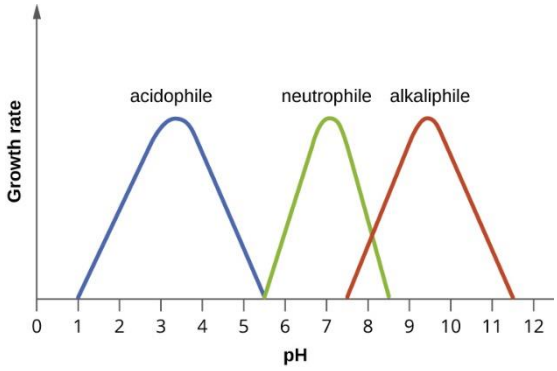
a) Temperature: Generally,an increase in temperature will increase enzyme activity. But if temperatures get too high, enzyme activity will diminish and the protein (the enzyme) will denature. On the other hand, lowering temperature will decrease enzyme activity. At freezing temperatures enzyme activity 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

# PRINCIPLES OF HACCP AND ITS 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 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.

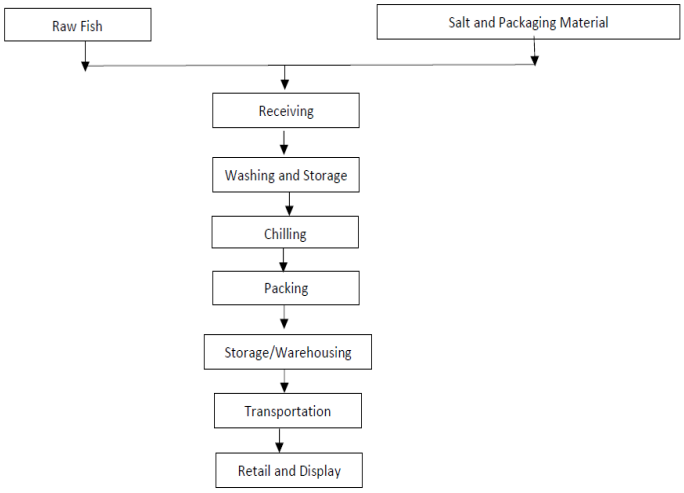


Fig 1. Example of flowchart of chilled fish

### *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:

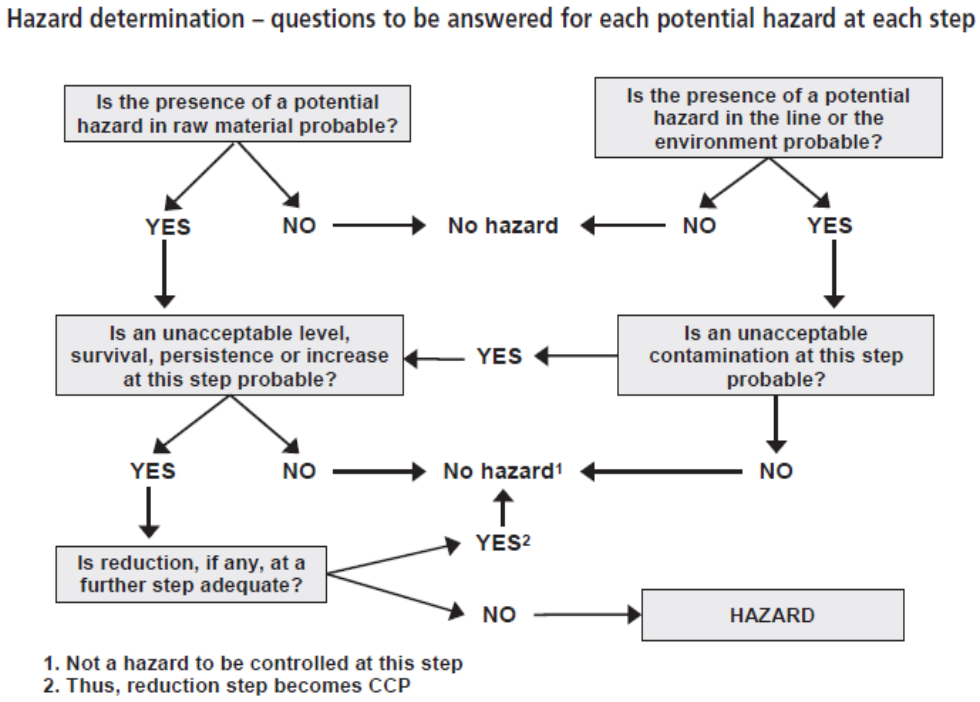


Fig 2. Hazard determination decision tree

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.

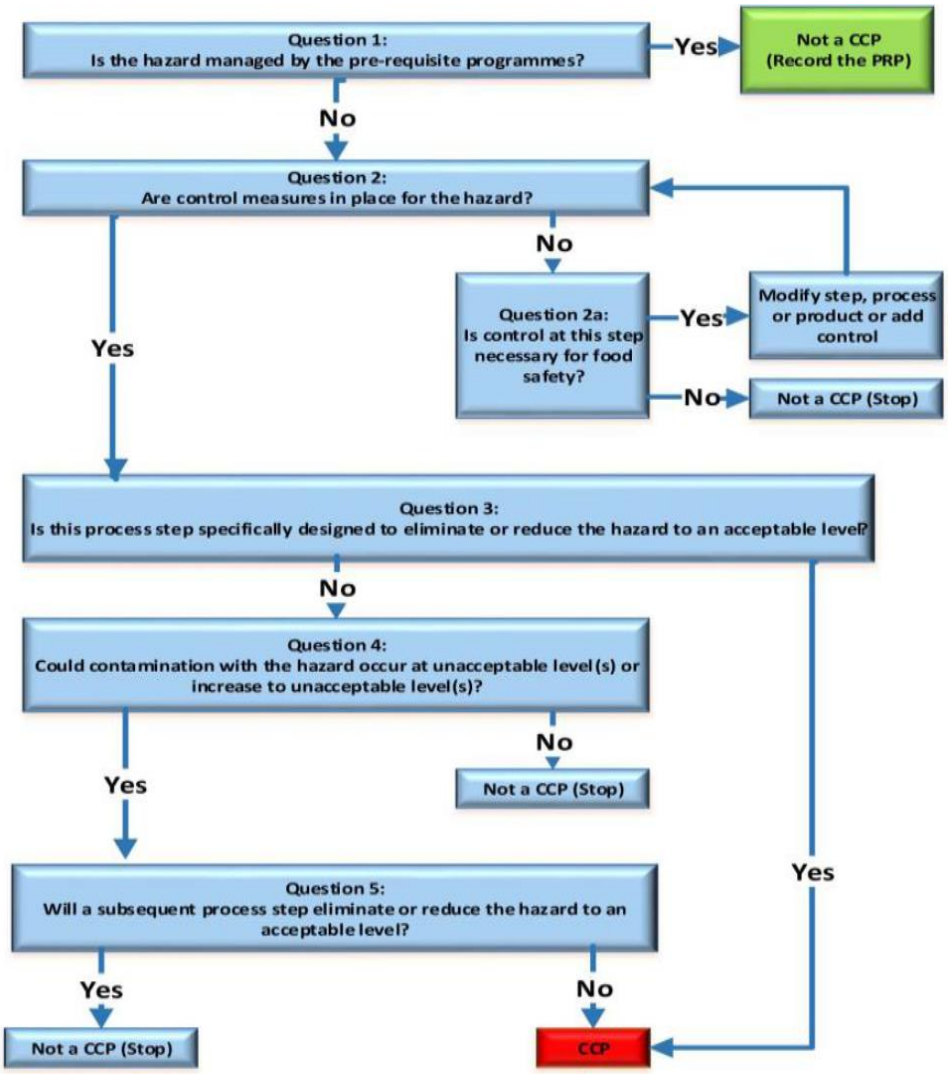


Fig 3. CCP decision tree

### *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**

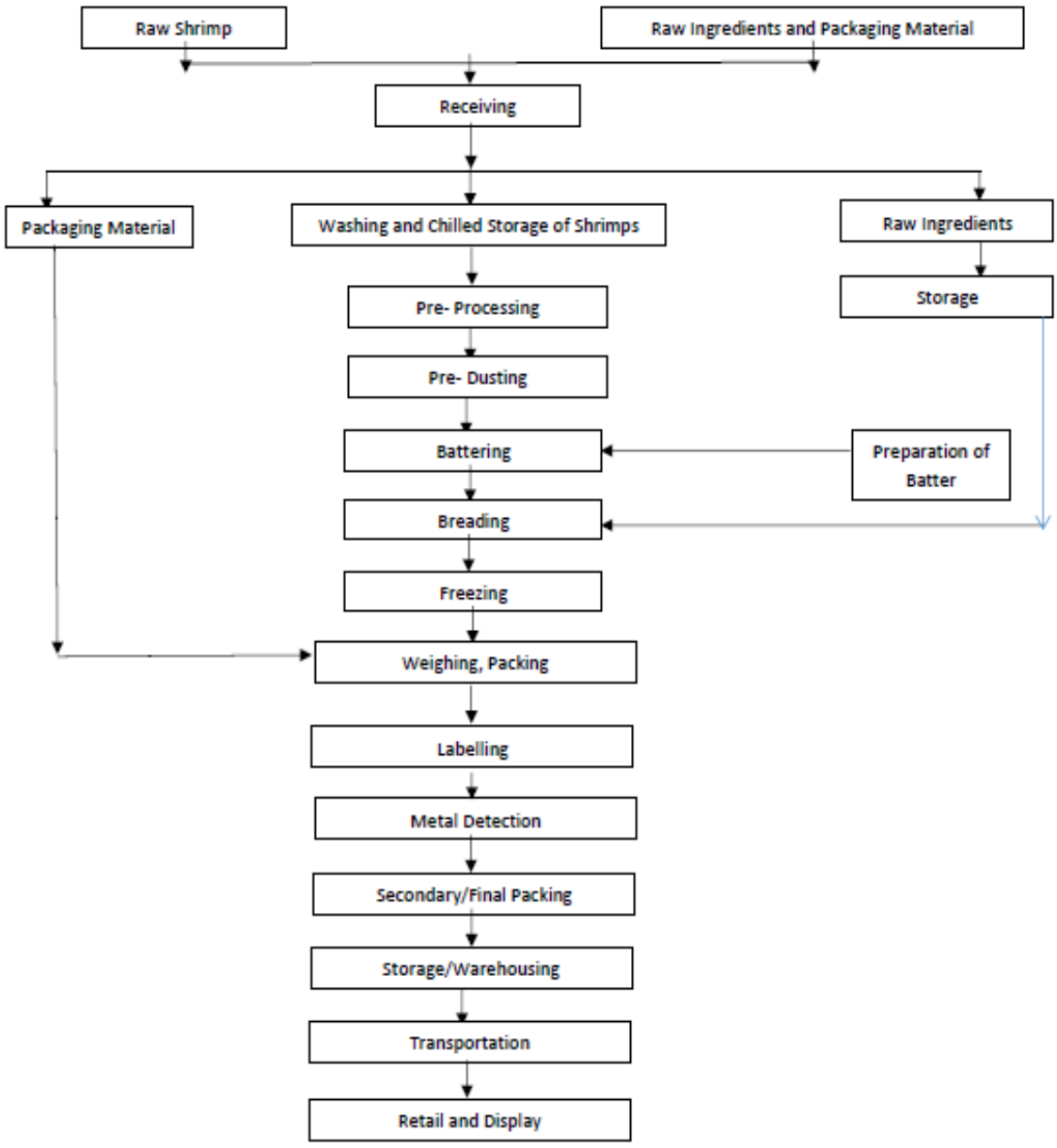


Fig 4. Example: IQF breaded shrimp

**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<br>Supplier's guarantee that sulphiting agents are not used and the raw product is free from pesticide residues.<br>Supplier's gurantee taking into account withdrawal period | Y | | | | | | N | Supplier's declaration<br>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.<br>Packaging material used must be food grade. |
| 2. | Washing | Biological | Microbial Pathogens | M | L | ML | Taken care by PRPs and eliminated during retorting stage<br>Use only potable water for washing | Y | - | - | - | - | - | N | Microbial pathogens are reduced or eliminated in the subsequent pre-cooking and retorting stage.<br>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<br>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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# ADVANCED MICROBIOLOGICAL SYSTEMS

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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.

# AUTHENTICITY AND TRACEABILITY OF SEAFOOD

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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 (Food Information legislation) | Meat, fish , composite foods |
| 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)<br>○ 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.<br>○ Metabolomics uses mostly nuclear magnetic resonance (NMR) and mass spectrometry (MS) analytical technologies<br>○ 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).<br>○ Microflora was found to be specific of the production system and microbial fingerprints were shown to differ between organically and conventionally grown fruits<br>○ 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.<br>○ 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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# OVERVIEW 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:**

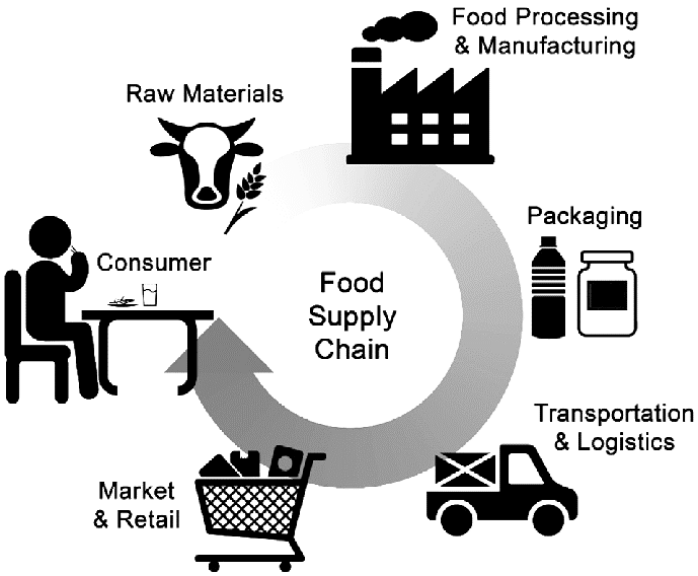


Fig. 1. Food Supply Chain

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.

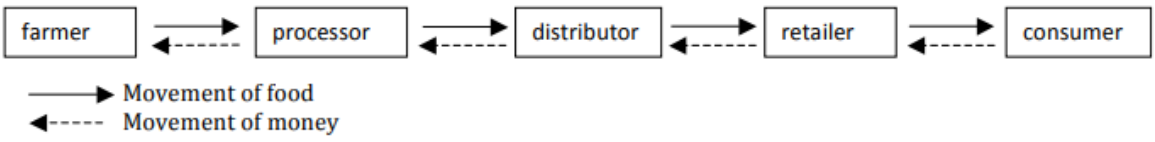


Fig.2.Movements of food and money in a simple food supply chain

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).

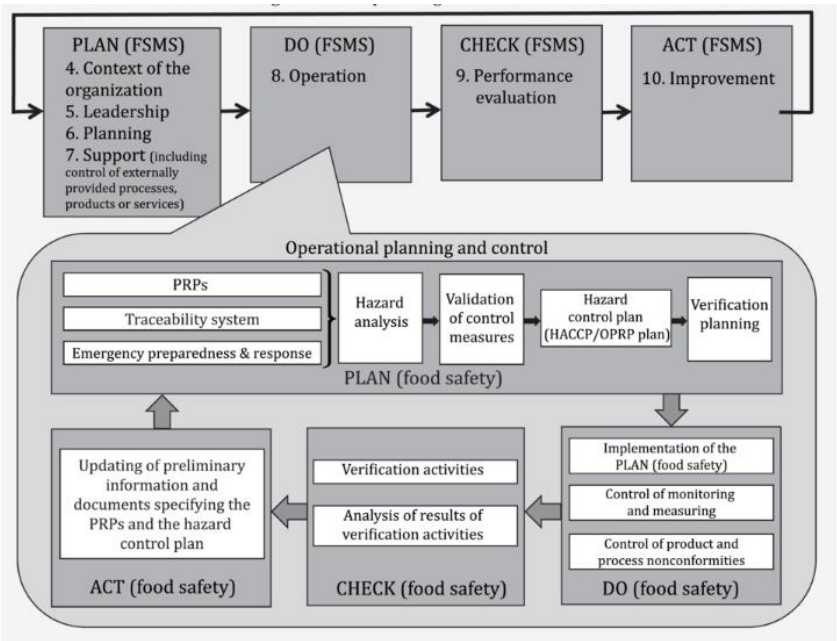


Fig Organizational planning and control of ISO 22000:2018

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

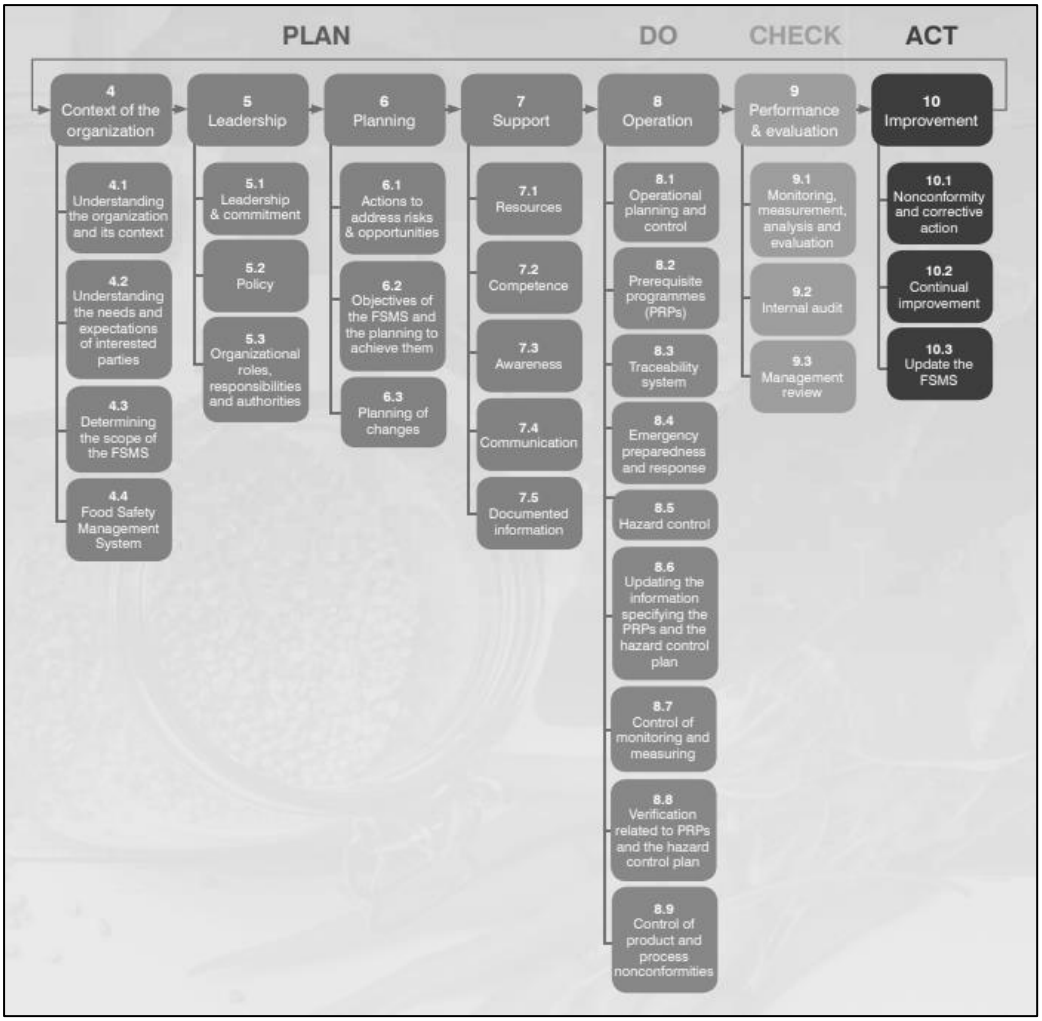


Fig.3. PDCA cycle of ISO22000:2018 (*source: NQA-ISO-22000-Implementation-Guide)*

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

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# IMPLEMENTATION OF ISO 22000:2018 FOOD SAFETY MANAGEMENT SYSTEM

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## Introduction

The International Organization for Standardization (ISO) was established in 1947 and ISO is an independent, non - governmental international organization with a membership of 164 national standard bodies. ISO employs a system of technical committees and working groups to develop international standards. ISO 22000 is an international standard that specifies requirements for a food safety management system where an organization in the food chain needs to demonstrate compliance with food safety requirements. 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.

ISO 22000 is the food safety management system that can be easily applicable to any organization in the food chain. ISO 22000 was initially developed on September 1st 2005 by the ISO/TC 34/SC 17 as the first truly international FSMS standard. Food safety hazards can occur at any stage in the food chain making adequate control throughout the food chain essential. By combining PDCA and risk-based thinking to manage business risk with HACCP to identify, prevent and control food safety hazards, ISO 22000 helps organizations to reduce exposure to risk and improve safety. ISO 22000 is aligned with the requirements of ISO 9001 in order to enhance the compatibility of the two standards and to ease their joint or integrated implementation.

The potential benefits to an organization of implementing a FSMS are:

- ability to consistently provide safe foods and products and services that meet customer and applicable statutory and regulatory requirements;
- addressing risks associated with its objectives;
- the ability to demonstrate conformity to specified FSMS requirements.

ISO 22000 combines generally recognized key elements to ensure food safety along the food chain:

- Interactive communication

- HACCP principles
- System management
- Prerequisite programmes

Covers 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.

An ISO 22000 food safety management system (FSMS) can be implemented in small, medium and large-sized food organizations from all aspects of the food chain:

- Food and ingredient manufacturers
- Retailers
- Wholesalers
- Agricultural producers
- Transport, logistics and storage providers
- Packers
- Equipment and packaging manufacturers
- Caterers

**Key requirements of ISO 22000**

**Clause 1: Scope**

This clause details the scope of the international standard. This includes requirements about planning, implementation, maintaining and updating an FSMS as well as effective communications.

**Clause 2: Normative references**

There are no normative references within the standard.

**Clause 3:** Terms and definitions - 45 definitions have been elucidated for proper understanding and implementation.

**Clause 4: Context of the organization**

4.1 Understanding the organization and its context - determine external and internal issues that are relevant

4.2 Understanding the needs and expectations of interested parties – as per statutory, regulatory and customer requirements

4.3 Determining the scope of the food safety management system – determine scope based on product, services and processes as per external and internal issues & requirements

4.4 Food safety management system - establish, implement, maintain and continually improve the FSMS in accordance with the requirements of the standard.

**Clause 5: Leadership**

5.1 Leadership and commitment - Top management shall demonstrate leadership and commitment with respect to the FSMS. Ensure a food safety policy and objectives, integration of FSMS requirements with business

5.2 Policy - establish, implement and maintain a food safety policy appropriate to the purpose and context of organization which satisfy the requirements, communicate the policy

5.3 Organizational roles, responsibilities and authorities –

5.3.1 Top management shall ensure that the responsibilities and authorities for relevant roles are assigned, communicated and understood within the organization.

5.3.2 The food safety team leader shall be responsible for ensuring the FSMS is established, implemented, maintained and updated, ensure relevant training and competencies for food safety team

**Clause 6: Planning**

Organization plans actions to address both the risks and opportunities identified in Clause 4. It focuses on the development and use of a planning process, rather than a procedure to address both a range of factors and the risk associated with such factors.

6.1 Actions to address risks and opportunities

6.1.1 determine the risks and opportunities of FSMS that need to be addressed

6.1.2 The organization shall plan actions to address these risks and opportunities of FSMS

6.1.3 Consider requirements and impacts when selecting FSMS actions

6.2 Objectives of the food safety management system and planning to achieve them

6.2.1 The organization shall establish objectives for the FSMS

The objectives of the FSMS shall:

a) be consistent with the food safety policy;

b) be measurable (if practicable);

c) take into account applicable food safety requirements, including statutory, regulatory and customer requirements;

d) be monitored and verified;

e) be communicated;

f) be maintained and updated as appropriate

6.2.2 Plan how to achieve your organization's FSMS objectives

6.3 Planning of changes

**Clause 7: Support**

This clause is all about the execution of the plans and processes that will enable your organization to successfully complete their FSMS responsibilities. This is a very powerful requirement covering all management system resource needs.

7.1 Resources

7.1.1 General - The organization shall determine and provide the resources needed for the establishment, implementation, maintenance, update and continual improvement of the FSMS.

7.1.2 People – competence of persons necessary to operate and maintain an effective FSMS

7.1.3 Infrastructure - provide the resources the FSMS need to have

7.1.4 Work environment - provide and maintain the resources for the establishment,

management and maintenance of the work environment necessary

7.1.5 Externally developed elements of the food safety management system - organization establishes, maintains, updates and continually improves its FSMS by using externally

developed elements of a FSMS, including PRPs, the hazard analysis and the hazard control plan ensure conformance with the requirements

7.1.6 Control of externally provided processes, products or services – apply criteria for evaluation, selection and monitoring of performance

7.2 Competence - ensure that these persons, including the food safety team and those responsible for the operation of the hazard control plan, are competent on the basis of appropriate education, training and/or experience

7.3 Awareness - The organization shall ensure that all relevant persons doing work shall be aware of:

a) the food safety policy;

b) the objectives of the FSMS

c) Individual contribution

d) Implications of non conforming the requirements

7.4 Communication - Support your FSMS by controlling relevant communications

7.4.1 General - Support FSMS by establishing communication systems

7.4.2 External communication - Support your FSMS by facilitating external communication

7.4.3 Internal communication - Support your FSMS by encouraging internal communication

7.5 Documented information - documented information of the FSMS and food safety requirements required by statutory, regulatory authorities and customers. Creating, updating and control of documents depending upon product, process and services

7.5.1 General

7.5.2 Creating and updating

7.5.3 Control of documented information

**Clause 8: Operation**

8.1 Operational planning and control - The organization shall plan, implement, control, maintain and update the processes needed to meet requirements for the realization of safe products, and to implement the actions determined.

8.2 Prerequisite programmes (PRPs) - to facilitate the prevention and/or reduction of contaminants (including food safety hazards) in the products, product processing and work environment – appropriate to the size, type and nature of the products handled

8.2.1 Make sure that prerequisite programmes are developed

8.2.2 Make sure that prerequisite programmes are implemented

8.2.3 Make sure that prerequisite programmes are acceptable

8.2.4 Make sure that prerequisite programmes are suitable

8.3 Traceability system - uniquely identify incoming material from the suppliers and the first stage of the distribution route of the end product as per requirements

8.4 Emergency preparedness and response - ensure procedures are in place to respond to potential emergency situations or incidents

8.5 Hazard control

8.5.1 Preliminary steps to enable hazard analysis

8.5.1.1 General - preliminary documented information shall be collected, maintained and updated by the food safety team related to product, process and equipment and also as per requirements

8.5.1.2 Characteristics of raw materials, ingredients and product contact materials – conduct hazard analysis on

a) biological, chemical and physical characteristics;

b) composition of formulated ingredients, including additives and processing aids;

c) source (e.g. animal, mineral or vegetable);

d) place of origin (provenance);

e) method of production;

f) method of packaging and delivery;

g) storage conditions and shelf life;

h) preparation and/or handling before use or processing;

i) acceptance criteria related to food safety or specifications of purchased materials and ingredients appropriate to their intended use.

8.5.1.3 Characteristics of end products

a) product name or similar identification; b) composition; c) biological, chemical and physical characteristics relevant for food safety; d) intended shelf life and storage conditions; e) packaging; f) labelling relating to food safety and/or instructions for handling, preparation and intended use; g) method(s) of distribution and delivery.

8.5.1.4 Intended use - groups of consumers/users and vulnerable group

8.5.1.5 Flow diagrams and description of processes

8.5.1.5.1 Preparation of the flow diagrams

8.5.1.5.2 On-site confirmation of flow diagrams

8.5.1.5.3 Description of processes and process environment - existing PRPs, process parameters, control measures and follow requirements

8.5.2 Hazard analysis - food safety team shall conduct a hazard analysis, based on the preliminary information, This shall ensure food safety and, where appropriate, a combination of control measures shall be used.

8.5.2.2 Hazard identification and determination of acceptable levels

a) the preliminary information and data collected in accordance with 8.5.1;

b) experience;

c) internal and external information including, to the extent possible, epidemiological, scientific and other historical data;

d) information from the food chain on food safety hazards related to the safety of the end products, intermediate products and the food at the time of consumption;

e) statutory, regulatory and customer requirements.

8.5.2.2.2 The organization shall identify step(s) (eg. receiving raw materials, processing, distribution and delivery) at which each food safety hazard can be present, be introduced, increase or persist.

8.5.2.2.3 The organization shall determine the acceptable level in the end product of each food safety Hazard

8.5.2.3 Hazard assessment – likelihood of occurrence prior to control and severity of adverse health effects

8.5.2.4 Selection and categorization of control measure(s)

8.5.2.4.1The organization shall categorize the selected identified control measure(s) to be managed as OPRP(s) or at CCPs

8.5.2.4.2 for each control measure establish measurable critical limits and/or measurable/observable action criteria

8.5.3 Validation of control measure(s) and combinations of control measures - validation shall be done prior to implementation of control measure

8.5.4 Hazard control plan (HACCP/OPRP plan) establish, implement and maintain a hazard control plan

8.5.4.1 General

a) food safety hazard(s) to be controlled at the CCP or by the OPRP;

b) critical limit(s) at CCP or action criteria for OPRP;

c) monitoring procedure(s);

d) correction(s) to be made if critical limits or action criteria are not met;

e) responsibilities and authorities;

f) records of monitoring.

8.5.4.2 Determination of critical limits and action criteria - Critical limits at CCPs shall be measurable. Action criteria for OPRPs shall be measurable or observable

8.5.4.3 Monitoring systems at CCPs and for OPRP – monitoring of failure of control measure relative to critical limit and action criteria

a) measurements or observations that provide results within an adequate time frame;

b) monitoring methods or devices used;

c) applicable calibration methods or, for OPRPs, equivalent methods for verification of reliable measurements or observations

d) monitoring frequency;

e) monitoring results;

f) responsibility and authority related to monitoring

g) responsibility and authority related to evaluation of monitoring results.

At each CCP, the monitoring method and frequency shall be capable of timely detection of any failure to remain within critical limits, to allow timely isolation and evaluation of the product. For each OPRP, the monitoring method and frequency shall be proportionate to the likelihood of failure and the severity of consequences.

8.5.4.4 Actions when critical limits or action criteria are not met - specify corrective actions and ensure

a) the potentially unsafe products are not released

b) the cause of nonconformity is identified;

c) the parameter(s) controlled at the CCP or by the OPRP is (are) returned within the critical limits or action criteria;

d) recurrence is prevented.

8.5.4.5 Implementation of the hazard control plan

8.6 Updating the information specifying the PRPs and the hazard control plan update the following information, if necessary:

a) characteristics of raw materials, ingredients and product-contact materials;

b) characteristics of end products;

c) intended use;

d) flow diagrams and descriptions of processes and process environment

8.7 Control of monitoring and measuring

The monitoring and measuring equipment used shall be:

a) calibrated or verified at specified intervals prior to use;

b) adjusted or re-adjusted as necessary;

c) identified to enable the calibration status to be determined;

d) safeguarded from adjustments that would invalidate the measurement results;

e) protected from damage and deterioration.

8.8 Verification related to PRPs and the hazard control plan

8.8.1 Verification

The verification activities shall confirm that:

a) the PRP(s) are implemented and effective;

b) the hazard control plan is implemented and effective;

c) hazard levels are within identified acceptable levels;

d) input to the hazard analysis is updated;

e) other actions determined by the organization are implemented and effective.

8.8.2 Analysis of results of verification activities

8.9 Control of product and process nonconformities

8.9.1 General - The organization shall ensure that data derived from the monitoring of OPRPs and at CCPs are evaluated by designated persons who are competent and have the authority to initiate corrections and corrective actions.

8.9.2 Corrections

8.9.2.1 The organization shall ensure that when critical limits at CCP(s) and/or action criteria for OPRPs are not met, the products affected are identified and controlled with regard to their use and release.

8.9.2.2 When critical limits at CCPs are not met, affected products shall be identified and handled as potentially unsafe products

8.9.2.3 Where action criteria for an OPRP are not met, the following shall be carried out:

a) determination of the consequences of that failure with respect to food safety;

b) determination of the cause(s) of failure;

c) identification of the affected products and handling in accordance with 8.9.4

8.9.2.4 Documented information shall be retained to describe corrections made on nonconforming products and processes, including:

a) the nature of the nonconformity;

b) the cause(s) of the failure;

c) the consequences as a result of the nonconformity

8.9.3 Corrective actions – when critical limit or action criteria are not met

a) reviewing nonconformities identified by customer or regulatory

b) reviewing trends in monitoring results that can indicate loss of control;

c) determining the cause(s) of nonconformities;

d) determining and implementing actions to ensure that nonconformities do not recur;

e) documenting the results of corrective actions taken;

f) verifying corrective actions taken to ensure that they are effective. The organization shall retain documented information on all corrective actions.

8.9.4 Handling of potentially unsafe products

8.9.4.1 General - The organization shall take action(s) to prevent potentially unsafe products from entering the food chain

8.9.4.2 Evaluation for release - Each lot of products affected by the nonconformity shall be evaluated. Products affected by failure to remain within critical limits at CCPs shall not be released, but shall be disposed

8.9.4.3 Disposition of nonconforming products

a) reprocessed or further processed within or outside the organization to ensure that the food safety hazard is reduced to acceptable levels; or

b) redirected for other use as long as food safety in the food chain is not affected; or

c) destroyed and/or disposed as waste.

8.9.5 Withdrawal/recall - The organization shall be able to ensure the timely withdrawal/recall of lots of end products that have been identified as potentially unsafe, by appointing competent person(s)

**Clause 9: Performance evaluation**

This is all about measuring and evaluating your food safety management system to ensure that it's effective and helps you to continually improve.

9.1 Monitoring, measurement, analysis and evaluation - analyse and evaluate appropriate data and information arising from monitoring, measurement, verification activities

9.2 Internal audit - conduct internal audits at planned intervals, plan, establish, implement and maintain (an) audit programme(s), including the frequency, methods, responsibilities, planning requirements and reporting,

9.3 Management review - Top management shall review the organization's FSMS, at planned intervals, to ensure its continuing suitability, adequacy and effectiveness.

a) the status of actions from previous management reviews;

b) changes in external and internal issues that are relevant to the FSMS, including changes in the organization and its context

c) information on the performance and the effectiveness of the FSMS includes

1)Results of system updating

2) monitoring and measurement results;

3) analysis of the results of verification activities

4) nonconformities and corrective actions;

5) audit results (internal and external);

6) inspections (e.g. regulatory, customer);

7) the performance of external providers;

8) the review of risks and opportunities and of the effectiveness of actions taken to address

9) the extent to which objectives of the FSMS have been met;

d) the adequacy of resources;

e) any emergency situation, incident or withdrawal/recall

f) relevant information obtained through external and internal communication,

g) opportunities for continual improvement.

**Clause 10: Improvement**

This clause requires organizations to determine and identify opportunities for continual improvement of the management system.

10.1 Nonconformity and corrective action - When a nonconformity occurs, the organization shall react to the nonconformity and, as applicable:

1) take action to control and correct it;

2) deal with the consequences

10.2 Continual improvement - The organization shall continually improve the suitability, adequacy and effectiveness of the FSMS.

10.3 Update of the food safety management system - Top management shall ensure that the FSMS is continually updated – based on external and internal inputs, verification, management review etc.

**Reference**


- ISO, 2018. ISO 22000:2018 Food safety management systems — Requirements for any organization in the food chain. Second edition, International Organization for Standardization, Geneva, Switzerland.


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# NATIONAL AND INTERNATIONAL REGULATIONS FOR SEAFOOD SAFETY

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Food Safety has been the buzz word in recent days as there are increasing consumer awareness on hazards present in food as well as the ombudsmen role played by independent media. Although regulatory regime across the world has taken proactive steps, in most of the cases it has been a knee-jerk reaction to the impending crisis. Defining the actual goal of food safety has been an arduous task as there are umpteen interrelated factors that influence the intended goals. Some of the definitions on food safety put forward by international agencies are as follows:

- Concept that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use (ISO 22000:2005)
- A suitable product which when consumed orally either by a human or an animal does not cause health risk to consumer (USDA-FSIS)
- Range of food related activities from prevention and surveillance to detection and control (ASTHO)

Food Safety also encompasses many aspects of handling, preparation and storage that introduces or controls chemical, microphysical and microbiological hazards. Quality of raw material, presence of pathogens, processing methods, climate change and cross-contamination also significantly impacts any food safety measure.

Seafood is always in news as it is proclaimed to be most nutritious and healthy food as well as being linked to increasing number of foodborne outbreaks across the globe. In the nutritional front, fish accounts for 17 percent of the global population intake of animal protein and 6.7% of all protein consumed (FAO, 2016). The world per capita consumption of fish and fishery products has increased from 9.9 Kg in 1960s to 20 Kg in 2014.

Seafood trade apart from being highly volatile accounts for 10 percent of total agricultural exports and 1 percent of world merchandise trade in value terms. In 2010, the quantum of seafood trade has crossed US$109 billion. Ninety percent of global trade in fish and fishery products consists of processed products, where 39% of the total quantity is traded as frozen.

This trend indicates high mobility of the fishery products across the globe, which demands stringent traceability system in place to track the movement of the commodity from harvest to consumers. Nearly 75% of the volume of seafood in international trade is imported by developed nations and 50% of that is exported by developing nations. Hence, food safety issues concerned with seafood is no more local or restricted to a particular geographical location, but has acquired global dimension. Some of the major food safety concerns linked to seafood are:

- presence of Ciguatera toxin in reef dwelling finfish
- histamine fish poisoning
- norovirus and *Vibrio parahaemolyticus* in raw shellfish
- *Salmonella* in shrimp products
- *Clostridium botulinum* in processed products
- high level of environmental pollutants
- mercury, cadmium, lead
- polychlorinated biphenyls and pesticides
- antimicrobial residues in aquaculture products

Apart from the above mentioned concerns which are mostly global, there are regional issues like use of adulterants like formaldehyde to retard decomposition process, ammonia to mask spoilage, use of un-approved additives (preservatives), high level of pesticides in dry fish and presence of emerging pathogens in fisheries environs.

The most challenging task for the policy makers has been to link incidences of foodborne illnesses with a particular food commodity. It needs a strong surveillance and monitoring mechanism to unequivocally attribute a particular food commodity. In USA, Centre for Disease Control (CDC) does the massive work of source tracking for major foodborne pathogens through pulse net programmes. The recent report by CDC (Scallan et al., 2011) indicates that 31 major pathogens reported in the United States caused 9.4 million episodes of foodborne illness, 55,961 hospitalizations and 1,351 deaths during 2. Most (58%) illnesses were caused by norovirus, followed bynon-typhoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (9%). Leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *Toxoplasma gondii* (8%). Leading causes of death were non-typhoidal *Salmonella* spp. (28%), *T. gondii* (24%), *Listeria monocytogenes* (19%), and norovirus (11%). In India, the recently established National Centre for Disease Control (formerly, National Institute of Communicable Diseases), Ministry

of Health and Family Welfare, Government of India has a similar mandate to undertake activities on outbreak investigation and provide referral diagnostic services.

In absence of etiological data linked to seafood, the export rejection figures provides an indirect account of food safety hazards associated with seafood. Import refusals and rejections from countries like USA, Japan, Russia and EU are on the rise because of presence of biological and chemical hazards in seafood, leading to heavy economic loss by seafood industries. The most common import refusal of seafood by USA is due to presence of *Salmonella*, *Listeria*, filth or illegal veterinary drugs. The RASFF portal of EU indicates alert notifications due to presence of veterinary drug residues, heavy metals, histamine, foreign bodies, biotoxin, defective packaging, incorrect labelling, improper health certificate, unapproved colour and additives and organoleptic aspects. In recent months most of the rejections from Japan had been due to presence of furazolidone (AOZ) and Ethoxyquin in shrimp. Seafood rejections from Russia are mostly due to presence of high load of mesophilic bacteria, coliforms, pathogens and presence of crystal violet.

**Genesis of Food Safety Standards and Regulations**

Food safety standards can be classified as regulatory, voluntary, Government/Statutory, private, domestic, international or benchmarked depending upon its scope and range of application. Most of these standards have evolved based upon sanitary and phyto-sanitary (SPS) requirements, economic interest, risk analysis or as precautionary approach. The precautionary approach mostly relies on perception i.e. equivalent level of protection, appropriate level of protection (ALOP) or as low as reasonably achievable (ALARA).

In international trade, sanitary and phytosanitary measures are envisioned to be based on sound scientific principles that ensure food safety and do not anyway compromise the production potential and resources of a particular country. These measures should not be linked to prevent market access based on non-scientific reasons, and are requirements but not sufficient condition of trade. As per the Annex A of WTO Agreement, Sanitary and phytosanitary measures are applied to (i) protect animal or plant life or health within the territory of the Member from risks arising from the entry, establishment or spread of pests, diseases, disease-carrying organisms or disease-causing organisms (ii) to protect human or animal life or health within the territory of the Member from risks arising from additives, contaminants, toxins or disease-causing organisms in foods, beverages or feedstuffs (iii) from

risks arising from diseases carried by animals, plants or products thereof, or from the entry, establishment or spread of pests and (iv) to prevent or limit other damage within the territory of the Member from the entry, establishment or spread of pests. WTO encourages members to use accepted International standards by Codex Alimentarius Commission, OIE (World Organization for Animal Health) and IPPC (International Plant Protection Convention). Countries may introduce or maintain SPS measures that provide higher level of protection than the current international or Codex standards.

**Salient features of some Export regulations related to Seafood European Union**

European Union is the biggest importer of fish and fishery products in the world. The food safety regulations set by EU is harmonised, gets periodically updated, transparent and based on principles of risk assessment. The key elements of EU requirements for import of seafood are (a) certification by a competent authority (b) compliance to hygiene and public health requirements in terms of structure of vessels, landing sites, processing establishments and on operational processes, freezing and storage (c) certified production area for bivalves (d) national control plan on heavy metals, contaminants, residues of pesticides and veterinary drugs (e) approval of establishments.

The legal acts of EU are managed through regulations, directives, decision, recommendations and opinions.

Regulation: A binding legislative act applied in entirety across EU

Directives: A "directive" is a legislative act that sets out a goal that all EU countries must achieve.

Decision: A "decision" is binding on those to whom it is addressed (e.g. an EU country or an individual company) and is directly applicable.

Recommendations: A "recommendation" is not binding act that allows the institutions to make their views known and to suggest a line of action without imposing any legal obligation on those to whom it is addressed.

Opinions: An "opinion" is an instrument that allows the institutions to make a statement in a non-binding fashion, in other words without imposing any legal obligation on those to whom it is addressed.

Some of the important EU legislations related to food safety issues of fish and fishery products are as follows:

Regulation (EC) No 178/2002: General principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

Regulation (EC) No 852/2004: Hygiene of foodstuffs.

Regulation (EC) No 853/2004: Specific hygiene rules for food of animal origin

Regulation (EC) No 854/2004: Specific rules for the organisation of official controls on products of animal origin intended for human consumption

Regulation (EC) No 2073/2005: Microbiological criteria for foodstuffs

Regulation (EC) No 882/2004: Official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules

Regulation (EC) No 1881/2006: Maximum levels for certain contaminants in foodstuffs

Regulation (EC) No 333/2007: Methods of sampling and analysis for the official controls for the levels of lead, cadmium, mercury, inorganic tin, 3- MCPD and benzo(a)pyrene in foodstuffs

Regulation (EC) No 1883/2006: Methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs

Regulation (EC) No 396/2005: Maximum residue levels of pesticides in or on food and feed of plant and animal origin

Council Directive 96/23/EC: Measures to monitor certain substances and residues thereof in live animals and animal products

Commission Decision (2005/34/EC): Harmonised standards for the testing for certain residues in products of animal origin imported from third countries

Commission Decision (2002/657/EC): Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results

Commission Decision (98/179/EC): Official sampling for the monitoring of certain substances and residues thereof in live animals and animal products

Commission Decision (2004/432/EC): Approval of residue monitoring plans submitted by third countries in accordance with Council Directive 96/23/EC

Council Directive 96/22/EC: Prohibition on the use in stock farming of certain substances having a hormonal or thyrostatic action and of betaagonists

Regulation (EC) No 470/2009: Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin

Commission Regulation (EU) No 37/2010: Pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

Commission Regulation (EC) No 2023/2006: Good manufacturing practice for materials and articles intended to come into contact with food

Commission Regulation (EC) No 1935/2004: Materials and articles intended to come into contact with food

Commission Regulation (EU) No 1129/2011: Amendment to Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the

Council by establishing a Union list of food additives Commission Regulation (EC) No 1333/2008 : Food Additives

Commission Regulation (EC) No 1334/2008: Flavourings and certain food ingredients with flavouring properties for use in and on foods

Commission Regulation (EC) No 1331/2008: Establishing a common authorisation procedure for food additives, food enzymes and food flavourings

Directive 2000/13/EC: Labelling, presentation and advertising of foodstuffs (until 12 December 2014)

Commission Regulation (EU) No 1169/2011: Provision of food information to consumers, amending Regulations

Commission Regulation (EU) No 1379/2013: Common organisation of the markets in fishery and aquaculture products

**USA**

In USA both Federal and State Regulatory agencies are involved in ensuring safety and quality of seafood. Multiple federal agencies are involved in regulatory oversight of seafood for both importation and export.

United States Department of Agriculture (USDA) oversees the implementation of country-of-origin labelling (COOL) regulation enacted under the Farm Security and Rural Investment Act of 2002. This law requires that all retailers, such as full-line grocery stores or supermarkets must notify their customers with information regarding the source of certain foods. The COOL regulation for fish and shellfish (7 CFR Part 60) came into force in 2005. Apart from the country of origin, all fish and shellfish covered commodities must be labelled to indicate whether they are wild caught or farm-raised.

United States Fisheries and Wildlife Service (USFWS) is also involved in regulation of import and export of shellfish and fishery products through Convention on International Trade in Endangered Species (CITES) act (50 CFR Part 23), Endangered Species Act (50 CFR Part 17), General Permit Procedures (50 CFR Part 13), Lacey Act (injurious wildlife) (50 CFR Part 16), Marine Mammal Protection Act (50 CFR Part 18) and Wildlife (import/export/transport) act (50 CFR Part 14). Live farm-raised fish and farm-raised fish eggs are exempted from export declaration and licensing requirements. Imports or exports of any sturgeon or paddlefish product, including meat, caviar, and cosmetics made from sturgeon eggs, dead uneviscerated salmon, trout and char and live fertilized eggs from these salmonid fish require a permit. Aquatic invertebrates and other animals that are imported or exported for human or animal consumption but that do not meet the definition of shellfish such as squid, octopus, cuttlefish, land snails, sea urchins, sea cucumbers and frogs are also covered under these provisions.

National Oceanic and Atmospheric Administration (NOAA) functioning under the United States Department of Commerce (USDC) provides voluntary seafood inspection program for fish, shellfish, and fishery products to the industry as per the 1946 Agricultural Marketing Act. The NOAA Seafood Inspection Programme often referred to as the U.S. Department of Commerce (USDC) Seafood Inspection Programme provides services such as establishment sanitation inspection, system and process audits, product inspection and grading, product lot inspection, laboratory analyses, training, consultation and export certification. NOAA Fisheries is the Competent Authority for export health certification and IUU catch documentation for US seafood products meant for export to EU and non-EU countries.

The U.S. Food and Drug Administration (USFDA) is vested with the primary Federal responsibility for the safety of seafood products in the United States. It operates a mandatory safety program for all fish and fishery products under the provisions of the Federal Food, Drug and Cosmetic (FD&C) Act, the Public Health Service Act, and related regulations. The most

important regulation enacted by USFDA was "Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products" published as final rule 21 CFR 123 on 18th December 1995 and came into force on 18th December 1997. It required processors to adopt the preventive system of food safety controls known as HACCP (Hazard Analysis and Critical Control Point). Seafood was the first food commodity in the U.S. to adopt HACCP in USA. For screening imports, USFDA uses a tool "Predictive Risk-based Evaluation for Dynamic Import Compliance Targeting (PREDICT)", that targets higher risk products for examination and sampling and minimizes the delay in shipments of lower risk products.

Food Safety and Modernization Act (FSMA) is the most important milestone event in the food safety scenario in USA. It was signed in to law on 4th January 2011 which sifted the focus from responding to a contamination to prevention of the actual cause. The salient features of FSMA act are as follows:

Sec. 103.Hazard analysis and risk-based preventive controls

(HARPC): Requires human and animal food facilities to

- evaluate hazards that could affect food safety;
- Identify and implement preventive controls to prevent hazards;
- Monitor controls and maintain monitoring records; and
- Conduct verification activities

Sec. 106.Protection against intentional adulteration

Sec. 111. Sanitary Transportation of Food

Sec. 301. Foreign supplier verification program

• Requires importers to verify their suppliers use risk-based preventive controls that provide same level of protection as U.S. requirements.

Sec. 302. Voluntary qualified importer program

Allows for expedited review and entry; facility certification required

Sec. 303. Certification for high-risk food imports

• FDA has discretionary authority to require assurances of compliance for high-risk foods

Sec. 304. Prior notice of imported food shipments

• Requires information on prior refusals to be added to prior notice submission

• Effective July 3, 2011

Sec. 307. Accreditation of third-party auditors

• FDA can rely on accredited third parties to certify that foreign food facilities meet U.S. requirements

Sec. 308. Foreign Offices of the Food and Drug Administration.

• Establish offices in foreign countries to provide assistance on food safety measures for food exported to the U.S.

Sec. 309. Smuggled Food

• In coordination with DHS, better identify and prevent entry of smuggled food

• Rules on anti-smuggling strategy is already framed

**China**

In recent years China has strengthened its SPS measures and has taken a number of precautionary steps to ensure safety to its population. Some of the important regulations enacted by Peoples Republic of China are as follows:

- GB 2763—2012: National food safety standard on Maximum residue limits for pesticides in food
- GB 2762—2012: National food safety standard on Contaminants in Food
- GB-2010: National Food Safety Standard for Pathogen Limits in Food (GAIN Report No. 12063)
- GB 2733-2005: Hygienic Standard for Fresh and Frozen Marine Products of Animal Origin
- GB 2760-2011 additives
- GB 10136-1988 Hygienic standard for salt & liquor-saturated aquatic products of animal origin

**Russia**

Russia has a comprehensive regulatory framework for fish and fishery products. The hygienic requirements are different from other countries as some of the microbiological parameters are

expressed as absent in 0.001g or 0.01g. Also some different nomenclature like QMAFAnM is followed instead of APC. The Russian regulation currently in force pertaining to fish and

fishery products are as follows:

- Hygienic requirements for safety and nutrition value of food products. Sanitary and epidemiological rules and regulations, sanpin 2.3.2.1078-01

**Japan**

Compared to other countries, SPS measures followed by Japan is very stringent. Many additives which are in the approved list of Codex are banned or prohibited in Japan. Japan uses a positive list system for MRL of agricultural chemicals in foods. A uniform limit of 0.01 ppm is followed for the compounds for which no risk assessment is done but which are included in the positive list (MHLW Notification No. 497, 2005). MHLW uses a toxicological threshold of 1.5 μg/day as the basis to determine the uniform limit. Substances having no potential to cause damage to human health are specified by MHLW Notification No.498. 2005. The MRL list is mentioned as compositional specification of foods (MHW Notification, No. 370, 1959, amendment No.499 2005, updated as on March 15, 2013). The relevant food safety acts of Japan as enacted by Ministry of Health, Labour and Welfare and other agencies are as follows:

- Food Sanitation Act (Act No.233, 1947): Latest Revision on June 5, 2009, Act No. 49)
- Specifications and Standards for Food and Food Additives, Latest Revision on September 6, 2010, MHLW Notification No. 336
- Japan's Specifications and Standards for Food Additives" (Eighth Edition). Published by the Ministry of Health, Labour and Welfare in 2007
- Food Safety Basic Act (Act No. 48, 2003)
- Agricultural Chemicals Regulation Law (Law No. 82, 1948)

**Codex Alimentarius Commission**

The Codex Alimentarius Commission (CAC) was established in 1961- 1963 by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) to implement their Joint FAO/WHO Food Standards Programme. CAC has the mandate to formulate food standards, code of practice, guidelines and recommendations to protect health of consumers, ensure fair practices in food trade and to promote coordination of all food standards work undertaken by international governmental and non-governmental

organizations. Codex operates through thee standing expert scientific bodies convened under the auspices of FAO and WHO to generate food data and provide risk-assessment type advice:

- Joint Expert Committee on Food Additives (JECFA)
- Joint Meeting on Pesticide Residues (JMPR)
- Joint Meeting on Microbiological Risk Assessment (JEMRA)

Different subject committees and commodity committees, adhoc intergovernmental task forces and regional coordinating committees function and under codex. Codex Committee on Fish and Fisheries Products (CCFFP) is entrusted with the task of formulating standards for different product categories. Although Codex standards on Fish and Fishery Products specifically do not address food safety requirements, but provide a strong framework for production, hygienic requirements and sampling.

**Available Codex Standard for Fish and Fishery Products**

| | | |
|---|---|---|
| 1. | Standard for Canned Salmon | CODEX STAN 3-1981 |
| 2. | Standard for Quick Frozen Finfish, Eviscerated or Uneviscerated | CODEX STAN 36-1981 |
| 3. | Standard for Canned Shrimps or Prawns | CODEX STAN 37-1981 |
| 4. | Standard for Canned Tuna and Bonito | CODEX STAN 70-1981 |
| 5. | Standard for Canned Crab Meat | CODEX STAN 90-1981 |
| 6. | Standard for Quick Frozen Shrimps or Prawns | CODEX STAN 92-1981 |
| 7. | Standard for Sardines and Sardine-Type Products | CODEX STAN 94-1981 |
| 8. | Standard for Quick Frozen Lobsters | CODEX STAN 95-1981 |
| 9. | Standard for Canned Finfish | CODEX STAN 119-1981 |
| 10 | Standard for Quick Frozen Blocks of Fish Fillets, Minced Fish Flesh and Mixtures of Fillets and Minced Fish Flesh | CODEX STAN 165-1989 |
| 11 | Standard for Quick Frozen Fish Sticks (Fish Fingers), Fish Portions and Fish Fillets - Breaded or in Batter | CODEX STAN 166-1989 |
| 12 | Standard for Salted Fish and Dried Salted Fish of the Gadidae Family of Fishes | CODEX STAN 167-1989 |
| 13 | Standard for Dried Shark Fins | CODEX STAN 189-1993 |
| 14 | General Standard for Quick Frozen Fish Fillets | CODEX STAN 190-1995 |
| 15 | Standard for Quick Frozen Raw Squid | CODEX STAN 191-1995 |
| 16 | Standard for Crackers from Marine and Freshwater Fish, Crustaceans and Molluscan Shellfish | CODEX STAN 222-2001 |
| 17 | Standard for Boiled Dried Salted Anchovies | CODEX STAN 236-2003 |
| 18 | Standard for Salted Atlantic Herring and Salted Sprat | CODEX STAN 244-2004 |
| 19 | Standard for Sturgeon Caviar | CODEX STAN 291-2010 |
| 20 | Standard for Live and Raw Bivalve Molluscs | CODEX STAN 292-2008 |
| 21 | Standard for Fish Sauce | CODEX STAN 302-2011 |

**Code of Practice**

| | |
|---|---|
| Code of Practice for Fish and Fishery Products | CAC/RCP 52-2003 |

**Guidelines**

| | |
|---|---|
| Guidelines for the Sensory Evaluation of Fish and Shellfish in Laboratories | CAC/GL 31-1999 |
| Guidelines on the Application of General Principles of Food Hygiene to the Control of Pathogenic Vibrio Species in Seafood | CAC/GL 73-2010 |
| Guidelines on the Application of General Principles of Food Hygiene to the Control of Viruses in Food | CAC/GL 79-2012 |
| Model Certificate for Fish and Fishery Products | CAC/GL 48-2004 |
| Guideline Procedures for the Visual Inspection of Lots of Canned Foods for Unacceptable Defects | CAC/GL 17-1993 |
| Guidelines on Good Laboratory Practice in Pesticide Residue Analysis | CAC/GL 40-1993 |
| General guidelines on sampling | CAC/GL 50-2004 |
| Guidelines on the Use of Mass Spectrometry (MS) for Identification, Confirmation and Quantitative Determination of Residues | CAC/GL 56-2005 |

**Codex standard applicable to Fish and Fishery Products**

| | |
|---|---|
| General Standard for Contaminants and Toxins in Food and Feed | CODEX STAN 193-1995 |
| General Standard for the Labelling of Prepackaged Foods | CODEX STAN 1-1985 |
| Standard for Food Grade Salt | CODEX STAN 150-1985 |
| General Standard for Food Additives | CODEX STAN 192-1995 |
| General Methods of Analysis for Contaminants | CODEX STAN 228-2001 |
| Recommended Methods of Analysis and Sampling | CODEX STAN 234-1999 |
| General Methods of Analysis for Food Additives | CODEX STAN 239-2003 |

## Bureau of Indian Standards (BIS)

Bureau of Indian Standards (BIS) functioning under the Ministry of Consumer Affairs, Food and Public Distribution, Government of India. It came into existence on 01 April 1987 through an Act of Parliament on 26 November 1986. It was functioning previously as Indian Standards Institution which was established on 06 January 1947. BIS has so far formulated 64 standards related to fish and fishery products, out of which 33 are active. All these standards are voluntary, which addresses method of production, quality and safety requirements. It also stipulates the method of testing and sampling. There is an attempt by FSSAI to re-draft all BIS standards related to fish and fishery products as most of the food safety requirements are not in sync with the current national standards.

**BIS Standards on Fish and Fishery Products**

| | | |
|---|---|---|
| IS 2168 | 1971 | Pomfret Canned in Oil |
| IS 2236 | 1968 | Prawns/Shrimp Caned in Brine |
| IS 2237 | 1997 | Prawns (Shrimps) - Frozen |
| IS 3336 | 1965 | Shark Liver Oil for Veterinary Use |
| IS 3892 | 1975 | Frozen Lobster Tails |
| IS 4304 | 1976 | Tuna Canned in Oil |
| IS 4780 | 1978 | Pomfret, Fresh |
| IS 4793 | 1997 | Whole Pomfret - Frozen |
| IS 5734 | 1970 | Sardine Oil |
| IS 6121 | 1985 | *Lactarius* sp Canned in Oil |
| IS 6122 | 1997 | Seer Fish (*Scomberomorus* Sp.) - Frozen |
| IS 6123 | 1971 | Seer Fish (*Scomberomorus* spp.), Fresh |
| IS 7143 | 1973 | Crab Meat Canned in Brine |
| IS 7313 | 1974 | Glossary of Important Fish Species of India |
| IS 7582 | 1975 | Crab Meat, Solid Packed |
| IS 8076 | 2000 | Frozen Cuttlefish and Squid |
| IS 9808 | 1981 | Fish Protein Concentrate |
| IS 10059 | 1981 | Edible Fish Powder |
| IS 10760 | 1983 | Mussels Canned in Oil |
| IS 10762 | 1983 | Tuna Canned in Curry |
| IS 10763 | 1983 | Frozen Minced Fish Meat |
| IS 11427 | 2001 | Fish and Fisheries Products - Sampling |
| IS 14513 | 1998 | Beche-de-mer |
| IS 14514 | 1998 | Clam Meat - Frozen |
| IS 14515 | 1998 | Fish Pickles |
| IS 14516 | 1998 | Cured fish and fisheries products - Processing and storage - Code of Practice |
| IS 14517 | 1998 | Fish Processing Industry - Water and Ice - Technical Requirements |
| IS 14520 | 1998 | Fish Industry - Operational Cleanliness and layout of market - Guidelines (Amalgamated Revision of IS 5735, 7581 and 8082) |
| IS 14890 | 2001 | Sardines - Fresh, Frozen and Canned (Amalgamated revision of IS 2421, 6677,8652,8653, 9750 and 10761 |
| 4891 | 2001 | Mackerel - Fresh, Frozen and Canned (Amalgamated Revision of IS 2420, 3849,6032, 6033 and 9312) |
| IS 14892 | 2000 | Threadfin - Fresh and Frozen |
| IS 14949 | 2001 | Accelerated Freeze Dried Prawns (Shrimps) (Amalgamated revision of IS 4781 and 4796 |
| IS 14950 | 2001 | Fish - Dried and Dry-Salted |

## Food Safety and Standards Authority of India (FSSAI)

The Food Safety and Standards Authority of India was established under the Food Safety and Standards Act, 2006 as a statutory body for laying down science based standards for articles of food and regulating manufacturing, processing, distribution, sale and import of

food so as to ensure safe and wholesome food for human consumption. Various central acts including the erstwhile Prevention of Food Adulteration Act (1954) were merged under this act The Food Safety and Standards Regulations (FSSR) came into force in 2011, which is divided to following sections:

FSS (Licensing and Registration of Food businesses) regulation, 2011

- FSS (Packaging and Labelling) regulation, 2011
- FSS (Food product standards and Food Additives) regulation, 2011 (part I)
  FSS (Food product standards and food additives) regulation, 2011
  (part II)
- FSS (Prohibition and Restriction on sales) regulation, 2011
- FSS (contaminants, toxins and residues) regulation, 2011
- FSS (Laboratory and sampling analysis) regulation, 2011

Recently, standards related to microbiological specifications of fish and fishery products, limit of heavy metals, PAH, PCBs and biotoxins have been incorporated in the FSSR.

**HACCP concept in seafood quality assurance**

Concept of HACCP was developed in the late 1950s and initiated in the early 1960s by the Pillsbury Company, in collaboration with NASA and the Natick Laboratories of the U.S. Army, and the U.S. Air Force Space Laboratory Project Group. The concepts designed were based on the principles of Failure Mode and Effect analysis (FEMA). It was first presented to regulatory community during National Conference on Food Protection in 1971 by Howard Bauman of the Pillsbury Company and first applied to low acid canned foods in 1974. In 1980s, other food processing companies embraced it voluntarily and at the same time FDA and USDA continued regulatory interest. HACCP gained regulatory approval from USFDA and USDA after it was endorsed by National Academy of Sciences and further by 9National Advisory Committee on Microbiological Specifications of Foods (NACMSF). On December 18, 1995, The Food and Drug Administration (FDA) published as a final rule 21 CFR 123, "Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products" that requires processors of fish and fishery products to develop and implement Hazard Analysis Critical Control Point (HACCP) systems for their operations. The regulation became effective December 18, 1997. HACCP was recommended by Codex Alimentarius Commission (CAC) in 1997 which is recognized as

"Recommended International Code of Practice-General Principles of Food Hygiene" (CAC/RCP 1-1969, Rev 3, 1997). In European countries, the EU Directive 93/43/EEC mandated the implementation of HACCP in all local legislation by December 1995. Subsequently the EC hygiene regulations 852/2004 and 853/2004 mandated that all food business operators should establish and operate food safety programmes and procedure based on HACCP principles. Since then HACCP has gained acceptance by many countries in Europe, Canada, New Zealand, Australia, Central and South America and many Asian countries. In India voluntary HACCP standards are given by Bureau of Indian Standards (IS 15000:1998).

**Hazard Analysis Critical Control Point (HACCP)**

The HACCP system is an internationally recognized system used to manage food safety. It has been endorsed by the Codex Alimentarius Commission as a tool that can be used to systematically identify hazards specific to individual products and processes and describe measures for their control to ensure the safety of fish and fish products. It is a dynamic system, capable of accommodating change in the system viz., changes in equipment design, processing procedures and technological advancements.

**HACCP is defined as a system which identifies, evaluates, and controls hazards which are significant for food safety**

HACCP is a structured, systematic approach for the control of food safety throughout the food system, from the farm to fork. It requires a good understanding of the relationship between cause and effect in order to be more pro-active. HACCP is supported by pre-requisite programmes like Good Manufacturing Practice (GMP), Good Hygienic Practices (GHP), SSOP (Sanitation standard operating procedures), Good Agricultural Practices (GAP), and Good Storage Practices (GSP), etc.

**Pre-requisite programmes**

Prerequisite programs provide a foundation for an effective HACCP system. They are often facility-wide programs rather than process or product specific. They reduce the likelihood of certain hazards. Prerequisite programs set the stage for a HACCP system and provide on-going support for the establishment's food safety system. They keep potential hazards from becoming serious enough to adversely impact the safety of foods produced.

Without clean working conditions free from microbiological, chemical, and physical contamination from many sources, a HACCP plan cannot be effective.

**Prerequisite programmes are practices and conditions needed prior to and during the implementation of HACCP and which are essential for food safety -WHO**

Some of the prerequisite programmes include GAP, GMP and GHP which must be working effectively within a commodity system before HACCP is applied. Establishments should revise their prerequisite programs, as necessary, to ensure their effectiveness, and should take appropriate corrective actions when they determine that their prerequisite programs may have failed to prevent contamination and/or adulteration of product. Good Agricultural Practices are "practices that address environmental, economic and social sustainability for on-farm processes, and result in safe and quality food and non-food agricultural products" (FAO).

The Good Manufacturing Practices commonly referred as current good manufacturing practices (cGMPs, 21 CFR 110) give details as to what specific procedures must be followed to comply with the regulation. Standard operating procedures (SOPs) are the steps your company takes to assure that the GMPs are met. They include stepwise procedures, employee training, monitoring methods, and records used by your company. Similarly, SSOP covers eight key sanitation conditions as required by USFDA. Good hygiene practices include all practices regarding the conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain.

**Basic principles of HACCP**

There are seven discrete activities that are necessary to establish, implement and maintain a HACCP plan, and these are referred to as the 'seven principles' in the Codex Guideline (1997). The seven Principles of HACCP are

**Principle 1:** Conduct a hazard analysis.

Hazard: A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

Hazard analysis: The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.

**Principle 2**: Determine the Critical Control Points (CCPs)

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.

**Principle 3**: Establish critical limits.

A criterion which separates acceptability from unacceptability, when monitoring a critical control point.

**Principle 4**: Establish a monitoring system

The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.

**Principle 5**: Establish a procedure for corrective action,

Any action to be taken when the results of monitoring at the CCP indicate a loss of control.

**Principle 6**: Establish procedures for verification

The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan.

**Principle 7**: Establish documentation concerning all procedures and records appropriate to these principles and their application

**Developing a HACCP plan (FAO guidelines)**

The all-important principles form the essential requirements of a food safety system and are designed to ensure that enough precaution is taken so that any hazard which can interfere with consumer health is addressed. The first principle of HACCP is hazard analysis. But understanding the product thoroughly is extremely important to get an idea on the possible hazards which could be associated with the product so that appropriate action can be taken to control or minimize the hazard. The seven principles of HACCP are usually carried out in twelve steps, as given below.

**Step 1 - Establish a HACCP team**

Hazard profile is related to the commodity. Therefore in order to understand fully the commodity, to identify the hazards associated, the CCP and to work out a control measures it is pertinent to have a team which has the knowledge about the product or commodity,

its production process and shelf-life. This would facilitate the proper implementation of HACCP for the production of the product. Therefore, it is important that the HACCP team is made up of people from a wide range of disciplines. The team should include:

- A team leader to lead the group and direct the team to carry out the work as per the system requirements. He should be well versed with the techniques and manage the team members to contribute to the cause.
- A person conversant with the production system who knows full details of the flow of production.
- Persons from varied field viz., biochemist, microbiologist, toxicologist, quality control manager or an engineer with an understanding of particular hazards and associated risks.
- Others who are involved in the varied activates of the system viz., packaging specialists, raw material buyers, distribution staff or production staff, farmers, brokers, who are involved with the process, and have working knowledge of it in order to provide expert opinion.
- Possibly one person to help the team with secretarial requirements.

**Task 2 - Describe the product**

Understanding the product is the important step as the hazard associated with depends on the product. To start a hazard analysis, a full description of the product, including customer specification, should be prepared. This should include information relevant to safety regulation/target level, and composition, physical/chemical properties of the raw materials and the final product, the water activity of the product (aw), the pH etc. There should information on the packaging, storage and distribution as well as information on the temperature of storage, distribution, labelling information and shelf-life of the product. This information helps the audit team to understand the possible hazards and their control measures.

**Task 3 - Identify the product's intended use**

Information on the intended use of the commodity or product as well as the information on the mode of consumption viz., direct consumption, cooked before hazard analysis will have bearing on the hazard analysis. The nature of the target group for the product may also be relevant, particularly if it includes susceptible groups such as infants, the elderly, and the

malnourished. The likelihood of misuse of a product should also be considered, such as the use of pet food as a human food, either by accident or design.

**Task 4 - Draw up the commodity flow diagram**

The first function of the team is inspecting the detailed commodity flow diagram (CFD) of the commodity system and the expertise of the production manager or product expert is important at this stage as far as hazard analysis is concerned.

**Task 5 - On site confirmation of flow diagram**

After studying the commodity flow diagram the team should visit the system where HACCP is implemented or proposed to be implemented which may include any step in the production viz., procurement of raw material, store, production area, packaging area, storage section where the product is kept before distribution, nature of distribution, conditions of distribution etc. The is known as 'walking the line ', a step by step checking to get information on whether relevant requirements of the system are considered while making the production line. The site for which the HACCP plan is being designed should be visited as many times as possible to ensure that all relevant information has been collected.

**Task 6 - Identify and analyse hazard(s) - (Principle 1)**

Effective hazard identification and hazard analysis are the keys to a successful HACCP Plan. All real or potential hazards that may occur in each ingredient and at each stage of the commodity system should be considered. Food safety hazards for HACCP programmes have been classified into three types of hazards:

- Biological: typically foodborne bacterial pathogens such as Salmonella, Listeria and E. coli, also viruses, algae, parasites and fungi.
- Chemical: There are three principle types of chemical toxins found in foods: naturally occurring chemicals, e.g. cyanides in some root crops, and allergenic compounds in peanuts; toxins produced by microorganisms, e.g. mycotoxins, and algal toxins; and chemicals added to the commodity by man to control an identified problem, e.g fungicidesor insecticides.
- Physical: contaminants such as broken glass, metal fragments, insects or stones.

The probability that a hazard will occur is called a risk. The risk may take a value from zero to one depending on the degree of certainty that the hazard will be absent or that it will be present.

After hazard identification, a hazard analysis must be conducted to understand the relative health risk to man or animal posed by the hazard. It is a way of organizing and analysing the available scientific information on the nature and size of the health risk associated with the hazard. The risk may have to be assessed subjectively and simply classified as low, medium, or high. Once a food safety hazard has been identified, then appropriate control measures should be considered. These are any action or activity that can be used to control the identified hazard, such that it is prevented, eliminated, or reduced to an acceptable level. The control measure may also include training of personnel for a particular operation, covered by GAP, GMP, and GHP.

**Task 7 - Determine the critical control points (CCPs) - (Principle 2).**

Each step in the commodity flow diagram, within the scope of the HACCP study, should be taken in turn and the relevance of each identified hazard should be considered. The team must determine whether the hazard can occur at this step, and if so whether control measures exist. If the hazard can be controlled adequately, and is not best controlled at another step, and is essential for food safety, then this step is a CCP for the specified hazard. If a step is identified where a food safety hazard exists, but no adequate control measures can be put in place either at this step or subsequently, then the product is unsafe for human consumption. Production should cease until control measures are available and a CCP can be introduced.

**Task 8 - Establish critical limits for each CCP - (Principle 3)**

Critical limits must be specified and validated for each CCP. Criteria often used include measurements of temperature, time, moisture level, pH, water activity, and sensory parameters such as visual appearance. All critical limits, and the associated permissible tolerances, must be documented in the HACCP Plan Worksheet, and included as specifications in operating procedures and work instructions.

**Task 9 - Establish a monitoring procedure - (Principle 4)**

Monitoring is the mechanism for confirming that critical limits at each CCP are being met. The method chosen for monitoring must be sensitive and produce a rapid result so that trained operatives are able to detect any loss of control of the step. This is imperative so that corrective action can be taken as quickly as possible so that loss of product will be avoided or minimized. Monitoring can be carried out by observation or by measurement, on samples taken in

accordance with a statistically based sampling plan. Monitoring by visual observation is basic but gives rapid results, and can therefore be acted upon quickly. The most common measurements taken are time, temperature and moisture content.

**Task 10 - Establish corrective action - (Principle 5)**

If monitoring indicates that critical limits are not being met, thus demonstrating that the process is out of control, corrective action must be taken immediately. The corrective action should take into account the worst case scenario, but must also be based on the assessment of hazards, risk and severity, and on the final use of the product. Operatives responsible for monitoring CCPs should be familiar with and have received comprehensive training in how to effect a corrective action. Corrective actions must ensure that the CCP has been brought back under control. Corrective action can then be applied to pre-empt a deviation and prevent the need for any product disposition.

**Task 11 - Verify the HACCP plan - (Principle 6)**

Once the HACCP plan has been drawn up, and all of the CCPs have been validated, then the complete plan must be verified. Once the HACCP plan is in routine operation, it must be verified and reviewed at regular intervals. This should be a task of the person charged with the responsibility for that particular component of the commodity system. The appropriateness of CCPs and control measures can thus be determined, and the extent and effectiveness of monitoring can be verified. Microbiological and/or alternative chemical tests can be used to confirm that the plan is in control and the product is meeting customer specifications. A formal internal auditing plan of the system will also demonstrate an ongoing commitment to keep the HACCP plan up to date, as well as representing an essential verification activity.

**Task 12 - Keep record - (Principle 7)**

Record keeping is an essential part of the HACCP process. It demonstrates that the correct procedures have been followed from the start to the end of the process, offering product traceability. It provides a record of compliance with the critical limits set, and can be used to identify problem areas. Records that should be kept include: all processes and procedures linked to CCP monitoring, deviations, and corrective actions.

**Steps involved in developing HACCP system**

(Based on Codex 1997)

| | | |
|---|---|---|
| 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 | 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 |

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 Global Standard for Food, SQF 2000, IFS, etc. Hence understanding concepts of HACCP would help in easy implementation of any food safety standard(s) deemed necessary to ensure safety of fish and fishery products.

**Definitions in HACCP**

**Control (verb):** To take all necessary actions to ensure and maintain compliance with criteria established in the HACCP plan.

**Control (noun):** The state wherein correct procedures are being followed and criteria are being met.

**Control measure:** Any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

**Corrective action**: Any action to be taken when the results of monitoring at the CCP indicate a loss of control.

**Critical Control Point (CCP):** 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.

**Critical limit:** A criterion which separates acceptability from unacceptability, when monitoring a critical control point.

**Deviation:** Failure to meet a critical limit.

**Flow diagram**: A systematic representation of the sequence of steps or operations used in the production or manufacture of a particular food item.

**HACCP plan**: A document prepared in accordance with the principles of HACCP to ensure control of hazards which are significant for food safety in the segment of the food chain under consideration.

**Hazard**: A biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect.

**Hazard analysis**: The process of collecting and evaluating information on hazards and conditions leading to their presence to decide which are significant for food safety and therefore should be addressed in the HACCP plan.

**Monitor**: The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a CCP is under control.

**Step**: A point, procedure, operation or stage in the food chain including raw materials, from primary production to final consumption.

**Validation**: Obtaining evidence that the elements of the HACCP plan are effective.

**Verification**: The application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan.

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# SAMPLING OF FISH & FISHERY PRODUCTS FOR INTERNATIONAL COMPLIANCE

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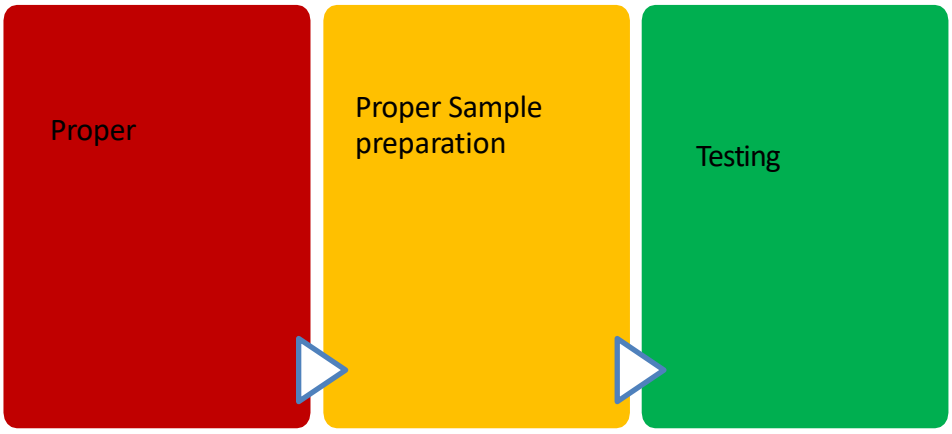
## Introduction

Samples for the testing has prime importance. Their adequacy and condition received by a laboratory determines the authenticity of results produced. If samples are improperly collected or representative of the sampled lot are not appropriate, the laboratory results can be questionable. Because interpretations about a large consignment of food are based on a relatively small sample of the lot, established sampling procedures must be applied uniformly. A representative sample is much needed as we know pathogens or any harmful substance are sparsely distributed within the food. Shipments of food consignments depends on the results presented in line with the legal standard for that particular commodity. The number making a representative sample from a designated lot of a food product must be statistically significant. The composition and nature of each lot affects the homogeneity and uniformity of the total sample mass. The proper statistical sampling procedure, according to whether the food is solid, semisolid, viscous, or liquid, must be determined by the sampler at the time of sampling. Because of the large volume of seafood consumed, any inspection system would have to involve sampling. Even if each boatload of fish were inspected, it would still not be reasonable to inspect each fish. Furthermore, much of the inspection is of a destructive nature. Therefore, it is important to investigate the statistical properties of the sampling procedures being used, as well as of any proposed procedures.

## Significance of sampling

Proper sampling/sample preparation is the foundation of quality testing.

Reliability on test result depends on following factors




Improper/wrong sampling procedures nullify the whole analytical chain of analysis and invalidate the results. No analytical method to correct the error made during sampling step. Measurement Uncertainty (MU) associated with sampling is not calculated. Only few grams of food samples are needed for testing. Hence sample must be a representative of the whole consignment.

- Improper / wrong sampling procedures nullify the whole analytical chain of analysis and invalidate the results.
- No analytical method to correct the error made during sampling step.
- Measurement Uncertainty (MU) associated with sampling is not calculated.
- Only few grams of food samples are needed for testing.

Hence sample must be a representative of the whole consignment.

**Official control**

Mandatory sampling and testing by EIA:

- Aquaculture Shrimps to EU, Japan, Korea for testing Antibiotics and other chemicals ( if applicable)
- All F&FP consignments to Saudi Arabia for testing Vibrio cholerae
- All F & FPs Products to South Africa for testing Vibrio spp.

Pre-Export Testing (PET) Sampling & Testing by EIC Approved Laboratory

- Cephalopods (Cadmium / Lead) / Histamine Forming Fish (Histamine).

**Sampling personnel**

- ✓ The sampler shall strictly adhere to the sampling **procedure**, sampling **scale** as per the requirement of EIC and importing countries.
- ✓ Sampler should have a clear knowledge and the purpose **of sampling**, including the sampling strategies to be followed.
- ✓ Sampler must be authorized by EIC

**Sampling strategy**

For making composite sample

- Type wise (Raw/Blanched/Cooked)
- Variety wise (Shrimps/Cephalopods /Fish)
- Source Wise (Wild caught /Aquaculture)
- Type of product (Fresh/Chilled & Frozen)
- Statistical formula $(\sqrt{n}+1)/2=x$

  Where, 'n' is the total no. of cartons in one consignment covered in one Health Certificate.

  'x' is the no. of cartons to be drawn at random from which composite sample shall be made for testing.

EIC requirement as follows

- ➢ Traceability on master carton ( e.g. Farm/Pond ID for Aquaculture Shrimps)
- ➢ Max. 4 production codes can be made composite sample.
- ➢ Temperature of the Cold Storage .

- Core temperature of product ( - 18 $^0$C or below)
- Marking on export packages ( e.g. Quality Mark, Approval Number, Production code etc.)

Model consignment details

| Product | Frozen Headless Shell on Vannamei Shrimps Aquaculture | | | | | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|
| Packing | 6X1.8 KGS NET WEIGHT BLOCK FROZEN | | | | | | | | | |
| Grade | | 21/25 | 26/30 | 31/35 | 31/40 | 41/50 | 51/60 | 61/70 | 71/90 | |
| Code | Pond ID | | | | | | | | | |
| 1F29 | AP000265/001 | - | - | 12 | 350 | 105 | - | - | 18 | 485 |
| 1F30 | AP000265/001 | - | - | 414 | 122 | - | 25 | 11 | 32 | 604 |
| 1G22 | AP000265/001 | - | 17 | 17 | - | - | - | - | 24 | 58 |
| 1G23 | AP000285/001 | 16 | 19 | 362 | 35 | 7 | - | - | 34 | 473 |
| 1G24 | AP000265/001 | 3 | 3 | 140 | 39 | - | - | - | 10 | 195 |
| | AP000285/002 | 3 | 4 | 155 | 15 | - | - | - | 8 | 185 |
| TOTAL | --- | 22 | 43 | 1100 | 561 | 112 | 25 | 11 | 126 | 2000 |

| Product | Frozen Headless Shell on Vannamei Shrimps Aquaculture | | | | | | | | | TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|
| Packing | 6X1.8 KGS NET WEIGHT BLOCK FROZEN | | | | | | | | | |
| Grade | | 21/25 | 26/30 | 31/35 | 31/40 | 41/50 | 51/60 | 61/70 | 71/90 | |

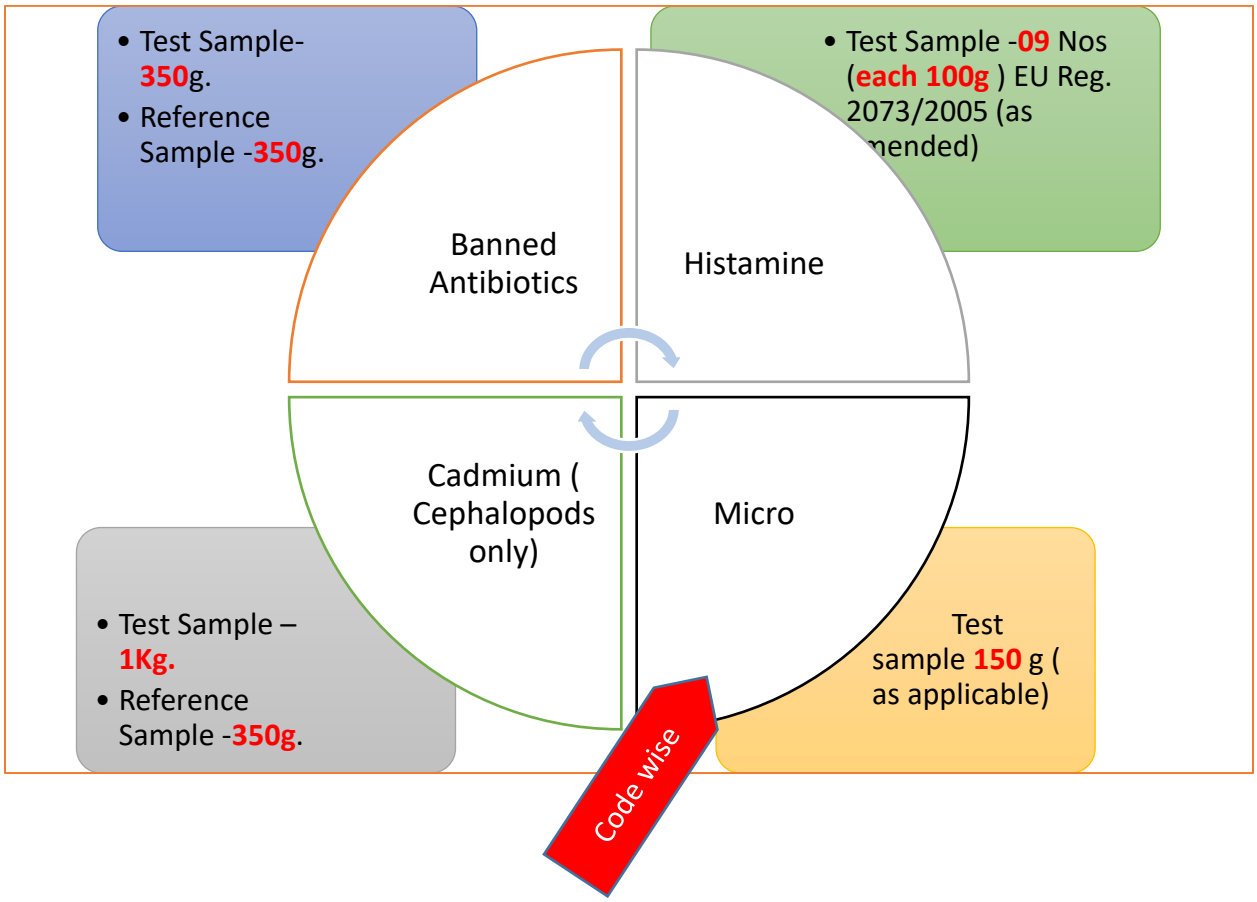
| Code | Pond ID | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1F29 | AP000265/001 | - | - | 12 | 350 | 105 | - | - | 18 | 485 |
| 1F30 | AP000265/001 | - | - | 414 | 122 | - | 25 | 11 | 32 | 604 |
| 1G22 | AP000265/001 | - | 17 | 17 | - | - | - | - | 24 | 58 |
| 1G23 | AP000285/001 | 16 | 19 | 362 | 35 | 7 | - | - | 34 | 473 |
| 1G24 | AP000265/001 | 3 | 3 | 140 | 39 | - | - | - | 10 | 195 |
| | AP000285/002 | 3 | 4 | 155 | 15 | - | - | - | 8 | 185 |
| TOTAL | --- | 22 | 43 | 1100 | 561 | 112 | 25 | 11 | 126 | 2000 |

23 Cartons or 40 Cartons ???

Decimal number ???

No. of composite Sample ???

4+1 or 3+2 ????



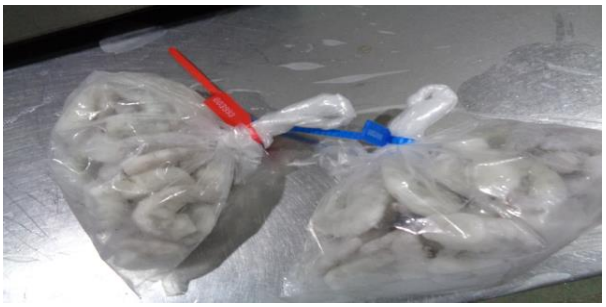


## Sealed master carton

The carton from which samples are drawn shall be sealed by the sampler with date and signature.


## Traceability

## SAMPLE HANDLING & TRANSPORTATION



Sealed Lab & Reference Samples

Packed with adequate Ice

Officially Sealed Sample Box

Note: To ensure that the samples are drawn, transported and received in lab by maintaining chain of custody to comply 98/179/EC.

**Observation during Audit**

- ✓ Inadequate / Not suitable Sampling tools.
- ✓ Reference samples were missing.
- ✓ Unaware of amended regulations of importing countries / EIC requirements.
- ✓ Samples were received with insufficient quantity / inadequate temperature.
- ✓ Seal numbers are not recorded for verification.
- ✓ Seal is not tampering proof.
- ✓ Deviation in sampling scale.

**Conclusion**

- Proper sampling and testing Results reduction of rejection from importing countries

**References**

https://www.fda.gov/food/laboratory-methods-food/bam-chapter-1-food-samplingpreparation-sample-homogenate

https://www.ncbi.nlm.nih.gov/books/NBK235721/

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# IMPORTING COUNTRIES REQUIREMENTS FOR FISH & FISHERY PRODUCTS

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## Introduction

Fish and fishery products are of prime importance of trade and nutritional security. Their contribution to livelihood security and export earnings, fisheries sector has rightfully gained prominence under government initiated programs. A major food safety issue in this highly perishable commodity includes high levels of pathogenic bacteria, parasitic infections, residues of agro-chemicals, veterinary drugs and heavy metal contamination. Inappropriate aquaculture practices, environmental pollution and cultural habits of food preparation and consumption also contribute to build-up of hazards in fishery products. Importing countries requirements are mainly based on food safety requirements of their country. Responsibility of CA is to ensure those food safety requirements are met before issuance of health certificate. Specific requirements for F&FP of EU, Japan, Russia, China, USA, South Africa, Korea, Australia, Saudi Arabia has been explained in this article.

## Country-wise Regulations

- EU Legislations : Regulation/Directives/Decision
- USregulation: 21CFR Part 556 (Residues of Drugs in Food (https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=556 ) & FDA Compliance Program Guidance Manual 7304.018 Chapter 04 – Pesticides And Chemical Contaminants https://www.fda.gov/media/71452/download
- Codex Alimentarius: Codex website for contaminant, residues, microbiological
- China: Food and Agricultural Import Regulations and Standards (e.g GAIN Report No. CH18025 - 2018)
- Russia: TR EAES 040/2016
- Japan: Japan Food Sanitation Law (JETRO)

The European Community (EC) has laid down joint conditions for imports of foodstuffs of animal or plant origin, taking into account of the need not only to protect consumer health but also to

protect the territory of the Union from the introduction of animal or plant diseases. The European Commission's Directorate-General for Health and Consumer Protection (DG SANCO) is responsible for food safety in the EU, it's import rules for fishery products seek to guarantee that all imports fulfil the same high standards as products from the EU Member States, with respect to hygiene and consumer safety and, if relevant, also the animal health status. Hence, it is very important that interested countries and business should understand the fundamental principles and philosophy of the European Food Law, which form the basis for EU import rules, in order to ensure that imports can take place smoothly and efficiently4 . The EU bases its systems on government-to-government assurances, without the intervention of any private type certification, neither standards such as ISO.

**EU Laws**

**Regulation:** It is a binding legislative act. It must be applied in its entirety across the EU. In general, Council adopted a regulation when EU wanted to make sure that there are common safeguards on goods imported from 3rd countries.

**Directives:** It is a legislative act that sets out a goal that all EU countries must achieve. However, it is up to the individual countries to devise their own laws on how to reach these goals.

**Decisions:** It is binding on those to whom it is addressed for a specific case and for specific country.

**EU food safety policy**

Four main areas of protection have been described in EU for food safety are

Food hygiene: food businesses (farms to restaurants) must comply with EU food laws including those imported to EU.

Animal health: sanitary controls and measures for pets, farmed animals and wildlife monitor and manage diseases, and trace the movement of all farm animals.

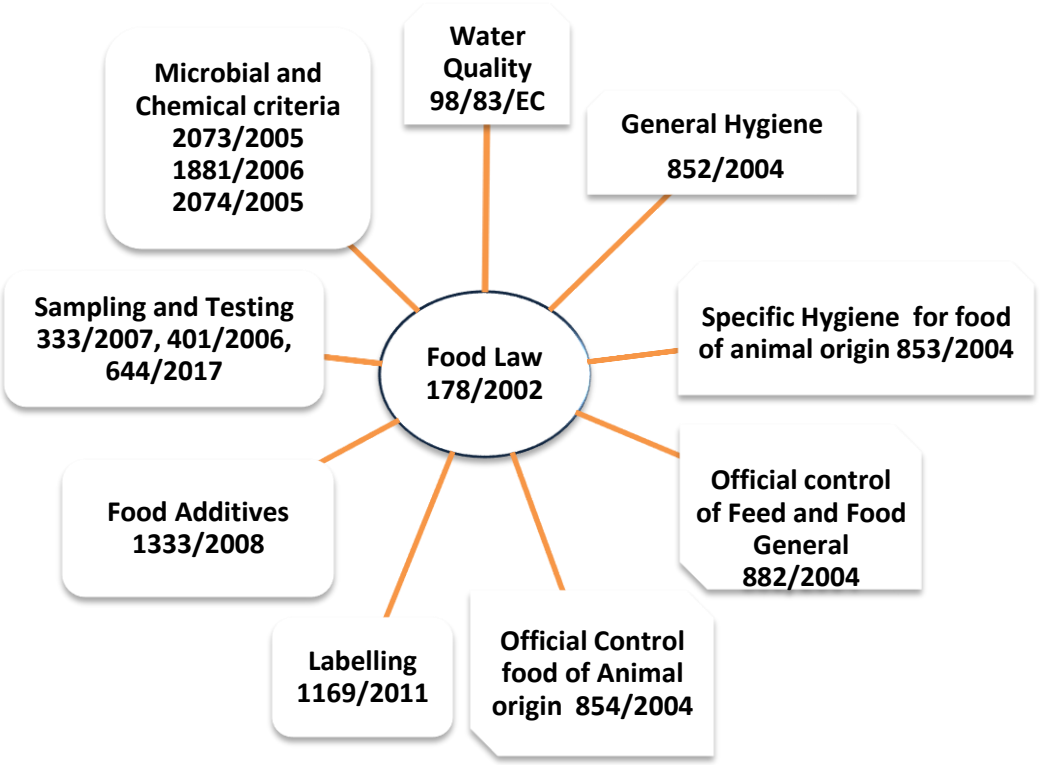
Plant health: detection and eradication of pests at an early stage prevents spreading and ensures healthy seeds.

Contaminants and residues: monitoring keeps contaminants away from food and animal feed. Maximum acceptable limits apply to domestic and imported food and feed products.

**Pre-requisite for import to EU**

- ✓ The country is authorized to export
- ✓ The CA is recognized ( Administrative power to exercice control on FBO and implément of RMP)
- ✓ The establishment has been approved ( approved by CA and en-listed in the EU site)
- ✓ Introduction into the EC via an approved BIP for veterinary checks.
- ✓ Consignment notified in advance
- ✓ Common Veterinary Entry Documents(CVED)
- ✓ Original Sanitary documents ( fax/copy not accepted)

**EU Legislations for food**




**178/2002/EC**

- General food law on the safety of food & feed in EU.
- It applies at all stages of the food chain (production, processing, transport and distribution)
- Foods dangerous to health or unfit for consumption shall not be put for sale.
- Traceability shall be maintained at all stages.
- Rapid Alert System for Food and Feed (RASFF).
- Set up the European Food Safety Authority (EFSA) to provide scientific and technical support on food safety and responsible for coordinating risk assessments, identifying emerging risks and advising EC on crisis management.

**2019/1381/EC**

- ✓ Transparency and sustainability of the EU risk assessment in the food chain amends mainly Regulation (EC) No 178/2002.
- ✓ Ensure more transparency: The public will have automatic access to all studies and information in support of risk assessment.
- ✓ Develop comprehensive risk communication: Ensure a comprehensive risk communication throughout the risk-analysis process, combined with open dialogue amongst all interested parties, verification for serious controversies /conflicting results, fact-finding missions to verify the compliance of laboratories, presenting overview report on outcome of the fact-finding missions etc.

**852/2004/EC**

- Ensure the hygiene of food at all stages of the production process, from the primary production stage (farming/fishing) to the final consumer.
- FBO (other than farming or fishing) shall implement HACCP.
- Where required by national or EU legislation, businesses in the food sector must be approved and all premises registered with the appropriate authority.

This EU law does not cover issues relating to nutrition, composition or quality, or the production or preparation of food in the home.

**853/2004/EC**

- Lays down specific hygiene rules for FBOs of food of animal origin both for processed and unprocessed.
- Aims to ensure a high level of food safety and public health.
- European Union (EU) countries must register and, where necessary, approve establishments handling products of animal origin.
- Rules are applied to all the steps of pre-processing & processing. e.g. Slaughterhouses/cutting and boning, storage, transport and maturation.
- Fishery sectors: harvesting, equipment, facilities, processing and transport.

**2073/2005/EC**

Microbiological criteria for foodstuffs with respect to the general and specific hygiene requirements as per 852/2004/EC on the hygiene of foodstuffs.

Provide objectives and reference points to assist FBO and CA to manage and monitor the safety of food. FBO to ensure that the food they handle, supply or process complies with two criteria.

- food safety criteria
- process hygiene criteria.

It specify which micro-organisms to be tested, sampling plan and analytical method etc.

**315/93/EC**

- Protect public health by prohibiting the marketing of foods containing an unacceptable amount of contaminants
- Contaminants are present in food as a result of treatment after production or through environmental contamination.
- The EU regulates the toxicologically acceptable levels of contaminants and keeps them at the lowest possible levels.
- Must not prohibit trade of foods that comply with this regulation.
- Extraneous matter, such as insect fragments, animal hair, etc. are not covered by this regulation.

**1881/2006/EC**

- ✓ Sets maximum levels for certain contaminants in food that have not been intentionally added to food but have arrived in the course of its production, packaging, transport, etc.
- ✓ Food with higher level of contaminants as specified shall not be sold.
- ✓ These limits cover the edible part of food.
- ✓ Foods complying with the maximum limits may not be mixed with other foods which exceed these limits.

**183/2005/EC**

- Ensure that animal feed is safe and of good quality by ensuring its traceability throughout the entire animal feed chain.
- Compulsory registration of all feed business operators by CA.
- Approval of feed additives, pre-mixtures and compound feeding stuffs.
- Good hygiene practice to be applied at all levels of production and use of feed and implementation of HACCP principles for the production.

**1169/2011/EC (Labeling of foodstuffs)**

- Responsibility lies with the manufacturer and the importer to ensure appropriate food information to the consumers.
- It merges the previous legislation, Directives 2000/13/EC on the labelling of foodstuffs and 90/496/EC on nutritional labeling.
- Mandatory information includes food's name, list of ingredients, net quantity, use by date, instructions for use if applicable, operator's name and address and a nutrition declaration.

**2017/625/EC**

- Official controls to ensure that food and feed laws are enforced to protect human health, animal health & welfare and plant health.
- Sets out a risk-based official control system by the to ensure food safety/integrity/ wholesomeness is maintained throughout production, processing and distribution.
- Sampling, analysis and testing for samples taken during official control activities
- Enforcement action in the event of non-compliance
- Training of staff of the CA and other related authorities.

**Some important recent regulations:**

- 2021/405/EC: Lists of third countries authorized for export to EU

- 2020/466/EC: Temporary measures to contain risks to human, animal and animal welfare due to coronavirus disease.
- 2020/2235/EC: Model official certificates for the entry into the EU and movements within the EU.
- 2019/1014/EC: Detailed rules on minimum requirements for BIP.
- 2019/1871/EC: Set reference point for action ( RPA) for the prohibited substances not having MRL as per 37/2010/EC. RPA would be effective from 28th November 2022
- 2019/624/EC: Specific rules for the performance of official controls on the production of meat and for production and relaying areas of live bivalve.
- 2016/429/EC: The competent authority shall include the certain information in the register of aquaculture establishments
- 396/2005/EC: Maximum residue levels of pesticides in food and feed

**EU - Testing requirements for F&FP**

- Aquaculture products: sampled and tested by EIA for CAP, NFM, TTC, OTC & CTC with 4 epimers.
- Cephalopods: sampled and tested for Cadmium and Lead by EIA/EIC approved labs.
- Histamine forming fishes: sampled & tested for histamine ( 9 samples) by EIA/EIC approved labs.
- Microbiological parameters shall be tested by using ISO methods as laid down in 2073/2005/EC.
- All consignments of Fish Meal / Fish Oil should be tested for Salmonella and enterabacteriaceae and DNA(Bovine, Ovine and Caprine)

**USA Requirement**

PROGRAM | 7304.018

**C. Target Testing Level (TTL)/ Regulatory Action Level (RAL)**

The following values are the current Target Testing Levels (TTL) or tolerance level (TL) for each chemotherapeutic agent. These levels are also considered as Regulatory Action Levels (RAL). However, TTL is not and should not be interpreted as a safe concentration or a tolerance level and it does not imply that an approval exists for that drug [21CFR530.3(g)].

| Animal Drug Residue | Target Testing Level or Tolerance Level (ppb) |
|---|---|
| Chloramphenicol[1] | 0.15 |
| Nitrofurans[1]<br>AOZ metabolite of Furazolidone<br>AMOZ metabolite of Furaltadone<br>SC metabolite of Nitrofurazone<br>AHD metabolite of Nitrofurantoin | <br>0.5<br>0.5<br>0.5<br>0.5 |

- **Salmonella ( No. of samples as per the category of food)**
- **Decomposition**
- **Filth**

**CU Requirements**

- Physical Facility (Sanitary rules and Norms SanPiN No. 6 dated 11-03-1996)
- Common Veterinary and sanitary requirements ( Decision by CU No. 317 dated 18-06-2010)
- Technical regulation( Decision No. 880 Dated 09-12-2011)

**Testing requirements of CU:**

| **Annual testing** | **Consignment wise** |
|---|---|
| PCB, Dioxine and Radionuclide | Heavy metals: Cd, Pb, Hg |
| | Dyes: Crystal violet & leuco cv |
| | Antibiotics: CAP, NFM & TTC |
| | Microbioligical:<br>QMAFAnM (Quantity of mesophilic aerobic and facultative anaerobic microorganisms), *Salmonella, Listeria monocytogenes,*<br>*V. cholerae, Staph aureus, (absent/0.01gm), Coliforms ( absent /0.001gm), V. parahaemolyticus & E.coli* |

**Japan Requirements**

- The main laws controlling entry of food products are the Food Sanitation Law and Quarantine Law.
- Consignment of aquaculture shrimps meant for export to Japan shall be sampled and tested by EIA for CAP, NFM, Pendimethalin and Ethoxyquin**.**

**Saudi Arabia requirements**

- Establishment must be enlisted in the site.
- Product must be sampled and tested by EIA for *Vibrio cholera* (consignment wise 5 composite samples)
- Aquaculture shrimps shall be tested for YHV & WSSV
- In case of fresh/chilled fishery products it must on post facto basis

**Vietnam requirement**

Registration of establishments with the National Agro-Forestry -Fisheries Quality Assurance Department (NAFFQAD), Vietnam

**China- Requirements**

- Establishment must be enlisted in the Chinese site
- Testing Requirements (GAIN report 2018)

  HM ( Cd, Pb, Methyl mercury)

  Banned antibiotics ( only for aquaculture)

Microbiological parameters

- Chemical parameters shall be tested at EIA lab only

**Australia Requirements**

- Product shall be inspected by CA and declaration in the Health Certificate that *"the fish were inspected under the supervision of the Competent Authority"*
- Approval separately for uncooked prawns
- Aquaculture raw shrimps shall be YHV & WSSV tested ( 13 samples each 5 pcs must be drawn for the test)

**Korea Requirements**

- Mandatory health certificate for export of chilled, frozen and live fish
- Aquaculture shrimps shall be sampled and tested by EIA for **Nitrofuran metabolites**
- Aquaculture shrimps shall be tested for **WSSV & YHV**

**South Africa Requirements**

*Product to be tested for Vibrio cholerae, vulnificus & mimicus*

**References:**

https://www.intracen.org/uploadedFiles/intracenorg/Content/Exporters/Exporting_Better/Quality_Management/Redesign/EQB84_Rev%201_eng_Exporting%20Seafood%20to%20the%20EU_FINAL_11.08_Blaha.pdf

http://iifpt.edu.in/olapp/pmfme/upload/mt_handbook_fish.pdf

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# PACKAGING OF FISHERY PRODUCTS

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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.

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# PRIVATE FOOD SAFETY STANDARDS

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Foodborne illnesses and consequent outbreaks have threatened human health since time immemorial. In the last century, with the systematic evolution of food science as a scientific discipline, major emphasis has been made to understand the food safety issues in a holistic manner. With the abundance of scientific and technological progress made in the areas of industrial food manufacturing, biochemical and microbiological aspects of food preservation and especially in the areas of risk analysis, there are cornucopia of food control measures exist today linked to every production process.

Traditional inspection methods that relied heavily on the end product quality assessment has been relegated as an antiquated practice. A gradual shift from reactive to proactive food safety principles has been the hallmark of modern food regulatory landscape. With the emergence of HACCP in 1959, an anticipated risk based quality assurance system was ushered and soon received wide-spread acceptance across the globe. With the ever increasing scale and complexity of the global food supply chain, the regulation of food safety has become a responsibility of both government and private sector. In this context, may private food safety standards have evolved over the years for implementation by seafood industry.

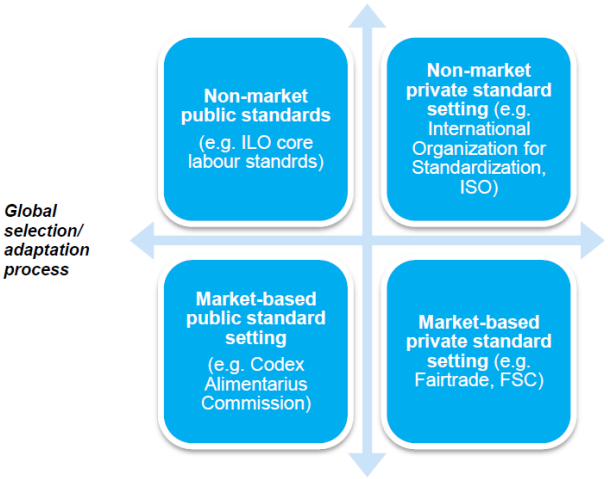
**Role of private food safety standards**

- *Barriers to trade* or *potential catalysts for trade:* The private food safety standards can be both trade facilitating and trade restricting in nature
- *Access new markets* by modernizing supply chains, and implementing management and good production practices

**The standard setting Process**

The international public standard setting process takes into account the following agreements in to consideration:

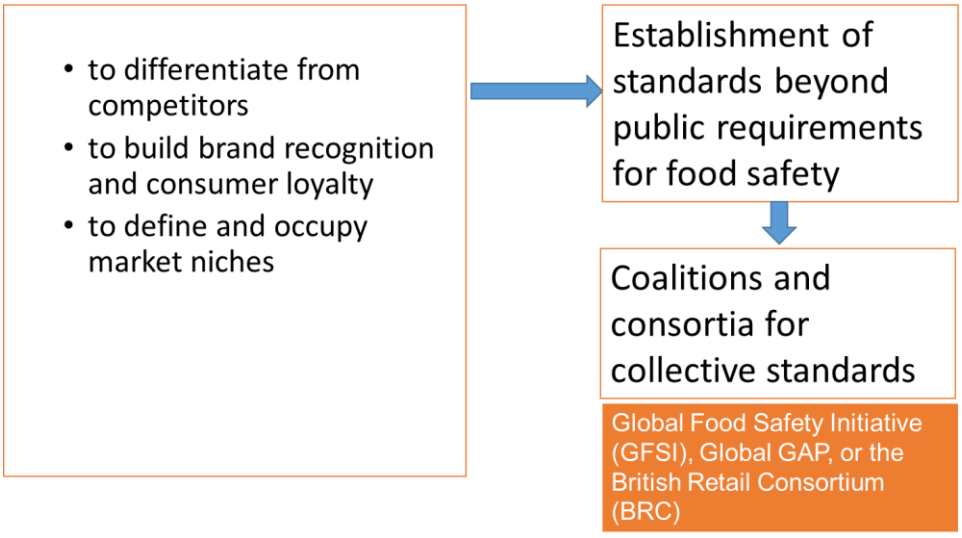
- Sanitary and Phytosanitary (SPS) Agreement
- Technical Barriers to Trade (TBT) Agreement
- Trade Related Intellectual Property Rights (TRIPS) Agreement




**Drivers for the development of private standards**

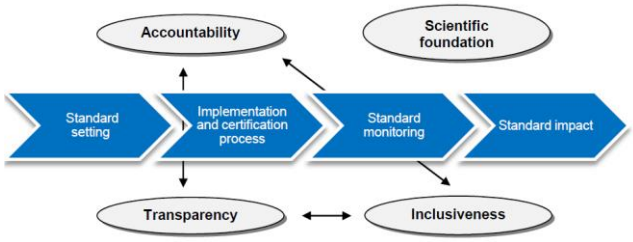
- Increased consumer awareness of the impact of food on health
- Food quality and due diligence requirements assigned to food chain operators
- Growing societal and consumer demand for more responsibly produced goods and information about the production and processing conditions of products.

**Evolution of Private standards**



**Legitimacy of the standards**

The legitimacy of private food safety standards are based upon TRANSPARENCY, INCLUSIVENESS and ACCOUNTABILITY at the different stages of




- standards setting,
- standard implementation and the certification process,
- standard monitoring, and
- the impacts of standards

Private Food Safety Standards in operation

**Manufacturing**

• BRC (British Retail Consortium) Global Standard Version 5

• Dutch HACCP (option B)

• FSSC 22000 (Food Safety System Certification)

• Global Aquaculture Alliance BAP, issue 2 (GAA Seafood Processing Standard)

• Global Red Meat Standard Version 3

• IFS (International Featured Standards) Version 5

• SQF (Safe Quality Food) 2000 Level 2

• Synergy 22000

**Primary production**

- GlobalGAP IFA Scheme Version 3
- Canada Gap
- SQF (Safe Quality Food) 1000 Level
- QS (Qualität und Sicherheit für Lebensmittel vom Erzeuger bis zum Verbraucher)
- SGF/IRMA and SGF/RQCS

**Feed Production**

- FAMI-QS Code of Practice
- GMP+
- TrusQ

**Manufacturing and primary production**:

• PrimusGFS

**Other private standards**

- Fair Trade
- Carbon Trust standard
- Rainforest Alliance
- Marine Stewardship Council
- UTZ certified
- EU logo Organic Farming
- Gluten free
- European V-label
- Halal food
- Kosher Food

**Benchmarking and certification with Food Safety Management System (FSMS)**

Global Food Safety Initiative (GFSI) is a private organization established and managed by the international trade association, the consumers Goods Forum under Belgian Law in 2000. This organization specifies the recognition of food safety certification programmes as per the benchmarked requirements.

**GFSI Recognised Certification Programmes**

- **Categories**

- AI - Farming of Animals
- AII - Farming of Fish
- BI - Farming of Plants
- BII - Farming of Grains and Pulses
- C - Animal Conversion
- D - Pre Processing Handling of Plant Products
- EI - Processing of Animal Perishable Products
- EII - Processing of Plant Perishable Products
- EIII - Processing of Animal and Plant Perishable Products (Mixed Products)
- EIV - Processing of Ambient Stable Products
- F - Production of Feed
- J - provision of Storage and Distribution Services
- L - Production of (Bio) Chemicals
- M - Production of Food Packaging

- Major GFSI Recognised Standards
    - SQF (SQF Code 7th Edition Level 2)
    - GLOBAL GAP (Global GAP Integrated Farm Assurance Scheme version 5, Produce Safety Standard Version 4, Harmonised Produce Standard)
    - PRIMUS GFS
    - IFS
    - FSSC 22000
    - Global Aquaculture Alliance
    - CANADAGAP
    - GRMS (Global Red Meat Standard)
    - BRC Global Standard
    - Global Seafood Alliance (BAP Certification)

# VALIDATION & VERIFICATION OF CHEMICAL TESTING METHODS

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## Introduction

Aquatic environment receives influx of pollutants from many point and non-point sources and considered ultimate sink in the pollutant transport cycle. Other than the environmental pollutants, pesticides and antibiotics are often directly used in aquaculture. Hence, a diverse range of anthropogenic chemicals such as Agricultural pesticides, Antibiotics, Vet drugs, Poly aromatic hydrocarbons, PCBs, Dioxins, Natural Toxins, Formaldehyde, PPCPs, PFAS, PBDE etc. are tested in various fish and fisheries products to ensure food safety and safeguard public health. A typical test method of chemical contaminants analysis involves extraction, cleanup, pre-concentration/dilution, and finally instrumental analysis. All sample preparation and processing procedures should be undertaken within the shortest time practicable to minimise sample decay and pesticide losses. Analyses for residues of very labile or volatile pesticides should be started, and the procedures which could lead to loss of analyte should be completed as soon as possible, but preferably on the day of sample receipt. Sample preparation, sample processing and sub-sampling to obtain portions should take place before any visible deterioration occurs. Only the edible part of the produce is sampled and tested. A clean-up, or dilution step may be necessary to reduce matrix interferences and reduce contamination of the instrument system leading to an improved selectivity and robustness.

Sample extracts are normally analysed using capillary gas chromatography (GC) and/or high performance or ultra-performance liquid chromatography (HPLC or UPLC) coupled to mass spectrometry (MS) for the identification and quantification of pesticides in food and feed samples. Various MS detection systems can be used, such as a single or triple quadrupole, ion trap, time of flight or orbitrap. Typical ionisation techniques are: electron ionisation (EI), chemical ionisation (CI), atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI). Different acquisition modes may be used such as full-scan, selected ion monitoring (SIM), selected reaction monitoring (SRM) and multiple reaction monitoring (MRM). Nowadays, selective detectors for GC (ECD, FPD, PFPD, NPD) and LC (DAD, fluorescence) are less widely used as they offer only limited specificity.

Since the validity of the test result is critical for regulatory control and protecting public health, it is important the test methods are rugged, repeatable, and reproducible. Laboratories often use official test methods of AOAC, AOCS, APHA, USEPA, FDA etc. or even use in house developed method. In house methods needs to be completely validated and official methods must be verified for the intended use in the laboratory. Hence, harmonized protocols for method validation and verification is crucial to ensure reliability of test results produced in different laboratories, in different parts of the world. The Document Nº SANTE/12682/2019, Commission Implementing Regulation (EU) 2021/808, and the Eurachem guideline for method validation are considered gold standards for method validation and verification of analytical food testing methods.

**What is Method Validation?**

Method validation is the process of confirming the method has performance capability as the application requires. It is a process of demonstrating or confirming that a method is suitable for its intended purpose which can be qualitative analysis, quantitative analysis, screening analysis, confirmatory analysis, limit tests, matrix extensions, platform extensions, and emergency/contingency operations. Validation includes demonstrating performance characteristics such as accuracy, precision, sensitivity, selectivity, limit of detection, limit of quantitation, linearity, range, and ruggedness, to ensure that results are meaningful and appropriate to make a decision. Following table lists the various definitions provided by different regulatory agencies.

| References | Validation Definition |
|---|---|
| **Codex CAC/GL 74** | Process to establish the **performance characteristics** and limitations of an analytical method: which analytes, in what kind of matrices, in the presence of which interference. Result = precision and trueness values of a certain analytical method under the examined conditions. |
| **ISO 16140-1** | Establishment of the **performance characteristics** of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled. |
| **USDA FSIS** | Process to measure **performance characteristics** of a particular test, with the goal of determining whether the test is equivalent to the reference test for the intended conditions of use. "Equivalent" = the performance characteristics are statistically indistinguishable. |

| | |
|---|---|
| **US FDA** | Demonstration that adequate confidence is provided when the results obtained by the alternative method i.e. the commercially available kit, are comparable to or exceed those obtained using the reference method using the **statistical criteria** contained in the approved validation protocol. |
| **Health Canada** | Evaluation of the **performance parameters** of a new method in comparison to an accepted reference method using paired or unpaired samples. In the context of relative validation, the results of the reference method are assumed to reflect the true microbiological status of the samples and the performance parameters of the alternative method are calculated in relation to this. |
| **ISO 17025:2005** | The confirmation by examination and the provision of objective evidence that the **particular requirements** for a specific intended use are fulfilled. |

**Identification of analytes and confirmation of results**

*Identification*

*Mass spectrometry coupled to chromatography*

Mass spectrometry coupled to a chromatographic separation system is a very powerful combination for identification of an analyte in the sample extract. It simultaneously provides retention time, mass/charge ratios and relative abundance (intensity) data.

*Requirements for chromatography*

The minimum acceptable retention time for the analyte(s) under examination should be at least twice the retention time corresponding to the void volume of the column. The retention time of the analyte in the extract should correspond to that of the calibration standard (may need to be matrix-matched) with a tolerance of ±0.1min, for both gas chromatography and liquid chromatography. Larger retention time deviations are acceptable where both retention time and peak shape of the analyte match with those of a suitable IL-IS, or evidence from validation studies is available. IL-IS can be particularly useful where the chromatographic procedure exhibits matrix induced retention time shifts or peak shape distortions. Overspiking with the analyte suspected to be present in the sample will also help to increase confidence in the identification.

*Requirements for mass spectrometry (MS)*

MS detection can provide mass spectra, isotope patterns, and/or signals for selected ions. Although mass spectra can be highly specific for an analyte, match values differ depending on the particular software used which makes it impossible to set generic guidance on match values for identification. This means that laboratories that use spectral matching for identification need to set their own criteria and demonstrate these are fit-for-purpose. Guidance for identification based on MS spectra is limited to some recommendations whereas for identification based on selected ions more detailed criteria are provided.

*Recommendations regarding identification using MS spectra*

Reference spectra for the analyte should be generated using the same instruments and conditions used for analysis of the samples. If major differences are evident between a published spectrum and the spectrum generated within the laboratory, the latter must be shown to be valid. To avoid distortion of ion ratios the concentration of the analyte ions must not overload the detector. The reference spectrum in the instrument software can originate from a previous injection (without matrix present), but is preferably obtained from the same analytical batch.

In case of full scan measurement, careful subtraction of background spectra, either manual or automatic, by deconvolution or other algorithms, may be required to ensure that the resultant spectrum from the chromatographic peak is representative. Whenever background correction is used, this must be applied uniformly throughout the batch and should be clearly recorded.

*Requirements for identification using selected ions*

Identification relies on the correct selection of ions. They must be sufficiently selective for the analyte in the matrix being analysed and in the relevant concentration range. Molecular ions, (de)protonated molecules or adduct ions are highly characteristic for the analyte and should be included in the measurement and identification procedure whenever possible. In general, and especially in single-stage MS, high m/z ions are more selective than low m/z ions (e.g. $m/z < 100$). However, high mass m/z ions arising from loss of water or loss of common moieties may be of little use. Although characteristic isotopic ions, especially Cl or Br clusters, may be particularly useful, the selected ions should not exclusively originate from the same part of the analyte molecule. The choice of ions for identification may change depending on background

interferences. In high resolution MS, the selectivity of an ion of the analyte is determined by the narrowness of the mass extraction window (MEW) that is used to obtain the extracted ion chromatogram. The narrower the MEW, the higher the selectivity. However, the minimum MEW that can be used relates to mass resolution. Extracted ion chromatograms of sample extracts should have peaks of similar retention time, peak shape and response ratio to those obtained from calibration standards analysed at comparable concentrations in the same batch. Chromatographic peaks from different selective ions for the analyte must fully overlap. Where an ion chromatogram shows evidence of significant chromatographic interference, it must not be relied upon for identification. Different types and modes of mass spectrometric detectors provide different degrees of selectivity , which relates to the confidence in identification. The requirements for identification are summarised in the following Table.

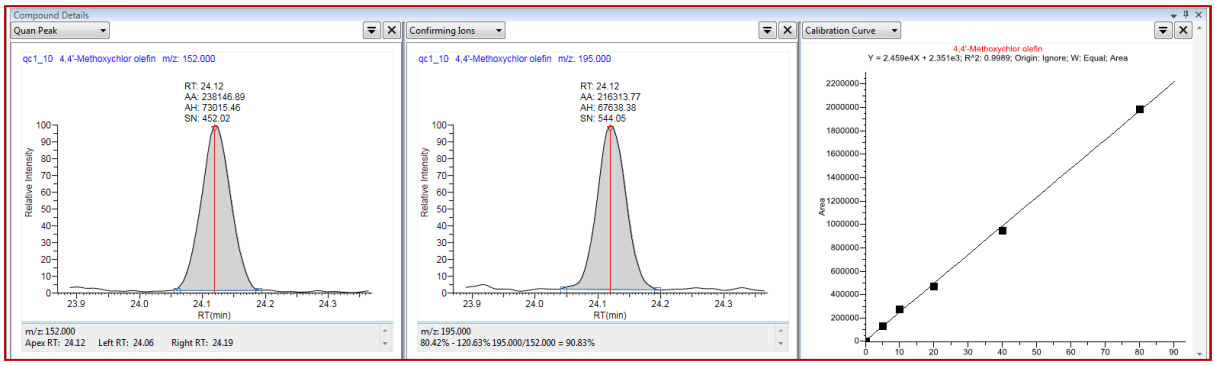
| MS detector/Characteristics | | Acquisition | Requirements for identification | |
|---|---|---|---|---|
| Resolution | Typical systems (examples) | | Minimum number of ions | other |
| Unit mass resolution | Single MS quadrupole, ion trap, TOF | full scan, limited m/z range, SIM | 3 ions | S/N ≥ 3<br>Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap.<br>Ion ratio from sample extracts should be within ±30 % (relative) of average of calibration standards from |
| | MS/MS triple quadrupole, ion trap, Q-trap, Q-TOF, Q-Orbitrap | selected or multiple reaction monitoring (SRM, MRM), mass resolution for precursor-ion isolation equal to or better than unit mass resolution | 2 product ions | |

| | | | | |
|---|---|---|---|---|
| | | | | same sequence |
| Accurate mass measurement | High resolution MS: (Q-)TOF (Q-)Orbitrap FT-ICR-MS sector MS | full scan, limited m/z range, SIM, fragmentation with or without precursor-ion selection, or combinations thereof | 2 ions with mass accuracy ≤ 5 ppm | S/N ≥ 3d Analyte peaks from precursor and/or product ion(s) in the extracted ion chromatograms must fully overlap |

**Method validation parameters and acceptance criteria**

*Sensitivity/Linearity*

Sensitivity of a method implies the lowest possible concentration the method can quantify with satisfactory repeatability and reproducibility. For quantitative analysis a calibration curve is prepared by injecting matrix matched or procedural standards spiked at different concentrations. The following Figure illustrates a typical example:




The lowest calibration level (LCL) must be equal to, or lower than, the calibration level corresponding to the RL. The RL must not be lower than the LOQ. Bracketing calibration must be used unless the determination system has been shown to be free from significant drift, e.g. by monitoring the response of an internal standard. The calibration standards should be injected at least at the start and end of a sample sequence. If the drift between two bracketing injections of the same calibration standard exceeds 30 % (taking the higher response as 100 %) the bracketed samples containing pesticide residues should be re-analysed. Results for those samples that do not contain any of those analytes showing unacceptable drift can be accepted

provided that the response at the calibration level corresponding to the RL remained measurable throughout the batch, to minimise the possibility of false negatives. If required, priming of the GC or LC system should be performed immediately prior to the first series of calibration standard solutions in a batch of analyses. The detector response from the analytes in the sample extract should lie within the range of responses from the calibration standard solutions injected. Where necessary, extracts containing high-level residues above the calibrated range must be diluted and re-injected. If the calibration standard solutions are matrix-matched, the matrix concentration in the calibration standard should also be diluted proportionately. Multi-level calibration (three or more concentrations) is preferred. An appropriate calibration function must be used (e.g. linear, quadratic, with or without weighing). The deviation of the back-calculated concentrations of the calibration standards from the true concentrations, using the calibration curve in the relevant region should not be more than ±20 %. Calibration by interpolation between two levels is acceptable providing the difference between the 2 levels is not greater than a factor of 10 and providing the response factors of the bracketing calibration standards are within acceptable limits. The response factor of bracketing calibration standards at each level should not differ by more than 20 % (taking the higher response as 100 %).

The acceptance criteria for this parameter is that the Deviation of back-calculated concentration from true concentration is ≤± 20 %.

Example:

(Back calculated concentration – True Concentration)*100/True Concentration

(10-9.8)*100/10 = 2%

*Matrix effect*

The matrix effect (ME) is evaluated by comparing peak areas of the matrix matched standards (peak area of post-extraction spike) with the corresponding peak areas of standards in solvent. The ME is quantified as the average percent suppression or enhancement in the peak area using the following equation:

$$\mathrm{ME}\ (\%) = \frac{\text{Peak area of matrix matched standard} - \text{peak area of solvent standard}) \times 100}{\text{Peak area of matrix matched standard}}$$

A negative value of ME signifies matrix induced signal suppression, whereas a positive value signifies an enhancement in signal intensity.

Determination of ME is important where a matrix matched standard is used for quantification, however use of procedural standard nullifies importance of matrix effect toa great extent is considered a better practice.

*Limit of Quantification*

Limit of Quantification (LOQ) is the lowest spike level meeting the identification and method performance criteria for recovery and precision. The LOQ should be less than or equal to MRL.

*Specificity*

Specificity corresponds to interfering signal of the target analyte in matrix blank or procedural blank. Ideally the matrix blank or procedural blank should be free from such interfering signal and that defines the specificity of the method. If such signals are present in the matrix blank, it should be less than or equal to 30% of the reporting limit. A specific method will not have any interference from the reagent blank, however matrix blank may have inherent contamination. In such case standard addition method can be adopted or a fresh set of calibration can be prepared using another related matrix blank.

*Recovery*

Spike recovery needs to determined at three different spike concentration, usually at half the MRL value, at MRL value, and at double the MRL value. The average recovery value of all the spike level tested should fall within 70 to 120%. However, recovery outside this range is acceptable when the repeatability relative standard deviation and reproducibility relative standard deviation is less than or equal to 20%.

*Precision*

Precision indicates the repeatability and reproducibility relative standard deviations of the analytical method. Repeatability $RSD_r$ for each spike level tested should be less than or equal to 20%. Whereas, within-laboratory reproducibility $RSD_{wR}$, derived from on-going method validation/verification should be less than or equal to 20%. $RSD_{wR}$ value are calculated from the recovery studies carried out on different days by different analysts.

*Ion ratio*

Ion ratio is an important criteria for quantitative analysis using mass spectrometers in multiple reaction monitoring mode. Percentage ion ratio is determined by the area ratio of qualifier and quantifier ion. The ion ratio of an analyte in the sample should fall within ±30 % of the average ion ratio of all the calibration levels.

**References for further reading**

1. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed: Document Nº SANTE/12682/2019.
2. Commission Implementing Regulation (EU) 2021/808 of 22 March 2021
   on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

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# Validation of Biological Testing Methods

***Dr. Satyen Kumar Panda***
ICAR- Central Institute of Fisheries Technology, Cochin-682 029
Email: satyenpanda@gmail.com

There are various biological testing methods in vogue and are associated with varied amount of complexities based up on their application.

- Screening and Confirmation methods
- Instrumental: Hybrid Methodology, alternate platforms
- Regulatory approved methods
- Elementary vs Technologically perplexed systems

Microbiological Testing Methods are prone to challenges such as logistical complexities in sampling; heterogeneous distribution of contaminant flora; high level of background flora; interfering ingredients; stress-Injury; viable but Non-culturable State; and high dependence on culture-based methods. The 50% of global testing in microbiology is still carried out in traditional media.

- Complexities of Target organisms
  - Bacteria
  - Fungi
  - Virus
  - Parasites

- Complexities of Methods
  - ISO
  - AOAC
  - USFDA-BAM
  - APHA
  - Health Canada
  - Country-specific NSBs

**Why do we need Validation/Verification of Rapid Food Testing Kits?**

- New tools must perform equal to or better than standard culture based methods
- Rapid tools perform better only in some food matrices

**Guidelines and Standards for Validation/Verification of Microbiological Rapid Food Testing Kits**

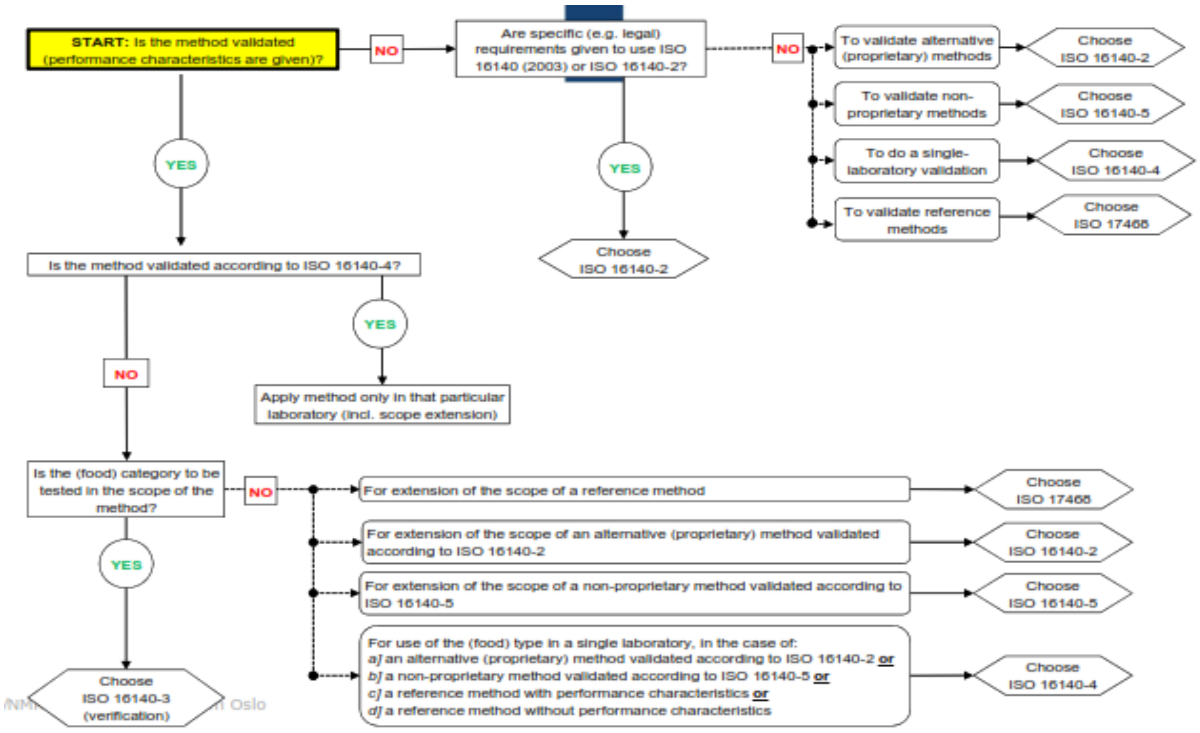
- Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds, Edition 3.0, U.S. Food and Drug Administration Foods Program, October 2019

- AOAC® Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces
- ISO 16140 series of standards

**ISO Standards on Microbiology Method Validation**

- ISO 16140-1:2016 Microbiology of the food chain — Method validation — Part 1: Vocabulary
- ISO 16140-2:2016 Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method
- ISO 16140-3: 2021 Microbiology of the food chain — Method validation — Part 3: Protocol for the verification of reference methods and validated alternative methods in a single laboratory
- ISO 16140-4:2020 Microbiology of the food chain — Method validation — Part 4: Protocol for method validation in a single laboratory
- ISO 16140-5:2020 Microbiology of the food chain — Method validation — Part 5: Protocol for factorial interlaboratory validation for non-proprietary methods
- ISO 16140-6:2019 Microbiology of the food chain — Method validation — Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures
- ISO 17468:2016 Microbiology of the food chain — Technical requirements and guidance on establishment or revision of a standardized reference method

**Selection of appropriate ISO standard for validation**




**Validation of Microbiological Testing Methods as per ISO 16140-2**

During validation comparison is made between a reference method and an alternative protocol. Both for Qualitative and Quantitative methods can be validated using this standard. This comprises of two phases:

Phase I: method comparison study: using diverse food matrices

Phase II: Interlaboratory study: using single food matrix (reproducibility)

**Qualitative Method Comparison Study**

- Paired/Unpaired study
- Sensitivity study
  - 5 food categories; 60 samples
- RLOD study
  - 1 matrix per category, 20 samples per matrix
- Inclusivity/exclusivity study
  - Inclusivity: 50 target cultures (100 for Salmonella)
  - Exclusivity: 30 non-target cultures

**Quantitative Method Comparison Study**

- Relative Trueness Study
  - 5 food categories; 15 samples/category
- Accuracy profile study
  - 5 food categories; 6 samples/category (2low, 2 medium, 2 high)
- Limit of quantification study
  - Used where indirect detection (fluorescence, turbidity); 10 blank
- Inclusivity/exclusivity study
  - Not required for TPC/Y&M count
  - Inclusivity: 50 /Exclusivity:30

**Performance of Interlaboratory Study**

- 10 collaborators; 10 valid data sets
- Three different levels of contamination
- Simulate sample stabilization/stress
- At least 8 blind replicates
- Calculate specificity, sensitivity, relative trueness, false positive ratio
- Interpret with respect to specified acceptability limit

भाकृअनुप - केन्द्रीय मात्स्यिकी प्रौद्योगिकी संस्थान
ICAR - CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY
सिफ्ट जंक्शन, विल्लिंडन आईलंड, मत्स्यपुरी पी.ओ., कोचिन, - 682 029, केरल, भारत।
CIFT Junction, Willingdon Island, Matsyapuri P.O., Cochin, - 682 029, Kerala, India.
*(ISO/IEC 17025: 2005 Accredited & ISO 9001: 2008 Certified)*

# INDIAN TECHNICAL ECONOMIC COOPERATION (ITEC) PROGRAMME
## on
# QUALITY ASSURANCE OF FISH AND FISHERY PRODUCTS

**19 September - 01 October, 2022**

## PROGRAMME SCHEDULE

| Date (Day) | Time (hr) | Topic | Resource person |
|---|---|---|---|
| 19.09. 2022 (Monday) | 10.30-11.00 | **INAUGURATION** | |
| | 11.00-11.30 | Virtual tour of CIFT | Dr. Chandrasekar V. Sr. Scientist ICAR-CIFT |
| | 11.30-12.15 | Introduction to fish preservation techniques | Zynudheen A. A. Principal ScientistICAR-CIFT |
| | 12.15-13.00 | Hygienic handling of fish | Viji P., Sr. Scientist ICAR-CIFT |
| 20.09.2022 (Tuesday ) | 10.30-11.10 | Post-mortem quality changes in fish | Femeena Hassan Principal Scientist ICAR-CIFT |
| | 11.10-11.50 | Spoilage indices in fish and shrimp | Jesmi Debbarma Sr. Scientist ICAR-CIFT |
| | 11.50-12.30 | Value addition in fish and fishery products | L. N. Murthy Principal Scientist ICAR-CIFT |
| | 12.30-13.10 | Sampling of fish & fishery products for international compliance | Sudhansu Sekhar Das EIA, Mangalapuram |
| 21.09.2022 (Wednesday) | 10.30-11.10 | Quality issues in production & export of freeze dried products | Laurette S. |
| | 11.10-11.50 | Tuna Processing: Quality and Safety Requirements | Venugopal T N. |
| | 11.50-12.30 | Development of seaweed-based products and relevant quality issues | Ashish Kumar Jha Sr. Scientist ICAR-CIFT |
| | 12.30-13.10 | Quality and Safety issues in coated fish products: Industry Perspective | Dr. George Ninan Principal Scientist ICAR-CIFT |

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|---|---|---|---|
| **22.09.2022 (Thursday)** | 10.30-11.10 | Good Aquaculture Practices (GAPs) | Varsha Mishra |
| | 11.10-11.50 | Quality issues in fish pickle | Anupama T. K. Scientist ICAR-CIFT |
| | 11.50-12.30 | Non thermal processing of fish | Sarika K., Scientist ICAR-CIFT |
| | 12.30-13.10 | Importing counties requirements for fish & fishery products | Sudhansu Sekhar Das EIA, Mangalapuram |
| **23.09.2022 (Friday)** | 10.30-11.10 | Thermal processing of fish | Mohan.C.O., Sr. Scientist ICAR-CIFT |
| | 11.10-11.50 | Quality issues in live/ fresh/chilled/frozen fish and fishery products | Satyen Kumar Panda Principal Scientist ICAR- CIFT |
| | 11.50-12.30 | Quality issues in dried fishery products | Priya E. R., Scientist ICAR-CIFT |
| | 12.30-13.10 | Quality issues in powdered fish-based products | Femeena Hassan Principal Scientist ICAR-CIFT |
| **24.09.2022 (Saturday)** | 10.30-11.10 | Quality issues in smoked fish products | Sreejith S. Scientist, ICAR-CIFT |
| | 11.10-11.50 | Quality issues in thermally processed fishery products | Remya S., Sr. Scientist, ICAR-CIFT |
| | 11.50-12.30 | Quality issues in fish mince and minced based products | Laly S. J. Sr. Scientist ICAR-CIFT |
| | 12.30-13.10 | Quality issues in convenience fishery products | Pankaj Kishore Scientist ICAR-CIFT |
| **26.09.2022 (Monday)** | 11.30-11.10 | Prerequisite programme (PRPs)- GMP | Femeena Hassan Principal Scientist ICAR-CIFT |
| | 11.10 - 11.50 | Chemical hazards in seafood – 1. Toxins, pesticides, antibiotic residues | Laly S.J Sr. Scientist ICAR-CIFT |
| | 11.50 - 12.30 | Biological hazards -1. Bacteria of Public Health Significance | Ranjit Kumar Nadella Scientist ICAR-CIFT |
| | 12.30 - 13.10 | Advanced microbiological techniques | Ranjit Kumar Nadella Scientist ICAR-CIFT |
| **27.09.2022 (Tuesday)** | 10.30-11.10 | Quality issues in fermented fishery products | Devananda Uchoi, Scientist ICAR-CIFT |
| | 11.10-11.50 | Traceability in Sea foods | Pankaj Kishore, Scientist ICAR- CIFT |
| | 11.50-12.30 | Chemical hazards in seafood – 2. Heavy metals, food additives, adulterants | Priya E.R., Scientist ICAR-CIFT |
| | 12.30-13.10 | Biological hazards in seafood- II | Pankaj Kishore Scientist ICAR- CIFT |
| **28.09.2022 (Wednesday)** | 10.30-11.10 | Physical hazards in seafood | Priya E. R. Scientist ICAR-CIFT |
| | 11.10-11.50 | Principles of HACCP & its implementation in the seafood industry | Devananda Uchoi Scientist ICAR- CIFT |
| | 11.50-12.30 | Overview of ISO 22000:2018 Food Safety Management System | Priya E. R. Scientist ICAR-CIFT |
| | 12.30-13.10 | Implementation of ISO 22000:2018 Food Safety Management System | Laly S.J. Scientist ICAR-CIFT |

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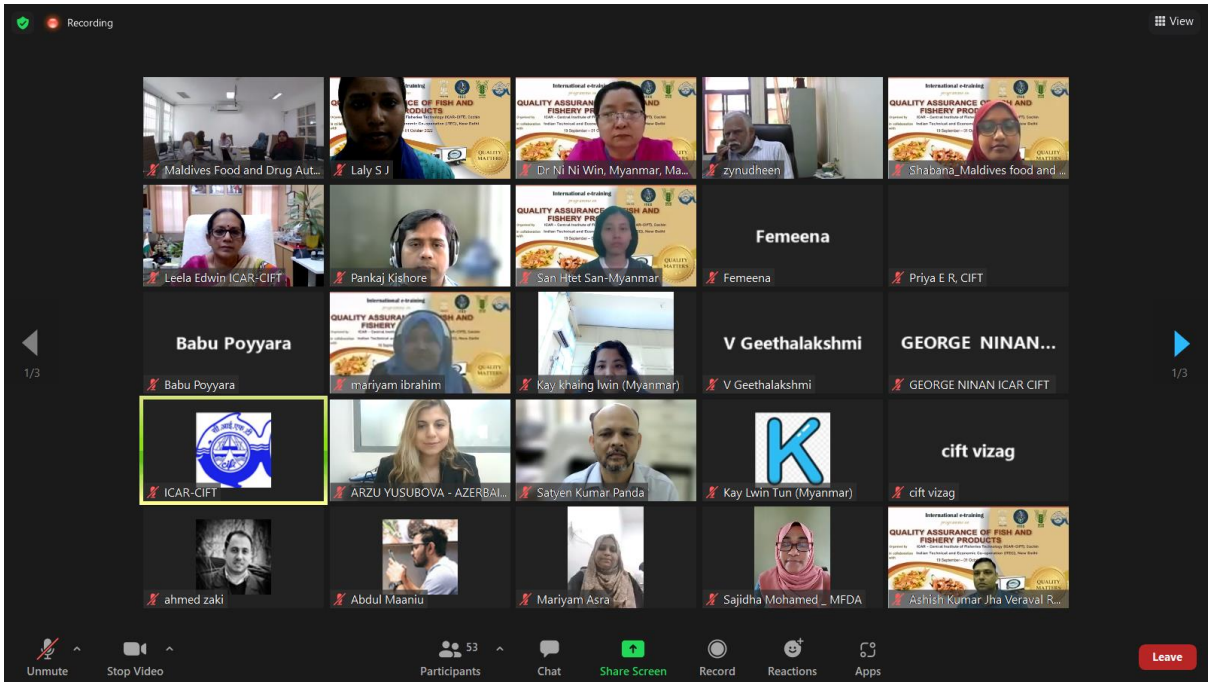
|---|---|---|---|
| **29.09.2022 (Thursday)** | 10.30-11.10 | Prerequisite Programs (SSOP) | Laly S.J<br>Sr. Scientist ICAR-CIFT |
| | 11.10-11.50 | National and international regulations for seafood safety | Satyen Kumar Panda<br>Principal Scientist ICAR-CIFT |
| | 11.50-12.30 | Seafood authenticity | Pankaj Kishore<br>Scientist ICAR-CIFT |
| | 12.30-13.10 | Validation & verification of chemical testing methods | Niladri Sekhar Chatterjee<br>Scientist ICAR-CIFT |
| **30.09.2022 (Friday)** | 10.30-11.10 | Private food safety standards | Satyen Kumar Panda<br>Principal ScientistICAR-CIFT |
| | 11.10-11.50 | Quality issues in fishery byproducts | Zynudheen A.A.<br>Principal Scientist ICAR-CIFT |
| | 11.50-12.30 | Packaging & Labelling requirements of fish products as per international regulations | Bindu J.<br>Principal Scientist ICAR-CIFT |
| | 12.30-13.10 | Validation & verification of Biological testing methods | Satyen Kumar Panda<br>Principal Scientist ICAR-CIFT |
| **01.10.2022 (Saturday)** | 10.30-11.50 | Feedback and VALEDICTORY | |

**** Course schedule is liable to be inter-changed in case of any exigencies.***

| **Course Directors** | |
|---|---|
| **Dr. Leela Edwin**<br>Director, ICAR-Central Institute of Fisheries Technology (CIFT) | |
| **Dr. Zynudheen A.A.**<br>Head, QAM Division, ICAR-CIFT | |
| **Dr. A. K. Mohanty**<br>Head, EIS Division, ICAR-CIFT | |
| **Course Coordinators** | |
| **Dr.Femeena Hassan**<br>Principal Scientist, QAM division | **Dr. Satyen Kumar Panda**<br>Principal Scientist, QAM division |
| **Dr. Laly. S. J**<br>Sr. Scientist, QAM Division | **Dr. Pankaj Kishore**<br>Scientist, QAM Division |
| **Dr.Ranjit Kumar Nadella**<br>Scientist, QAM Division | **Dr. Devananda Uchoi**<br>Scientist, QAM Division |
| **Mrs. Priya E.R.**<br>Scientist, QAM Division | **Dr. Chandrasekar V.**<br>Scientist, EIS Division. |
| Leela Edwin<br>**(Director)**<br>ICAR-CIFT, Cochin, Kerala | |




Inaugural session


ITEC session in progress

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